e-ISSN 2544-1361

European Journal of Clinical and Experimental Medicine

Formerly: Medical Review

Quarterly

Vol. 20, No. 3

Publication date: September 2022

Rzeszów, Poland 2022

EDITOR-IN-CHIEF
Rafał Filip

DEPUTY EDITOR-IN-CHIEF
Justyna Wyszyńska

EXECUTIVE SUBJECT EDITOR
Artur Mazur

LANGUAGE EDITOR
David Aebisher

STATISTICAL EDITOR
Marek Biesiadecki

EDITORIAL ASSISTANT
Sabina Galiniak

EDITIORAL BOARD
Halina Bartosik-Psujek
Dorota Bartusik Aebisher
Ewelina Czenczek-Lewandowska
Małgorzata Nagórska
Łukasz Ożóg

SUBJECT EDITORS

Anthropology: Anna Radochońska (Faculty of Education, Rzeszow University, Rzeszow, Poland)
Cell biology and cell line research: Sara Rosińska (French Institute of Health and Medical Research, Nantes, France)
Chronobiology and sleep disturbance: Tomoko Wakamura (School of Health Sciences, Faculty of Medicine, Kyoto University, Kyoto, Japan)
Clinical psychology and psychopathology: Mieczysław Radochoński (Faculty of Education, Rzeszow University, Rzeszow, Poland)
Ethics: Andrzej Garbarz (Faculty of Education, Rzeszow University, Rzeszow, Poland)
Experimental study and colorectal surgery: Omer Faruk Ozkan (Umraniye Research and Education Hospital, Istanbul, Turkey)
Gastroenterology, hepatology and eating disorders: Józef Ryżko (Carpathian State University in Krosno, Krosno, Poland)
Genetics and molecular biology: Izabela Zawlik (Institute of Medical Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Gynecology, obstetrics and surgery: Grzegorz Raba (Institute of Medical Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Health promotion: Oleh Lyubinets (Department for Public Health Management, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine)
History of medicine: Sławomir Jandziś (Institute of Health Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Human nutrition: Katarzyna Dereń (Institute of Health Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Immunology and experimental treatment: Jacek Tabarkiewicz (Institute of Medical Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Internal medicine: Marek Grzywa (Institute of Medical Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Medical biology: Ahmet Kiziltunc (Department of Biochemistry, Faculty of Medicine, Atatürk University, Erzurum, Turkey)
Medical chemistry: Dorota Bartusik-Aebisher (Institute of Medical Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Medical statistics: Muhammad Asif (Department of Statistics, Government Associate College Qadir Pur Raan Multan, Multan, Pakistan)
Midwifery: Serap Ejder Apay (Health Science Faculty, Atatürk University, Erzurum, Turkey)
Neurology and neurosurgery: Andrzej Maciejczak (Institute of Medical Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Nursing science: Valerie Tothova (Faculty of Health and Social Sciences, University of South Bohemia, Czech Republic)
Occupational therapy: Hanneke Van Bruggen (Occupational Therapy Programme, Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia)
Oncology: Bożenna Karczmarek-Borowska (Institute of Medical Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Oral surgery and dental surge: Bogumił Lewandowski (Institute of Medical Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Orthopedics: Sławomir Snela (Institute of Medical Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Quantitative social research: Attila Sarvary (Faculty of Health Sciences at Nyíregyháza, University of Debrecen, Debrecen, Hungary)
Pediatrics: Bartosz Korczowski (Institute of Medical Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Photochemistry and photobiology: David Aebisher (Institute of Medical Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Physical culture: Piotr Matłosz (Institute of Health Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Public health: Paweł Januszewicz (Institute of Health Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Rehabilitation: Andrzej Kwolek (Institute of Health Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Social medicine: Anna Wilmowska-Pietruszyńska (Institute of Health Sciences, Medical College of Rzeszow University, Rzeszow, Poland)
Sport sciences and pain medicine: Gladson Ricardo Flor Bertolini (Center for Biological and Health Sciences, Western Paraná State University, Cascavel, Paraná, Brazil)

SCIENTIFIC BOARD

Heiner Austrup (Department of Orthopedics, Waldklinik Jesteburg Center for Rehabilitation, Jesteburg, Germany)
Oleg Bilyanskiy (Lviv State University of Physical Culture, Lviv, Ukraine)
Katarzyna Błochowiak (Department of Dental Surgery and Periodontology, Medical University of Poznań, Poland)
Tetyana Boychuk (National University of Vasyl Stefanyk in Ivano-Frankivsk, Ivano-Frankivsk, Ukraine)
Janusz Cwanek (Department of Physiotherapy, University of Information Technology and Management, Rzeszów, Poland)
Jan Czernicki (Department of Rehabilitation, Branch in Piotrków Trybunalski of Jan Kochanowski University in Kielce, Piotrków Trybunalski, Poland)
Ewa Demczuk-Włodarczyk (Faculty of Physiotherapy, Wroclaw University of Health and Sport Sciences, Wroclaw, Poland)
Ulrich Dockweiler (FA Neurology and Psychiatry/Psychotherapy, FA Psychosomatic Medicine and Psychotherapy, Bad Salzuflen, Germany)
Yevhen Dzis (Department of Internal Medicine, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine)

Jakub Gąsior (Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland)
Jean-Michel Gracies (Hospital Henri Mondor, Creteil, France)
Zuzana Hudáková (Department of Physiotherapy, Catholic University in Ružomberok, Ružomberok, Slovakia)
Fuyong Jiao (Children's Hospital of Shaanxi Provincial People's Hospital of Xi'an Jiaotong University, Xi'an, China)
Piotr Kaliciński (Department of Pediatric Surgery and Organ Transplantation, Children's Memorial Health Institute, Warsaw, Poland)
Bartłomiej Kamiński (Otolaryngology Ward of Maria Skłodowska-Curie District Hospital in Skarżysko-Kamienna, Poland)
Andrzej Kawecki (Medical College of Rzeszów University, University of Rzeszow, Rzeszow, Poland)
Andrzej Kleinrok (Department of Nursing, University of Information Technology and Management, Zamość, Poland)
Krzysztof Stanisław Klukowski (Faculty of Rehabilitation, Józef Piłsudski University of Physical Education, Warsaw, Poland)
Romuald Krajewski (Medical College of Rzeszów University, University of Rzeszow, Rzeszow, Poland)
Krystyna Księżopolska-Orłowska (Department of Rheumatology Rehabilitation, Institute of Rheumatology in Warsaw, Poland)
Jolanta Kujawa (Faculty of Health Sciences, Medical University of Lodz, Łódź, Poland)
Maciej Machaczka (Department of Hematology, Karolinska University Hospital, Stockholm, Sweden)
Bartosz Małkiewicz (Department of Minimally Invasive and Robotic Urology, Wroclaw Medical University, Wroclaw, Poland)
Anna Marchewka (Faculty of Rehabilitation, University of Physical Education in Krakow, Krakow, Poland)
Kas Mazurek (Faculty of Education, University of Lethbridge, Lethbridge, Canada)
Gil Mor (Department of Physiology, Wayne State University, Detroit, Michigan, USA)
Serhiy Nyankovskyy (Danylo Halytsky Lviv National Medical University, Lviv, Ukraine)
Grzegorz Panek (Department of Obstetrics and Gynecology, Medical University of Warsaw, Warsaw, Poland)
Marek Pieniążek (Faculty of Rehabilitation, University of Physical Education in Krakow, Krakow, Poland)
Ľudmila Podracká (Department of Medical Chemistry and Biochemistry, P.J. Safárik University, Košice, Slovakia)
Oliver Racz (Pavol Jozef Šafárik University in Košice, Košice, Slovakia)
Jerzy Reymond (Department of Maxillofacial Surgery, Specialist Hospital in Radom, Radom, Poland)
Marek Rudnicki (Ross University School of Medicine, Miramar, Florida, USA)
Ludwika Sadowska (Faculty of Health Sciences, Wroclaw Medical University, Wroclaw, Poland)
Piotr Sałustowicz (University of Applied Sciences, Bielefeld, Germany)
Victor Shatylo (National Academy of Sciences of Ukraine, Kyiv, Ukraine)
Jarosław Sławek (Faculty of Health Sciences, Medical Univeristy of Gdańsk, Gdansk, Poland)
Jerzy Socha (Medical College of Rzeszów University, University of Rzeszow, Rzeszow, Poland)
Carolyn Summerbell (Department of Sport and Exercise Sciences, Durham University, Durham, United Kingdom)
Zbigniew Śliwiński (Faculty of Health Sciences, Collegium Medicum of Jan Kochanowski University in Kielce, Kielce, Poland)
Peter Takač (Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia)
Grzegorz Telega (Medical College of Wisconsin, Milwaukee, USA)
Oleksandra Tomashevska (Danylo Halytsky Lviv National Medical University, Lviv, Ukraine)
Andriy Vovkanych (Lviv State University of Physical Culture, Lviv, Ukraine)
Edward Walczuk (Republican Scientific-Practical Center of Expertise and Medical Rehabilitation, Minsk, Belarus)
Marta Waliszewska-Prosół (Department of Neurology, Wroclaw Medical University, Wroclaw, Poland)
Jerzy Widuchowski (Department of Physiotherapy, The Higher School of Physiotherapy in Wroclaw, Wroclaw, Poland)
Margret A. Winzer (Faculty of Education, University of Lethbridge, Lethbridge, Canada)
Marek Woźniewski (Faculty of Physiotherapy, Wroclaw University of Health and Sport Sciences, Wroclaw, Poland)
Zbigniew K. Wszołek (Mayo Clinic College of Medicine, Rochester, USA)

Technical development, layout and interior design: Wojciech Pączek
Cover design: Wiesław Grzegorczyk

ICV 2021: 100.00
MEiN: 20.00

Indexing:
Ministry of Science and Higher Education (Poland)
Index Copernicus
The Central EuropeanJournal of SocialSciences and Humanities (CEJSH)
POL-Index
Central Medical Library (Poland)
ARIANTA – Science and branch Polish electronic journals
J-Gate
The Directory of Open Access Journals (DOAJ)
Main Medical Library in Warszawa
EBCSO
Scopus

e-ISSN 2544-1361

EDITORIAL CORRESPONDENCE
European Journal of Clinical and Experimental Medicine Editorial Office
35-959 Rzeszów, ul. Kopisto 2A,
tel. 17 851 68 38, fax 17 872 19 30
http://www.ejcem.ur.edu.pl
e-mail: ejcemur@gmail.com
https://mc04.manuscriptcentral.com/pmur

PUBLISHER: PUBLISHING OFFICE OF THE UNIVERSITY OF RZESZÓW
35-959 Rzeszów, ul. prof. S. Pigonia 6,
tel./fax 17 872 14 26, e-mail: wydaw@ur.edu.pl

The graphic form and content of this publication is a work protected by copyright law. Any use of the whole or parts of this form without permission of the publisher constitutes copyright infringement involving criminal and civil prosecution (Article 78,79 et seq. and Article 115 et seq. of the Act of February 4th 1994 on Copyright and Related Rights), regardless of the protection provided by the legislation against unfair competition. It is possible to reprint summaries. The editorial board is not responsible for the content of advertisements.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 250

Contents

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.1

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 251–259

REVIEW PAPER

Monitoring of infliximab treatment in inflammatory bowel diseases – basic knowledge and current data based on clinical trials in a population of Polish patients

Anna Pękala

Department of Gastroenterology, IBD Unit of Clinical Hospital 2, Rzeszow, Poland

ABSTRACT

Introduction and aim. Infliximab is the oldest biological drug belonging to the group of tumor necrosis factor antagonists. Despite the availability of many new biological therapies, this drug still plays an important role in the treatment of inflammatory bowel diseases. However, a significant problem related to pharmacotherapy is the high inter-individual variability of the response.

Material and methods. This study presents results of the research on the treatment with infliximab in the inflammatory bowel disease (IBD) patients including our own experience in Polish IBD patients.

Analysis of the literature. Therapeutic failure while using infliximab can be attributed partly to inadequate serum concentrations of the drug and the development of anti-drug antibodies. Many studies have attempted to find a relationship between the specific level of infliximab and the achieved healing effect. These analyses show that the optimal level of the drug differs depending on the type of disease, its phenotype, and therapeutic goal and that the optimization of infliximab therapy remains an open topic. Two studies involving the population of Polish IBD patients examined the level of infliximab during and after induction, as well as the frequency of anti-drug antibodies. Two studies involving a population of Polish IBD patients examined the level of infliximab during and after induction, as well as the frequency of anti-drug antibodies. These studies demonstrated the need for monitoring infliximab treatment at weeks 6 and 14.

Conclusion. Reactive monitoring is believed to enable the most rational treatment decisions; however, experts also recommend that proactive monitoring should measure infliximab concentrations at the end of induction and at least once during maintenance treatment.

Keywords. infliximab, Crohn's disease, therapeutic drug monitoring, ulcerative colitis

Introduction

The term inflammatory bowel disease (IBD) includes Crohn's disease (CD) and ulcerative colitis (UC). These are chronic disorders of the gastrointestinal tract, and their incidence and prevalence is increasing worldwide. Obtaining disease remission is still a major challenge since no causal therapy is currently available.[1] The pathogenesis of IBD is complex and multifactorial. Disturbance of the immune system and an abnormal response to the intestinal microbiota are the main contributors to pathogenesis, in addition to the influence of environmental factors in a genetically susceptible host. Dysregulation of the immune system leads to epithelial damage and increased inflammation, which is sustained by intestinal bacteria and activated inflammatory cells.[2]

Corresponding author: Anna Pękala, e-mail: apekala@onet.pl

Received: 26.05.2022 / Accepted: 22.06.2022 / Published: 30.09.2022

Pękala A. *Monitoring of infliximab treatment in inflammatory bowel diseases – basic knowledge and current data based on clinical trials in a population of Polish patients*. Eur J Clin Exp Med. 2022;20(3):251–259. doi: 10.15584/ejcem.2022.3.1.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

Conventional therapies for IBD include agents which affect many elements of the inflammatory cascade within the intestines. These include corticosteroids, immunosuppressants such as thiopurines – [azathioprine (AZA), 6-mercaptopurine (6-MP)] and methotrexate (MTX)), and 5-aminosalicylic acid (5-ASA). However, only some patients receiving this treatment are responsive to treatment and achieve remission. A breakthrough in the treatment of inflammatory bowel disease appeared after the introduction of biological drugs having a strong immunomodulatory effect. These are monoclonal antibodies which selectively suppress some cytokines of the inflammatory pathway such as tumor necrosis factor α (TNF-α), some adhesion molecules, or interleukins.[3]

The oldest biological drug is infliximab (IFX), which has been in use for two decades, and is a monoclonal antibody directed against the cytokine TNF-α. Tumor necrosis factor-α is one of the most investigated proinflammatory mediators involved in the pathogenesis of IBD.[4] This cytokine stimulates the acute phase response, cachexia, cytotoxicity, influences the production of interleukins, increases the expression of adhesive molecules, and stimulates the fibroblast proliferation.[5] Studies have shown that levels of TNF are increased in blood, stool, and intestinal mucosa samples of IBD patients and that these levels depend on the clinical disease activity. Anti-TNF monoclonal antibodies induce IBD remission in some patients. Furthermore, Anti-TNF blockade can promote apoptosis of activated T cells, and restore the proper functioning of the intestinal barrier by protecting epithelial cells from apoptosis and tight junction compromise in the gastrointestinal epithelium.[6]

Infliximab and other TNF-α antagonists are safe and highly effective for the induction and maintenance of remission for both UC and CD; however, for most of these drugs, a high inter-individual variability in response is observed.[7] Up to approximately one-third of patients receiving IFX do not respond to induction therapy, while in primary responders, up to approximately 50% lose response to the drug over time and require dose intensification or treatment discontinuation.[8] This severe limitation of TNF antagonist therapy has led to attempts to overcome treatment resistance through Therapeutic Drug Monitoring (TDM), which allows individualization of therapy. Measurement of drug concentrations and optimization of the dosing regimen increases the chance of treatment response. Moreover, it also allows us to avoid unnecessary interventions when the drug concentration is optimal.

Aim

Reactive TDM is recommended in all cases of loss of response (LOR), whereas the role of proactive monitoring is still under investigation. At the same time, more studies have demonstrated its usefulness. This article provides a review of infliximab treatment monitoring information, which is important in clinical practice. Data has been derived from recommendations of gastroenterological societies and from many clinical studies, including studies conducted on Polish IBD patients.

Material and methods

The study analyses numerous articles describing clinical trials and review papers on monitoring infliximab treatment in inflammatory bowel diseases. Two original studies on a group of Polish IBD patients were also included. The study included 84 and 65 patients with IBD treated with the biosimilar infliximab CT-P13 (Remsima) in the 3rd degree IBD center in south-eastern Poland (the city of Rzeszów) between the year 2016 and 2019.

Analysis of the literature

Pharmacokinetics of infliximab

Infliximab is a chimeric human-mouse monoclonal antibody that binds with high affinity to both the soluble and transmembrane forms of human TNF-α. A single intravenous infusion of infliximab produces a dose-proportional increase in the maximum serum concentration (C_{max}).

In most patients, IFX is detectable in the serum within 8 to 12 weeks after a single dose. The mean half-life is 8 to 9.5 days. There are many factors which can affect the concentration of a drug by either increasing or decreasing its clearance. Increased clearance leading to decreased infliximab concentrations may be associated with anti-drug antibodies (ADA), increased inflammatory activity of the disease, increased fecal excretion, low serum albumin concentration, and reduced body mass. Decreased clearance of the drug may occur with concomitant immunosuppression.[9]

Dosage of infliximab and drug therapeutic window

Infliximab is administered at a fixed dose and intervals derived from previous dose-finding studies for IFX.[10,11] According to the summary of product characteristics for IFX and biosimilars, the dosage of IFX is 5 mg/kg of body mass during induction therapy at 0, 2, and 6 weeks followed by 5 mg/kg of body mass every 8 weeks for maintenance therapy. In Crohn's disease, dose escalation up to 10 mg/kg has been shown to restore treatment response. These data are based on the ACCENT I and II studies, which determined the dosing of IFX for Crohn's disease, and the ACT-1 and ACT-2 studies, which analyzed a population of UC patients treated with the original IFX.[12,13] Simultaneously, in a post-hoc analysis of the ACT data from UC patients, the mean serum concentration of IFX in both induction and maintenance therapy was significantly greater in patients with clinical response and mucosal healing than in other

patients. Additionally, subsequent and current studies have shown that increased exposure to IFX is associated with better outcomes in UC.[14]

A recent analysis of numerous studies examining the need for IFX dose escalation in IBD showed that patients with UC more often required dose escalation than patients with Crohn's diseas.[15] However, attempts to alter the dosing regimen and accelerate the induction strategy in severe UC have produced inconclusive results. A small retrospective analysis of 50 patients with severe UC showed that the accelerated induction strategy of IFX reduces the need for an early colectomy.[16] In contrast, a retrospective study and meta-analysis found no association between accelerated IFX induction therapy and lower rates of colectomy in patients with acute severe ulcerative colitis (ASUC) when compared to standard induction therapy.[17] Similarly, it was recently shown that the use of high-dose IFX therapy did not increase 3-month colectomy-free survival in this cohort.[18]

Generally, the therapeutic level of IFX is 3-7 μg/ml, an IFX level < 3 μg/ml is considered sub-therapeutic, while an IFX level > 7 μg/ml is supratherapeutic.[19] In contrast, American guidelines recognize IFX concentrations ≥ 5 μg/ml as target trough levels.[20] However, some patients may require greater levels of the drug. The target level of IFX to achieve endoscopic and clinical remission may range from 8-12 μg/ml. Even greater drug levels have been reported for fistula healing in Crohn's disease, ranging from 18-20 μg/ml.[21,22] Many studies have attempted to associate IFX concentration with a response or clinical, endoscopic, or histological remission. For example, one retrospective study of patients with CD showed that IFX concentrations > 9.8 μg/ml were associated with endoscopic and histological remission.[23] Another study of UC patients showed that greater drug levels were required to achieve histological remission. In that study, histological remission was achieved at a concentration > 10.5 μg/ml and endoscopic remission with an IFX concentration > 7.5 μg/ml.[21]

In a study involving a Polish population, the concentration of biosimilar IFX associated with clinical response and the absence of LOR during a year of treatment was 4.6 μg/ml for CD and 3.1 μg/ml for UC at 14 weeks.[24] For comparison, other studies in CD patients demonstrated a sustained clinical response with an IFX level of at least 3.5 μg/ml or at least 7 μg/ml at week 14.[25,26] In patients with UC, mucosal healing was associated with an IFX concentration of ≥ 5.1 μg/ml at week 14 and ≥ 2.3 μg/ml at week 30. Endoscopic remission was observed at IFX concentrations ≥ 6.7 μg/ml at week 14 and ≥ 3.8 μg/ml at week 30.[27] These differences seem to suggest that optimal IFX levels for response or remission may differ between patients.

During induction therapy, levels of IFX are significantly greater, but a therapeutic window has not yet been established. Large differences in target drug levels are reported depending on the type of disease, its phenotype, and the analyzed therapeutic targets.[28] For CD at week 2, a level of IFX above 16.9 μg/ml may be sufficient to achieve a clinical response and above 20.4 μg/ml for clinical remission at week 14. In contrast, UC patients demonstrated a clinical response to an IFX level > 11.5 μg/ml at 2 weeks and clinical remission at 14 weeks at a level > 15.3 μg/ml.[29]

Investigating the concentration of infliximab and anti-drug antibodies

Various methods exist to measure IFX levels, with the three most commonly used being enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and homogenous mobility shift assay (HMSA). The most common assay for quantifying biopharmaceuticals is ELISA, in which the drug is captured on a plate and detected using a secondary antibody.[30]

For the most reliable assessment of IFX concentrations, minimal measurements are taken, which are measurements occurring just before the next infusion of the drug. A single measurement of IFX concentration may not be sufficient in an individual patient, and it may be necessary to measure sequential trough levels and interpret them in relation to therapeutic response. At the same time, it is necessary to continuously take into account factors which may have influenced the variability of drug exposure, such as changes in the dosing schedule and changes in drug clearance.[31]

For measuring levels of anti-drug antibodies (ADA), different assay types are used, in which the ADA are detected using the labeled biopharmaceutical itself. Usually, IFX concentration is measured in the first stage. If the drug concentration is undetectable or sub-therapeutic, ADA testing is indicated in the second step.

Presence and significance of anti-drug antibodies

ADA may already be detected 2–4 weeks after the first administration of the drug. Two types of ADA can be distinguished: binding antibodies - BAb, which decrease the drug level by increasing drug clearance via immune complex formation and neutralizing antibodies - NAb, which block the pharmacologically active site of the drug. In practice, the distinction between binding and neutralizing antibodies does not matter, since almost all ADA are neutralizing and the available tests do not differentiate their type.[32] For the proper interpretation of the antibody measurement, information on the type of assay used for ADA measurement is needed, which may be a drug-sensitive or non-drug-sensitive assay. Usually, the most available test is ELISA, which is a drug-sensitive test. This test can only quantify the unbound excess of ADA and/or drug; however, it does not detect them when in they are bound with each other.[33] In contrast to

ELISA, drug-tolerant assays will provide a more accurate assessment and detect drug-associated ADA, regardless of IFX level. Because not all ADA lead to decreased drug levels, and clinical efficacy is primarily related to an adequate IFX level, the drug-sensitive ELISA appears to be sufficient in daily practice, although its limitations should be taken into consideration. The test usually gives a positive ADA result only when the drug level is low or undetectable, which in practice means that the drug has not reached a clinically effective threshold. However, obtaining a double-negative result of the drug and antibodies may be a false result, and only the use of a drug-insensitive test allows us to detect ADA.[32]

In a Polish study where the presence of the ADA was assessed via ELISA during induction and maintenance therapy, ADA were present in 20.4% of patients with non-therapeutic CT-P13 levels and 50% of patients with undetectable CT-P13 concentrations, with up to 100% of patients having undetectable drug levels at week 6 of treatment. Only one patient with detectable drug levels had antibodies simultaneously.[34] Reports of the prevalence of ADA against IFX are inconsistent due to the various assay formats used to monitor immunogenicity and the period of treatment in the clinical trial. In general, the reported detection rate for ADA covers a wide range (from 20 to 71.8%).[35] In our study of a polish population, a total of 84 patients with IBD received CT-P13 and were followed-up for an average of 7 months. Overall, 20.4% of patients with non-therapeutic levels of IFX had concomitant antibodies. The percentage of patients with ADA detected during induction treatment was 11.3% compared to 9.6% during maintenance therapy; however, undetectable levels of IFX were a significant risk factor for antibody development and non-response at week 6 of therapy.[34]

Another important element in the assessment of IFX immunogenicity is the estimation of ADA titers. For 1st generation ELISA, a cut-off of 8 μg/ml was established, above which the titer is considered high. This corresponds to 374 ng/ml in 2nd generation ELISA and a cut-off of 119 ng/ml in ready-to-use ELISA kits.[36] A high titer was also defined for RIDASCREEN (R-Biofarm, Germany) and InformTx/Lisa Tracker (Theradiag, France), having a cut-off value of 200 ng/ml, and an antibody assay range of 10-200 ng/ml. For other tests, there are insufficient data to establish an appropriate cut-off value for high titers of anti-IFX antibodies.[37] A high antibody titer is of greater significance; however, some patients can generate an enduring high titer ADA response, while in some patients this response is only temporary. Studies have shown that ADA titers can decrease over time and that detection of ADA may be transient in IBD patients treated with IFX.[38]

ADA may reduce the efficacy of IFX therapy by neutralizing the drug, preventing it from binding to TNF, and by enhancing the clearance rate due to formation of complexes. The presence of ADA against IFX is also associated with a higher risk of infusion reactions. Infusion-related reactions after administration of IFX are the most common adverse effects of the drug and the reported incidence rate varies between 4–15%.[39]

A factor which appears to contribute to adverse events is the formation of very large TNFi-ADA complexes, which tend to be formed only at high ADA concentrations. Although the frequency of antibody production is relatively high, there have been relatively few cases of serious infusion-related reactions. This can be explained by the fact that the majority of TNFi-ADA complexes are small non-immune activating complexes.[40] The low frequency of antibody-related adverse events has also been confirmed by a Polish study. No allergic infusion-related reactions were observed in 9 patients who had antibodies over a broad range of 2.3 to 30 AU/ml and had received another infusion due to delayed antibody response.[34]

Risk factors and prevention of ADA

Infliximab has been shown to be the most immunogenic of all biologic drugs. For comparison, a large meta-analysis showed that of the patients using IFX, 25.3% developed ADAC compared to 14.1% using adalimumab, 6.9% using certolizumab, 3.8% using golimumab and 1.2% using etanercept.[41,42] Factors which increase the risk of formation of antibodies against IFX are a longer disease duration, a higher baseline activity, and not being TNF treatment-naïve.[42] The frequency of antibody and titer detection can vary depending on the IFX dosing regimen and the usage of other medication. Anti-drug antibody formation is also affected by the serum concentration of TNFi. In clinical practice, attempts to overcome immunogenicity led to higher trough levels of IFX . Sufficiently high drug levels have been shown to suppress the immune response toward TNFi, especially in the first three months of treatment.[43] It was demonstrated that upon dose intensification, low concentration ADAs (not detectable using a drug-sensitive assay) disappear in more than half of the patients and are not clinically relevant. Greater ADA concentrations require greater drug doses to maintain the therapeutic effect.[44] At the same time, it has been shown that with appropriately high antibody titers, optimization of the dosage is ineffective. In the absence of detectable IFX, high titers of ADA necessitate a change in therapy.[37]

Many studies have shown that concomitant use of immunosuppressive agents (methotrexate, 6-mercaptopurine, azathioprine, and others) during biological therapy reduced the probability of ADA formation and among biological drugs, this is especially true of IFX.[45] The pharmacokinetic benefits of combination therapy, which lead to greater anti-TNF drug levels and less

ADA production, are most important during the first 12 months of therapy; however, these benefits may also persist beyond this time. The benefits of adding an immunomodulator to anti-TNF therapy are also seen in patients who have previously failed immunomodulator treatment. Immunogenicity is reduced, which would lead to an increase in serum anti-TNF levels, and through concomitant therapy, may contribute to a reduction in disease activity.[46] Studies have shown that both thiopurines and MTX exert beneficial effects on the pharmacokinetics of anti-TNF agents when used in combination therapy with biological drugs.[47]

Reactive monitoring of infliximab treatment

Reactive monitoring of IFX treatment involves measuring drug concentrations in cases of non-response or a decrease in response, usually in a patient who initially responds to treatment and involves maintenance treatment. Knowledge of the IFX level makes it possible to distinguish between patients with normal levels of the drug and patients with non-therapeutic IFX levels, who additionally require measurement for the presence and concentration of ADA. Infliximab treatment algorithms make the management dependent on low or normal drug and antibody levels. Patients having symptoms of active disease and low IFX levels with concomitant high concentrations of antibodies against IFX should switch to another TNF antagonist or another biological drug. Patients having symptoms of active disease, low concentrations of IFX, and absence of antibodies (or having them in low titers) should undergo dosage intensification.[48] Patients having a therapeutic concentration of the drug should be evaluated to confirm the presence of active disease using objective methods such as endoscopic or radiologic examinations. If active disease is confirmed, anti-TNF therapy should be discontinued and a surgical treatment option should be considered. Many studies have confirmed the significant benefits of reactive monitoring during IFX treatment.[49]An alternative to reactive monitoring is empirical dose escalation based on clinical symptoms alone and this has also been shown to be relatively beneficial.[50] However, reactive TDM of biologics is ultimately recommended as the new standard of care as it enables the most rational therapeutic decisions to be undertaken.[37]

An important advantage of TDM is not only the possibility to determine the extent to which treatment should be escalated but also to identify patients who will not benefit from dosage increase due to normal drug levels or the presence of high antibody titers. In clinical practice, this also means a more rational choice for the next drug. Patients who have a secondary loss of IFX efficacy due to high antibody titers are most likely to respond well to another anti-TNF agent. Patients who have therapeutic levels of IFX are also likely to have sufficient levels of the drug to saturate all of the TNF-α, and their disease is mediated by a different inflammatory pathway that should be the new target for therapy. It was also shown that in the case of IFX efficacy loss in the absence of ADA, the response to a second anti-TNF agent is likely to be weaker.[51,52] However, in line with recommendations from the latest 2019 international gastroenterological consensus, IFX should not be discontinued in patients with active disease, unless drug levels exceed 10 μg/ml.[37]

Proactive monitoring in maintenance therapy

Proactive therapeutic concentration monitoring is the measurement of a drug concentration at a determined time point followed by drug titration to a target dose. It involves aiming for a specific serum drug level as an independent treatment target, regardless of the patient's disease activity or response status. It is not part of standard practice, but its use is intended to predict and prevent treatment failure, mainly to prevent secondary loss of response. Studies examining the benefits of conducting proactive TDM have yielded mixed results. A role for proactive TDM of IFX was explored in the landmark studies, TAXIT and TAILORIX; however, superiority over symptom-based dose optimization was not demonstrated. The TAXIT study showed that the 3–7 mg/mL trough concentration after dose escalations results in an improved response in CD patients at a lower drug cost due to dose de-escalations in some patients.[53,54] A prospective randomized trial of 122 biologic-naïve adult patients with active CD found that increasing the IFX dose based on a combination of symptoms, biomarkers, and serum drug concentrations did not lead to corticosteroid-free clinical remission in a larger proportion of patients than increasing the IFX dose based on clinical symptoms alone.[55] However, they observed that in IFX treatment, proactive TCM of IFX often identified patients with low or undetectable trough levels and increased the likelihood of maintaining treatment.[56,57]

At the same time, many analyses were carried out which showed that obtaining a response and remission in IBD is associated with a higher concentration of IFX in the serum.[58] Several later studies have shown not only the benefits but also the advantage of a proactive approach to monitoring IFX concentration in comparison to reactive TDM. These analyses showed that proactive monitoring was associated with better clinical outcomes, which meant greater durability of the drug, less need for IBD-related surgery or hospitalization, and a lower risk of anti-IFX antibodies, and was more cost-effective.[59] In addition, it has been shown that maintaining the therapeutic concentration of IFX allows us to obtain comparable results, regardless of the concomitant use of immunosuppression.[60] This observation suggests that patients receiving IFX monotherapy with

contraindications to immunosuppression will benefit significantly from proactive treatment monitoring. Experts believe that the minimum trough concentration of IFX in patients in remission should be greater than 3 μg/ml and recommend at least one measurement of IFX concentration during maintenance treatment.[37]

Proactive monitoring in infliximab induction therapy

Proactive monitoring in induction treatment involves measuring the concentration of IFX at weeks 2 and 6 before the second and third induction doses, and is indirectly related to the measurement of post-induction concentrations at 14 weeks of treatment. Large post-hoc analyses from ACT1 and 2 and TIALORIX showed that greater levels of IFX during induction therapy at weeks 2 and 6, in both UC and CD patients, are required to achieve endoscopic remission.[61,62]

However, TDM during induction therapy is much less used than in maintenance therapy, both in practice and in clinical trials. Two of our studies conducted on a population of Polish patients related mainly to monitoring during this treatment period. Sixty-five patients (32 with CD and 33 with UC) were recruited for the study with regular measurements during and after the induction period. In addition to the minimum measurements at 6 and 14 weeks, we also assessed the usefulness of indirect measurements at 10 and 12 weeks. Our study showed that with the standard IFX dosage of 5 mg/kg, only 57.6% of UC patients and 68.8% of CD patients achieved the IFX treatment minimum of 3 μg/ml at week 14, although over 80% of both groups showed primary treatment response. In the course of our follow-up, more than half of the UC patients with non-therapeutic drug levels and all CD patients experienced loss of response to treatment or required a dosage increase. No additional benefit was demonstrated from taking indirect measurements at weeks 10 and 12. Our results clearly suggest that patients with non-therapeutic drug levels at week 14 require further monitoring and supervision as they are at high risk of losing response.[25]

During induction, TDM is not currently considered the standard of care, although guidelines from gastroenterological societies indicate the advisability of measuring IFX levels at 14 weeks in all patients.[37]

It is currently recommended to aim for a target of 7-10 μg/ml. In patients with a high initial inflammatory load [e.g. ASUC or CD with anal fistulas], it is also recommended to measure IFX levels earlier in induction and aim for higher target drug concentrations at these time points: week 2 [20-25 μg/ml] and week 6 [10-15 μg/ml].[63] The possibility of early antibody detection is an additional benefit of proactive monitoring of IFX treatment during induction.

In a study involving a population of Polish IBD patients treated with biosimilar IFX (CT-P13), the presence of ADA detected by ELISA was examined during induction and maintenance treatment. A total of 84 IBD patients received CT-P13 and were followed on average for 7 months. The percentage of people with antibodies detected during induction treatment was 11.3% compared to 9.6% during maintenance treatment. The study showed a statistically significant relationship between undetectable levels of CT-P13 and the presence of ADA at week 6 of therapy (ADA was detected in all patients with undetectable levels of CT-P13, p=0.381). Patients with IBD and undetectable levels of CT-P13 prior to the third induction dose were at high risk for the presence of ADA as well as primary non-response.[34]

Conclusion

Use of therapeutic drug monitoring of biopharmaceuticals to personalize treatment is an important new standard having an impact on IBD therapy. Despite many studies on the determination of the therapeutic window for IFX, the optimal trough concentration of IFX remains unclear and falls within a very wide range, making effective monitoring-based therapy increasingly important. In everyday practice, a limitation may also be the availability of tests for measuring the level of IFX and antibodies, making it impossible to obtain a quick result. Recent developments in point-of-care testing are very promising, which determine the concentrations of IFX and ADA within minutes and will enable real-time TDM .

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, A.P.; Writing – Original Draft Preparation, A.P.; Writing – Review & Editing, A.P.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

References

1. Pasternak G, Aebisher D, Filip R, Bartusik-Aebisher D. Inflammatory bowel disease: clinical aspects. *Eur J Clin Exp Med*. 2018;16(4):341–345.
2. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature*. 2011;474(7351):307–317.
3. Queiroz NSF, Regueiro M. Safety considerations with biologics and new inflammatory bowel disease therapies. *Curr Opin Gastroenterol*. 2020;36(4):257–264.

4. Hemperly A, Vande Casteele N. Clinical Pharmacokinetics and Pharmacodynamics of Infliximab in the Treatment of Inflammatory Bowel Disease. *Clin Pharmacokinet*. 2018;57(8):929–942.
5. Baert FJ, Rutgeerts PR. Anti-TNF strategies in Crohn's disease: mechanisms, clinical effects, indications. *Int J Colorectal Dis*. 1999;14(1):47–51.
6. Ngo B, Farrell CP, Barr M. Tumor necrosis factor blockade for treatment of inflammatory bowel disease: efficacy and safety. *Curr Mol Pharmacol*. 2010;3(3):145–152.
7. Pękala A, Aebisher D, Pardak P, Filip R. Efficacy and safety of biosimilar IFX (CT-P13) and adalimumab in patients with active fistulizing perianal Crohn's disease naïve to anti-TNF therapy: preliminary results from the POLIBD study. *Eur J Clin Exp Med*. 2019;17(1):12–15.
8. Gisbert JP, Panés J. Loss of response and requirement of infliximab dose intensification in Crohn's disease. A review. *Am J Gastroenterol*. 2009;104:760–777.
9. Sandborn WJ. Tailoring treatment to the individual patient: drug monitoring. *Dig Dis*. 2014;32(Suppl 1):88–91.
10. Targan SR, Hanauer SB, van Deventer SJ, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N Engl J Med*. 1997;337:1029–1035.
11. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med*. 2005;353:2462–2476.
12. Kamm MA. Review article: biological drugs in Crohn's disease. *Food Pharmacy*. 2006;24(Suppl 3):80–89.
13. Adedokun O, Sandborn W, Feagan GB, et al. Association between serum concentration of infliximab and efficacy in adult patients with ulcerative colitis. *Gastroenterology*. 2014;147(6):1296–1307.
14. Vande Casteele N, Jeyarajah J, Jairath V, Feagan BG, Sandborn WJ. Infliximab Exposure-Response Relationship and Thresholds Associated With Endoscopic Healing in Patients With Ulcerative Colitis. *Clin Gastroenterol Hepatol*. 2019;17:1814–1821.
15. Guberna L, Nyssen O, Chaparro M, Gisbert J. Frequency and Effectiveness of Empirical Anti-TNF Dose Intensification in Inflammatory Bowel Disease: Systematic Review with Meta-Analysis. *J Clin Med*. 2021;10(10):2132.
16. Gibson DJ, Heetun ZS, Redmond CE, et al. An Accelerated Infliximab Induction Regimen Reduces the Need for Early Colectomy in Patients with Acute Severe Ulcerative Colitis. *Clin Gastroenterol Hepatol*. 2015;13:330–335.
17. Nalagatla H, Falloon K, Tran G, et al. Effect of Accelerated Infliximab Induction on Short -and Long- term Outcomes of Acute Severe Ulcerative Colitis: A Retrospective Multicenter Study and Meta-analysis. *Clin Gastroenterol Hepatol*. 2019;17(3):502–509.
18. Chao C, Al Khoury A, Aruljothy A, et al. High-Dose Infliximab Rescue Therapy for Hospitalized Acute Severe Ulcerative Colitis Does Not Improve Colectomy-Free Survival. *Dig Dis Sci*. 2019;64(2):518–523.
19. Moore C, Corbett G, Moss AC. Systematic review and meta-analysis: serum infliximab levels during maintenance therapy and outcomes in inflammatory bowel disease. *J Crohns Colitis*. 2016;10:619–625.
20. Feuerstein JD, Nguyen GC, Kupfer SS, Falck-Ytter Y, Singh S. American Gastroentero-logical Association Institute Clinical Guidelines Committee, American Gastroenterological Association Institute Guideline on Therapeutic Drug Monitoring in Inflammatory Bowel Disease. *Gastroenterology*. 2017;153:827–834.
21. Papamichael K, Rakowsky S, Rivera S, Cheifetz AS, Osterman MT. Infliximab trough concentrations during maintenance therapy are associated with endoscopic and histologic healing in ulcerative colitis. *Aliment Pharmacol Ther*. 2018,47(4):478–484.
22. Yarur AJ, Kanagala V, Stein DJ, et al. Higher infliximab trough levels are associated with perianal fistula healing in patients with Crohn's disease. *Aliment Pharmacol. Ther*. 2017;45:933–940.
23. Papamichael K, Rakowsky S, Rivera C, Cheifetz A, Osterman M. Association Between Serum Infliximab Trough Concentrations During Maintenance Therapy and Biochemical, Endoscopic, and Histologic Remission in Crohn's Disease. *Inflamm Bowel Dis*. 2018;15;24(10):2266–2271.
24. Pękala A, Filip R. Levels of Biosimilar Infliximab during and after Induction Treatment in Crohn's Disease and Ulcerative Colitis—A Prospective Polish Population Study. *J Clin Med*. 2021;10(22):5311.
25. Cornillie F, Hanauer SB, Diamond RH, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: A retrospective analysis of the ACCENT I trial. *Gut*. 2014;63:1721–1727.
26. Kennedy NA, Heap GA, Green HD, et al. Predictors of anti-TNF treatment failure in anti-TNF-naive patients with active luminal Crohn's disease: A prospective, multicentre, cohort study. *Lancet Gastroenterol Hepatol*. 2019;4:341–353.
27. Casteele NV, Jeyarajah J, Jairath V, Feagan BG, Sandborn WJ. Infliximab Exposure Response Relationship and Thresholds Associated with Endoscopic Healing in Patients with Ulcerative Colitis. *Clin Gastroenterol Hepatol*. 2019;17:1814–1821.
28. Papamichael K, Cheifetz A. Letter: Infliximab concentrations during induction therapy - one size doesn't fit all. *Aliment Pharmacol Ther*. 2018;47(9):1334–1335.
29. Gonczi L, Vegh Z, Golovics PA. Prediction of short- and medium-term efficacy of biosimilar infliximab therapy. Do trough levels and antidrug antibody levels or clinical and biochemical markers play the more important role? *J Crohns Colitis*. 2017;11:697–705.
30. Darrouzain F, Bian S, Desvignes C. Methods for measuring serum concentrations of monoclonal antibodies and anti-biopharmaceutical antibodies in patients; how

to measure accurately and clinically relevant levels. *Ther Drug Monit*. 2017;39(4):316–321.
31. Dreesen E, Bossuyt P, Mulleman D, Gils A, Pascual-Salcedo D. Practical recommendations for the use of therapeutic drug monitoring of biopharmaceuticals in inflammatory diseases. *Clin Pharmacol*. 2017;3(9):101–111.
32. Atiqi S, Hooijberg F, Loeff F, Rispens T, Wolbink GJ. Immunogenicity of TNF-Inhibitors. *Front Immunol*. 2020;11:312.
33. Nechansky A. HAHA–nothing to laugh about. Measuring the immunogenicity (human anti-human antibody response) induced by humanized monoclonal antibodies applying ELISA and SPR technology. *J Pharm Biomed Anal*. 2010;51:252–254.
34. Pękala A, Filip R, Aebisher D. Anti-Drug Antibodies in Patients with Inflammatory Bowel Diseases Treated with Biosimilar Infliximab: A Prospective Cohort Study. *J Clin Med*. 2021;10:2653.
35. Casteele VN, Khanna R, Levesque BG, et al. The relationship between infliximab concentrations, antibodies to infliximab and disease activity in Crohn's disease. *Gut*. 2015;6:1539–1545.
36. Imbrechts M, Van Stappen T, Compernolle G, Tops S, Gils A. Anti-infliximab antibodies: How to compare old and new data? *J Pharm Biomed Anal*. 2022;177:112842.
37. Papamichael K, Cheifetz AS, Melmed GY, et al. Appropriate Therapeutic Drug Monitoring of Biologic Agents for Patients With Inflammatory Bowel Diseases. *Clin Gastroenterol Hepatol*. 2019;17(9):1655–1668.
38. Vande Casteele N, Gils A, Singh S, et al. Antibody response to infliximab and its impact on pharmacokinetics can be transient. *Am J Gastroenterol*. 2013;108:962–971.
39. Bloem K, Hernandez-Breijo B, Martinez-Feito A, Rispens T. Immunogenicity of therapeutic antibodies: monitoring antidrug antibodies in a clinical context. *Ther Drug Monit*. 2017;39:327–332.
40. Van Schie KA, Kruithof S, Ooijevaar-de Heer P, et al. Restricted immune activation and internalisation of anti-idiotype complexes between drug and antidrug antibodies. *Ann Rheum Dis*. 2018;77:1471–1479.
41. Thomas SS, Borazan N, Barroso N, et al. Comparative immunogenicity of TNF inhibitors: impact on clinical efficacy and tolerability in the management of autoimmune diseases. a systematic review and meta-analysis. *Bio Drugs*. 2015;29:241–258.
42. Dirks NL, Meibohm B. Population pharmacokinetics of therapeutic monoclonal antibodies. *Clin Pharmacokinet*. 2010;49:633–659.
43. Drobne D, Bossuyt P, Breynaert C, et al, Withdrawal of immunomodulators after co-treatment does not reduce trough level of infliximab in patients with Crohn's disease. *Clin Gastroenterol Hepatol*. 2015;13(3):514–521.
44. Van Stappen T, Vande Casteele N, Van Assche G, Ferrante M, Vermeire S, Gils A. Clinical relevance of detecting anti-infliximab antibodies with a drug-tolerant assay: post hoc analysis of the TAXIT trial. *Gut*. 2018;67(5):818–826.
45. Ben-Horin S, Waterman M, Kopylov U, et al. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol*. 2013;11(4):444–447.
46. Ward MG, Irving PM, Sparrow MP. How should immunomodulators be optimized when used as combination therapy with anti-tumor necrosis factor agents in the management of inflammatory bowel disease? *World J Gastroenterol*. 2015;21(40):11331–11342.
47. Vermeire S, Noman M, Van Assche G, Baert F, D'Haens G, Rutgeerts P. Effectiveness of concomitant immunosuppressive therapy in suppressing the formation of antibodies to infliximab in Crohn's disease. *Gut*. 2007;56:1226–1231.
48. Sandborn WJ. Tailoring treatment to the individual patient: drug monitoring. *Dig Dis*. 2014;32(Suppl 1):88–91.
49. Guidi L, Pugliese D, Panici Tonucci T, et al. Therapeutic drug monitoring is more cost-effective than a clinically-based approach in the management of loss of response to infliximab in inflammatory bowel disease: an observational multi-centre study. *J Crohns Colitis*. 2018;12(9):1079–1088.
50. Guberna L, Nyssen OP, Chaparro M, Gisbert JP. Frequency and Effectiveness of Empirical Anti-TNF Dose Intensification in Inflammatory Bowel Disease: Systematic Review with Meta-Analysis. *J Clin Med*. 2021;10(10);2132.
51. Bartelds GM, Wijbrandts CA, Nurmohamed MT, et al. Anti-infliximab and anti-adalimumab antibodies in relation to response to adalimumab in infliximab switchers and anti-tumour necrosis factor naive patients: a cohort study. *Ann Rheum Dis*. 2010;69:817–821.
52. Qiu Y, Chen BL, Mao R, et al. Systematic review with meta-analysis: Loss of response and requirement of anti-TNF alpha dose intensification in Crohn's disease. *J Gastroenterol*. 2017;(52):535–554.
53. D'Haens GR, Vermeire S, Lambrecht G, et al. 692 drug-level based dosing versus symptom-based dose adaptation in patients with Crohn's disease: a prospective, randomized multicenter study (TAILORIX). *Gastroenterology*. 2016;150(4):143.
54. Vande Casteele NV, Ferrante M, Van Assche G, et al. Trough Concentrations of Infliximab Guide Dosing for Patients with Inflammatory Bowel Disease. *Gastroenterology*. 2015;148:1320–1329.e3.
55. D'Haens D, Vermeire S, Lambrecht G, et al. Increasing Infliximab Dose Based on Symptoms, Biomarkers, and Serum Drug Concentrations Does Not Increase Clinical, Endoscopic, and Corticosteroid-Free Remission in Patients With Active Luminal Crohn's Disease. *Gastroenterology*. 2018;154(5):1343-1351.e1.
56. Van Hoeve K, Dreesen E, Hoffman I, et al. Higher infliximab trough levels are associated with better outcome in

paediatric patients with inflammatory bowel disease. *J Crohns Colitis.* 2018;12:1316–1325.

57. Vande Casteele N, Jeyarajah J, Jairath V, Feagan BG, Sandborn W. Infliximab exposure-response relationship and thresholds associated with endoscopic healing in patients with ulcerative colitis. *Clin Gastroenterol Hepatol.* 2019;17(9):1814-1821.e1
58. Vaughn BP, Martinez-Vazquez M. Patwardhan VR, Moss AC, Sandborn WJ, Cheifetz AS, Proactive therapeutic concentration monitoring of infliximab may improve outcomes for patients with inflammatory bowel disease: results from a pilot observational study. *Inflamm Bowel Dis*. 2014;20(11):1996–2003.
59. Papamichael K, Chachu KA, Vajravelu RK, et al. Improved long-term outcomes of patients with inflammatory bowel disease receiving proactive compared with reactive monitoring of serum concentrations of infliximab. *Clin Gastroenterol Hepatol.* 2017;15:1580–1588.
60. Colombel JF, Adedokun OJ, Gasink C, et al. Combination therapy with infliximab and azathioprine improves infliximab pharmacokinetic features and efficacy-a post-hoc analysis. *Clin Gastroenterol Hepatol*. 2018;17(8):1525-1532.e1.
61. Dreesen E, Baert F, Laharie D, et al. Monitoring a Combination of Calprotectin and Infliximab Identifies Patients With Mucosal Healing of Crohn's Disease. *Clin Gastroenterol Hepatol*. 2020;18:637–646.
62. Vande Casteele N, Jeyarajah J, Jairath V, Feagan BG, Sandborn WJ. Infliximab exposure-response relationship and thresholds associated with endoscopic healing in patients with ulcerative colitis. *Clin Gastroenterol Hepatol.* 2019;17:1814–1821.
63. Sparrow MP, Papamichael K, Ward MG, et al. Therapeutic Drug Monitoring of Biologics During Induction to Prevent Primary Non-Response, *J Crohns Colitis*. 2020;14(4):542–556.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.2

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 260–265

REVIEW PAPER

Valeriana officinalis – a review

Wiktoria Mytych [1], David Aebisher [2]

[1] English Division Science Club, Medical College of The University of Rzeszow, Rzeszow, Poland
[2] Department of Photomedicine and Physical Chemistry, Medical College of The University of Rzeszow, Rzeszow, Poland

ABSTRACT

Introduction and aim. *Valeriana officinalis* has been recognized in traditional medicine and used since ancient times for a variety of health ailments. It is mainly appreciated for its sedative and sleeping properties. Currently, scientists are conducting numerous studies on the exact chemical composition of valerian and the properties they carry in the human body.

Material and methods. This paper presents a narrative review on valerian.

Analysis of the literature. The desire to summarize information on the uses and properties of *V. officinalis* is presented. *V. officinalis* exhibits sedative, sleep-inducing and antidepressant properties. Studies show broad effects on the human nervous system, for example, reducing stress.

Conclusion. By discovering new properties of valerian, its properties are expanding significantly day by day. Its main use is primarily in the treatment of sleep disorders and nervous system disorders. However, it is also used in headaches, depression, anti-cancer therapy, urinary and digestive disorders. More and more people are turning to valerian as an alternative to drugs that have more side effects.

Keywords. insomnia, valerian, valeriana, *valeriana officinalis*

Introduction

Valeriana officinalis was used as early as in ancient Greece and Rome by Galen and Hippocrates to treat various ailments such as digestive problems and urinary tract diseases, but also in the 16th century for accelerated heartbeat, headaches and nervousness (Fig. 1). According to the WHO, "Traditional medicine is used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (WHO, 2000).

The word valerian comes from valere (Latin), which translates as "to be in good health".[1] Over 200 different species of valerian are known worldwide. The most common in Europe and North America is *V. officinalis*, which is also known as valerian in colloquial speech. Valerian essence is obtained from the roots of this plant.[2] Nowadays, valerian is commonly used as a sleep aid and stress reliever.[1] This natural extract is often prescribed and recommended for sleep disorders. Insomnia, which affects more and more people in modern society, is a sleep disorder in which patients have difficulty falling asleep and staying asleep. The role of sleep is not completely understood. We know that it has a key function in the body's physiological processes, recovery and mood.[3] This problem affects approximately 30% worldwide.[4] In the treatment of insomnia, mainly sedative-hypnotic drugs are used. However, due to their numerous side effects they are very often replaced by herbs, which are a good alternative to the adverse effects of drugs.[2] This medicinal herb has sedative, hypnotic and anxiolytic effects that are supposed to improve the process of falling asleep and relieve tension in the

Corresponding author: Wiktoria Mytych, e-mail: wiktoriamytych@gmail.com

Received: 8.05.2022 / Revised: 30.06.2022 / Accepted: 10.07.2022 / Published: 30.09.2022

Mytych W, Aebisher D. *Valeriana officinalis – a review*. Eur J Clin Exp Med. 2022;20(3):260–265. doi: 10.15584/ejcem.2022.3.2.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

nervous system.[2,5] Mirzaee et al. in 2015 in their study also proved the effectiveness of valerian in migraine headaches.[6] Serotonergic effects similar to antidepressants have also been seen.[7] Researchers have been interested in the potential of valerian in cancer therapy. They believe it may affect cancer cell death.[8] Side effects of valerian are rare, mostly mild and short-lived. These mainly include dizziness or nausea.[1] However, the U.S. Food and Drug Administration recognized valerian as "generally safe" for use.[9] According to the European Medicine Agency, valerian root essence relieves nervous tension and sleep disorders.[2]

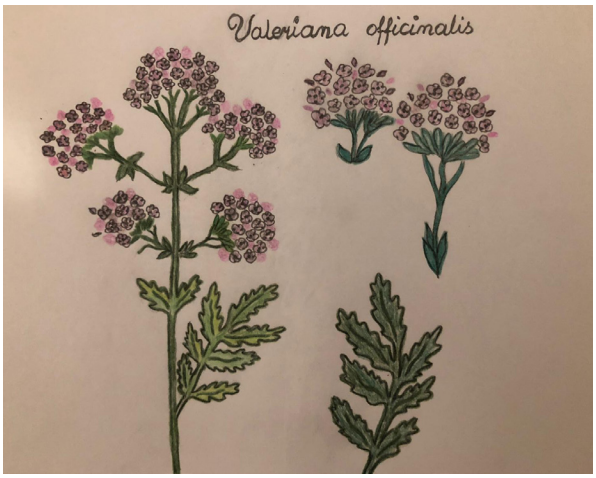

Fig. 1. *Valeriana officinalis*

Aim

The purpose of the article was to provide an overview of *Valeriana officinalis*.

Material and methods

This article is a review to discuss the latest progress made in *Valeriana officinalis* research. Scientific articles were reviewed by searching for information on valerian using the online database with scientific articles, including PubMed, Google Scholar and other available scientific databases. The following keywords were used to search for scientific articles: *Valeriana officinalis*, valerian, valeriana. Table 1 shows the steps of the literature review.

Table 1. Stages of literature search

Search stages	Search phrases
1	MeSH: *valeriana officinalis*, valerian, valeriana, *in vivo, in vitro*
2	Peer-reviewed articles
3	Available abstract

A literature review includes 47 selected scientific articles, published since 2004. Older articles were excluded due to presenting only the latest reports and knowledge base about *V. officinalis*. The exclusion criteria were taken into account when selecting the appropriate items shown in Table 2.

Table 2. Exclusion criteria used for the analysis

Exclusion criteria
– Languages of papier other than Polish, English and French
– Date of publication: published below 2004
– A short paper without details
– Unable to data extract

At the initial stage, 1057 publications were identified. Subsequently, after removal of duplicates, (n=1004) articles remained. Articles (n=887) were rejected at the inclusion and exclusion criteria stage. Articles (n=117) meeting the conditions were selected, and further (n=70) articles were rejected at the detailed analysis stage. By subjecting them to detailed analysis, finally (n=47) scientific papers were extracted. The process of reviewing the articles is shown in the PRISMA 2009 Flow diagram.

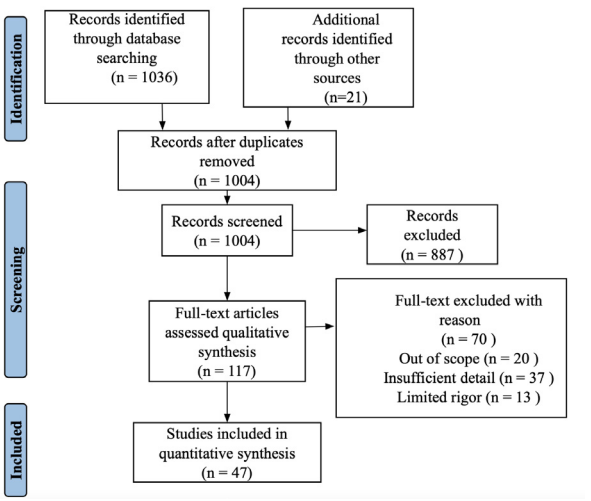

Fig. 2. PRISMA 2009 flow diagram

Analysis of literature

Chemical composition

V. officinalis has several biochemical components that act together to develop the full effectiveness of *V. officinalis*. Some of the main components are being listed below (Tab. 3):

Gamma-aminobutyric acid (GABA) is a nonstandard amino acid which is an amino acid that has undergone a chemical modification after the translational process. It affects the central nervous system as an inhibitory neurotransmitter that controls neurophysiological functions.[17] Studies have shown that higher levels of GABA in the body reduces anxiety and promotes relaxation. GABA acts when it enters the synaptic

cleft to bind on postsynaptic GABA receptors. Two major postsynaptic GABA receptors can be found on the postsynaptic neuron. The ionotropic receptors and metabotropic receptors. The binding of GABA to the ionotropic receptors, also called GABA-A receptor, opens channels so negatively charged chloride ions can enter. This results in a hyperpolarized cell and thus inhibits the creation of action potential. The binding of GABA to the metabotropic receptors, also called GABA-B receptor, leads to the exit of positively charged potassium ions in the cell through the potassium channels which also results in hyperpolarized cells and inhibits the conduction of action potential.[18,19] Simplified can be said that due to the binding of GABA and the receptors, action potential of neurons are being inhibited and this in return reduces the neuron excitability. So if there is not enough GABA in the body or if the binding process does not take place correctly, patients often suffer from diseases like epilepsy, anxiety, movement disorders and insomnia.[17,18,20]

Table 3. Classification of chemicals found in *V. Officinalis*

Chemical composition	
Gamma-amino-butyric acid	A neurotransmitter with inhibitory effects throughout the nervous system.[10]
Valepotriates	A group of unstable iridoids, exert a regulatory effect on the autonomic nervous system.[11]
Sesquiterpenes	Colorless lipophilic compounds.[12]
Flavonoids	A group of organic chemical compounds found in plants that act as dyes, antioxidants and natural insecticides and fungicides.[13]
Alkaloids	Complex organic molecules containing a heterocyclic nitrogen ring, which have been widely exploited for their diverse pharmacological properties.[14]
Triterpenes	Members of isoprenoids that are derived from a C30 precursor, squalene; the most abundant secondary metabolites present in marine organisms.[15]
Monoterpenes	A class of isoprenoids produced from geranyl diphosphate.[16]

Valepotriates: Is one of the active compounds in the extract of *V. officinalis*. This organic compound belongs to the group of esterified iridoids. Valepotriates have a sedative effect on the body, which is based on the increased release of the inhibitory neurotransmitter GABA. Examples for valepotriate are valtrate, didrovaltrate, and isovaleric acid. In which isovaleric acids are responsible for the smell of the plant.[21-23]

Sesquiterpenes: Are one of the main compounds in essential oil of valeriana. Sesquiterpenes belong to the group of terpenes that consist of fifteen carbon atoms (three isoprene units) which can be either acyclic or ring shaped. One of the components of sesquiterpenes is valerenic acid. Valerenic acid inhibits GABA breakdown and this in return leads to sedation.[24-27]

Flavonoids: are natural compounds with the function to control the activity of cells and to combat free radicals. Due to the antioxidant effect of flavonoids, free molecules can be destroyed which can be harmful for the body. Flavonoids are divided into six subgroups, based on the chemical structure flavones, flavanones, flavonols, flavan-3-ols, isoflavones, and anthocyanins.[28,29] Examples of flavonoids in valeriana are linarin and apigenin. Studies in which linarin was injected into the mice, had shown that linarin in *V. officinalis* has sedative and sleep induced effects. The actual mechanism of linarin in the brain has not been solved until today but it is assumed that sedative effects can amplify due to various combinations of linargin, hesperidin, valerenic acid and 6-methylapigenin.[30]

Alkaloids: Are organic compounds that contain nitrogen and have complex ring structures. They can act as poisoning, pain reliever or anesthetics, so the field of application of alkaloids is broad. Examples for alkaloids are actinide and valerine. For the synthesis of actinidine are the precursors lysine and quinolinic acid needed. Actinide belongs to the psychoactive group of alkaloids. It behaves agonistic in relation to benzodiazepine receptors and acts then on gamma-aminobutyric acid receptors so intracellular chloride can flow in. This process leads then to the inhibitory effect on the central nervous system.[23,31]

Triterpenes: Are chemical compounds that consist of three terpene units. Almost 200 different structures of triterpenes are known and they are being divided by the amount of rings that they contain. Triterpenes are used for their anti-inflammatory, antiviral and antitumoral effects. An example for triterpenes is ursolic acid. It inhibits the nuclear signaling of factor-kappa B in cancer cells, keeps inflammatory levels down and also increases the antioxidants in the brain, so stress on brain cells decreases.[32,33]

Monoterpenes: Belong to the class of terpenes with two isoprene units. Four different types of monoterpenes are known: acyclic, monocyclic, bicyclic and tricyclic, classified according to the shape. Monoterpenes show anti-inflammatory effects by regulating the increase of cytokine release. Furthermore, effects such as antiviral, antioxidant and antitumor have been observed. Examples for monoterpenes in *V. officinalis* are bornyl acetate, borneol. Bornyl acetate belongs to the group of bicyclic monomeres and it has already been used for skin care and natural antiseptic disinfectant. Anti-inflammatory effects such as suppressing the proinflammatory cytokines TNF-α and IL-1β release, which both are involved in the disease Atherosclerosis. Generally can be said that monoterpenes have a wide range of positive effects for the body.[34-36]

Valeriana officinalis in vitro on studies

Kara et al. evaluated oxidative stress and cytotoxicity on human hepatocellular carcinoma and human colorectal adenocarcinoma cell lines. The results showed that valerian root extract did not induce oxidative stress in HepG2 and Caco2 cell lines. Valerian is not an alternative for cancer treatment. But in tolerable concentrations, it can be recommended due to its property of not inducing oxidative stress.[8] De Brito et al. studied the interaction of valerian in cortical spreading depression and analyzed the protective effect against cytotoxic effects of rotenone in in vitro cultures of rat C6 glioma cells. *In vitro* studies on rat C6 glioma cells showed a protective effect against rotenone-induced cytotoxicity.[37] Shi et al. showed in their in vitro study that valerian extract reduces breast cancer cell proliferation. This could make valerian a therapeutic agent for breast cancer. The authors also suggest the potential effectiveness of valerian acid as an HDAC inhibitor.[38] Hellum et al. in their work on *in vitro* cells, they presented the effect of valerian on mechanism-based inhibition of CYP2D6 activity. The study demonstrated the action of valerian components as inhibitors on CYP2D6 enzyme activity. However, they note that this type of inhibition is irreversible, making it impossible to ignore the deleterious or toxic responses of valerian action in in vivo studies.[39] Hellum et al. presented the effect of valerian on CYP activity in human hepatocytes. In the results, we see that statistically significant inducing properties on CYP2D6 and CYP3A4 were found. The researchers suggested in their work that valerian is one herb whose data may be clinically relevant in future in vivo CYP studies.[40] In a subsequent study, the same authors evaluated the effect of the induction potential dose of commercially used herbal products on the metabolic activity of CYP2C19 and CYP2E1 in human hepatocyte cultures. In the case of valerian, the results showed that it is a weak inducer of CYP2C19 and in the case of CYP2E1 it did not show inducing properties.[41] Lefebvre et al. investigated the in vitro effects of products containing valerian root extracts on the metabolism and transport of P-glycoprotein via the cytochrome P450 CYP3A4 pathway. The results showed that valerian extracts have the ability to inhibit metabolism through the cytochrome P450 3A4 pathway and transport by P-glycoprotein.[42]

Valeriana officinalis in vivo on studies

Sudati et al. conducted an in vivo study on flies evaluating protection against the harmful effects of rotenone. The results confirmed the effectiveness of valerian in reducing rotenone-induced toxicity in *Drosophila melanogaster*. These authors also suggest the usefulness of the results obtained in future studies of movement disorders such as Parkinson's disease.[43] Bogacz et al. conducted an in vivo study of the effects of compounds in valerian root on CYP3A4 gene expression, as well as on nuclear receptors PXR, CAR, RXR, GR, and HNF-4a in male rats. The results showed decreased expression levels of CYP3A1 (homolog of human CYP3A4), RXR, and HNF-4a and increased for CAR. The data show an effect on decreasing CYP3A4 expression. They suggest further in vivo studies in evaluating the safety of pharmacotherapy by the possibility of interaction with synthetic drugs metabolized by this enzyme.[44] Benke et al. in their in vivo studies in mice, showed that neurons expressing GABA(A)-containing beta3 receptors are a major cellular substrate for the anxiolytic effects of valerian extracts.[45] Torres-Hernández et al. through the data obtained in an in vivo study using zebrafish larval swimming behavior, demonstrated the high psychoactivity of valerian extract with respect to a behavioral-molecular approach.[46] Dimpfel in his study, he used rats with implanted electrodes and monitored EEG wave frequencies and then administered valerian root extract in one of the test groups. Results compared to a matrix of synthetic drugs showed that valerian exhibited effects similar to physiological sleep. The changes that occurred resembled natural sleep and may suggest the effectiveness of valerian in acting on health.[47]

Conclusion

V. officinalis commonly known as herb is a very common species, accompanying us since ancient times. Over the centuries its composition, chemical properties, and effects on the human body have been studied in order to use it in herbal medicine as a sedative, anti-depressant, and sleep inducer. A single oral dose of *V. officinalis* modulates intraventricular facilitatory circuits by acting as an anti-anxiety agent. Other, lesser known applications of valerian should not be overlooked either, such as its diuretic and cognitive enhancing properties. The various substances contained in valerian provide a broad spectrum of the plant's medicinal properties. It is a good option for treating insomnia, which nowadays affects more and more people, and for relieving nervous tension connected with stress. Scientists have confirmed numerous aspects of valerian and its positive effects on the human body in their studies. Of particular value here is the GABA content, which inhibits the action potential of neurons, reducing their excitability, and valepotriates, which increase the release of GABA. The rhizome and root are available in the form of capsules, tablets or alcoholic extracts, so everyone can choose the form that is most convenient for them. Valerian can also be consumed in the form of valerian honey, which has a pleasant taste. In pharmacies or herbal stores it appears under different trade names. Note that consumption of valerian also carries gastrointestinal side effects such as nausea and abdominal cramps.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, W.M. and D.A.; Writing – Original Draft Preparation, W.M. and D.A.; Writing – Review & Editing, W.M. and D.A.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

References

1. *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury*. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012.
2. Shinjyo N, Waddell G, Green J. Valerian Root in Treating Sleep Problems and Associated Disorders-A Systematic Review and Meta-Analysis. *J Evid Based Integr Med*. 2020;25:2515690X20967323.
3. Halson SL. Sleep in elite athletes and nutritional interventions to enhance sleep. *Sports Med*. 2014;44(Suppl 1):S13–S23.
4. Bhaskar S, Hemavathy D, Prasad S. Prevalence of chronic insomnia in adult patients and its correlation with medical comorbidities. *J Family Med Prim Care*. 2016;5(4):780–784.
5. Bruni O, Ferini-Strambi L, Giacomoni E, Pellegrino P. Herbal Remedies and Their Possible Effect on the GABAergic System and Sleep. *Nutrients*. 2021;13(2):530.
6. Azizi H, Shojaii A, Hashem-Dabaghian F, et al. Effects of *Valeriana officinalis* (Valerian) on tension-type headache: A randomized, placebo-controlled, double-blind clinical trial. *Avicenna J Phytomed*. 2020;10(3):297–304.
7. Dietz BM, Hajirahimkhan A, Dunlap TL, Bolton JL. Botanicals and Their Bioactive Phytochemicals for Women's Health. *Pharmacol Rev*. 2016;68(4):1026–1073.
8. Kara M, Alparslan ED, Öztaş E, Erdoğan ÖN. *In Vitro* Cytotoxicity and Oxidative Stress Evaluation of Valerian *(Valeriana officinalis)* Methanolic Extract in Hepg2 and Caco2 Cells. *Turk J Pharm Sci*. 2021;18(5):604–608.
9. Valerian. In: *Drugs and Lactation Database (LactMed)*. Bethesda (MD): National Library of Medicine (US); May 17, 2021.
10. Hepsomali P, Groeger JA, Nishihira J, Scholey A. Effects of Oral Gamma-Aminobutyric Acid (GABA) Administration on Stress and Sleep in Humans: A Systematic Review. *Front Neurosci*. 2020;14:923.
11. Das G, Shin HS, Tundis R, et al. Plant Species of Sub-Family Valerianaceae-A Review on Its Effect on the Central Nervous System. *Plants (Basel)*. 2021;10(5):846.
12. Chadwick M, Trewin H, Gawthrop F, Wagstaff C. Sesquiterpenoids lactones: benefits to plants and people. *Int J Mol Sci*. 2013;14(6):12780–12805.
13. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. *J Nutr Sci*. 2016;5:e47.
14. Heinrich M, Mah J, Amirkia V. Alkaloids Used as Medicines: Structural Phytochemistry Meets Biodiversity-An Update and Forward Look. *Molecules*. 2021;26(7):1836.
15. Kushiro T, Ebizuka Y. *1.18 - Triterpenes. Comprehensive Natural Products II*. Elsevier; 2010: 673–708.
16. Zielińska-Błajet M, Feder-Kubis J. Monoterpenes and Their Derivatives-Recent Development in Biological and Medical Applications. *Int J Mol Sci*. 2020;21(19):7078.
17. Savage K, Firth J, Stough C, Sarris J. GABA-modulating phytomedicines for anxiety: A systematic review of preclinical and clinical evidence. *Phytother Res*. 2018;32(1):3–18.
18. Jewett BE, Sharma S. Physiology, GABA. *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2021:July 26.
19. Tarkowski ŁP, Signorelli S, Höfte M. γ-Aminobutyric acid and related amino acids in plant immune responses: Emerging mechanisms of action. *Plant Cell Environ*. 2020;43(5):1103–1116.
20. Pineau S, Legros C, Mattei C. The Medical use of Lemon Balm (Melissa officinalis) and Valerian (Valeriana officinalis) as Natural Sedatives: Insight into their Interactions with GABA Transmission. *Int J Clin Pharmacol Pharmacother*. 2016;1:112.
21. Orhan IE. A Review Focused on Molecular Mechanisms of Anxiolytic Effect of Valerina officinalis L. Connection with Its Phytochemistry through in vitro/in vivo Studies. *Curr Pharm Des*. 2021;27(28):3084–3090.
22. Murray MT, Pizzorno J. *Valeriana officinalis (Valerian), Textbook of Natural Medicine (Fifth Edition)*. St. Louis, Missouri: Elsevier; 2021:902-905.
23. Patočka J, Jákl J. Biomedically relevant chemical constituents of Valeriana officinalis. *Journal of Applied Biomedicine*. 2010;8:11–18.
24. Cho S, Shimizu M. *Natural Sleep Aids and Polyphenols as Treatments for Insomnia*. Academic Press, Elsevier; 2015:141–151.
25. Chadwick M, Trewin H, Gawthrop F, Wagstaff C. Sesquiterpenoids lactones: benefits to plants and people. *Int J Mol Sci*. 2013;14(6):12780–12805.
26. Chen HW, Wei BJ, He XH, Liu Y, Wang J. Chemical Components and Cardiovascular Activities of Valeriana spp. *Evid Based Complement Alternat Med*. 2015;2015:947619.
27. Murphy K, Kubin ZJ, Shepherd JN, Ettinger RH. Valeriana officinalis root extracts have potent anxiolytic effects in laboratory rats. *Phytomedicine*. 2010;17(8-9):674–678.
28. Mottaghipisheh J, Taghrir H, Boveiri Dehsheikh A, et al. Linarin, a Glycosylated Flavonoid, with Potential Therapeutic Attributes: A Comprehensive Review. *Pharmaceuticals (Basel)*. 2021;14(11):1104.
29. Li J, Hao L, Wu J, Zhang J, Su J. Linarin promotes osteogenic differentiation by activating the BMP-2/RUNX2

pathway via protein kinase A signaling. *Int J Mol Med*. 2016;37(4):901–910.
30. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*. 2010;4(8):118–126.
31. Morin CM, Beaulieu-Bonneau S, Cheung JMY. *Treatment of Insomnia. Handbook of Sleep Disorders in Medical Conditions.* Academic Press, Elsevier; 2019:27–50.
32. Ríos JL. Effects of triterpenes on the immune system. *J Ethnopharmacol*. 2010;128(1):1–14.
33. Seo DY, Lee SR, Heo JW, et al. Ursolic acid in health and disease. *Korean J Physiol Pharmacol*. 2018;22(3):235–248.
34. Quintans JSS, Shanmugam S, Heimfarth L, et al. Monoterpenes modulating cytokines - A review. *Food Chem Toxicol*. 2019;123:233–257.
35. Yang L, Liu J, Li Y, Qi G. Bornyl acetate suppresses ox-LDL-induced attachment of THP-1 monocytes to endothelial cells. *Biomed Pharmacother*. 2018;103:234–239.
36. Volcho KP, Anikeev VI. *Environmentally Benign Transformations of Monoterpenes and Monoterpenoids in Supercritical Fluids.* Elsevier; 2014:69–87.
37. Amaral de Brito AP, Galvão de Melo IMDS, El-Bachá RS, Guedes RCA. *Valeriana officinalis Counteracts* Rotenone Effects on Spreading Depression in the Rat Brain *in vivo* and Protects Against Rotenone Cytotoxicity Toward Rat Glioma C6 Cells *in vitro*. *Front Neurosci*. 2020;14:759.
38. Shi F, Li Y, Han R, et al. Valerian and valeric acid inhibit growth of breast cancer cells possibly by mediating epigenetic modifications. *Sci Rep*. 2021;11(1):2519.
39. Hellum BH, Nilsen OG. The in vitro inhibitory potential of trade herbal products on human CYP2D6-mediated metabolism and the influence of ethanol. *Basic Clin Pharmacol Toxicol*. 2007;101(5):350–358.
40. Hellum BH, Hu Z, Nilsen OG. The induction of CYP1A2, CYP2D6 and CYP3A4 by six trade herbal products in cultured primary human hepatocytes. *Basic Clin Pharmacol Toxicol*. 2007;100(1):23–30.
41. Hellum BH, Hu Z, Nilsen OG. Trade herbal products and induction of CYP2C19 and CYP2E1 in cultured human hepatocytes. *Basic Clin Pharmacol Toxicol*. 2009;105(1):58–63.
42. Lefebvre T, Foster BC, Drouin CE, Krantis A, Livesey JF, Jordan SA. In vitro activity of commercial valerian root extracts against human cytochrome P450 3A4. *J Pharm Pharm Sci*. 2004;7(2):265–273.
43. Sudati JH, Vieira FA, Pavin SS, et al. Valeriana officinalis attenuates the rotenone-induced toxicity in Drosophila melanogaster. *Neurotoxicology*. 2013;37:118–126.
44. Bogacz A, Mrozikiewicz PM, Karasiewicz M, et al. The influence of standardized Valeriana officinalis extract on the CYP3A1 gene expression by nuclear receptors in in vivo model. *Biomed Res Int*. 2014;2014:819093.
45. Benke D, Barberis A, Kopp S, et al. GABA A receptors as in vivo substrate for the anxiolytic action of valerenic acid, a major constituent of valerian root extracts. *Neuropharmacology*. 2009;56(1):174–181.
46. Torres-Hernández BA, Colón LR, Rosa-Falero C, et al. Reversal of pentylenetetrazole-altered swimming and neural activity-regulated gene expression in zebrafish larvae by valproic acid and valerian extract. *Psychopharmacology (Berl)*. 2016;233(13):2533–2547.
47. Dimpfel W. Pharmacological classification of herbal extracts by means of comparison to spectral EEG signatures induced by synthetic drugs in the freely moving rat. *J Ethnopharmacol*. 2013;149(2):583–589.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.3

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 266–271

REVIEW PAPER

Silybum marianum – properties and application in medicine – a review

Marta Sowińska , Magdalena Szpunar

Student's Biochemical Science Club URCell, Medical College of The University of Rzeszów, Rzeszów, Poland
supervisor: Sabina Galiniak, Dorota Bartusik-Aebisher

ABSTRACT

Introduction and aim. The subject of this article is milk thistle – a plant belonging to the Asteraceae family. Due to its properties, it has a beneficial effect on the functioning of important organs and systems of the human body. The aim of this paper was to review information on milk thistle, its use in medicine and the description of some of the chemical compounds due to which it owes its healing properties.

Material and methods. The article is a review of the publicly available literature on milk thistle, the chemical composition of its compounds and its use in medicine.

Analysis of the literature. Current information about *Silybum marianum* is presented.

Conclusion. Milk thistle is a valuable medicinal plant due to the presence of numerous antioxidant and anti-inflammatory compounds. The benefits of supplementation with ingredients derived from milk thistle, their negligible interaction with other drugs and the lack of significant side effects, known so far, indicate that the plant described in the article can be a good medicine and a preventive measure against diseases affecting our society.

Keywords. milk thistle, silybin, silymarin

Introduction

The subject of this article is milk thistle - a plant belonging to the Asteraceae family. Due to its properties, it has a beneficial effect on the functioning of important organs and systems of the human body.[1-2] So far, the focus has been on its detoxifying effect and supporting liver regeneration, but the latest research shows that the compounds obtained from milk thistle show a broader health-promoting effect.[3-4] Due to the high potential of milk thistle as a raw material for medical use, the article will be a kind of review in which the habitat of this plant and its habit will be presented, as well as its use in medicine, along with a description of some chemical compounds thanks to which it owes its healing properties.[5-10]

Aim

A general overview of information on milk thistle, with particular emphasis on its use in medicine and the description of some of the chemical compounds due to which it owes its healing properties.

Material and methods

The review is based on the available literature on milk thistle, the chemical composition of its compounds and its use in medicine. The time of publishing the articles ranges from 1996 to 2022, with the oldest article describing the general appearance of the plant. In contrast, information on the properties of chemicals and their clinical effects is based on articles that describe

Corresponding author: Magdalena Szpunar, e-mail: szpunarmag@gmail.com

Received: 8.05.2022 / Revised: 2.07.2022 / Accepted: 11.07.2022 / Published: 30.09.2022

Sowińska M, Szpunar M. *Silybum marianum – properties and application in medicine – a review*. Eur J Clin Exp Med. 2022;20(3):266–271. doi: 10.15584/ejcem.2022.3.3.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

research at the cellular level, animal studies, and clinical trials.

Analysis of the literature

Milk thistle (*Silybum marianum*) is a herbaceous, annual (under favourable conditions - biennial) plant belonging to the Asteraceae family and comes from the regions of the Mediterranean Sea, where it is most common today. However, it can be found all over the world, as it was introduced by man to various countries. In Poland, it occurs mainly as a plant cultivated to obtain valuable medicinal substances, sometimes it is treated as an ornamental plant, less often it occurs in a wild-growing form. Milk thistle is one of the plants that do not require special growing conditions, but its cultivation is favoured by a humid and warm climate and fertile soil. Under such conditions, *S. marianum* can reach a height of up to two metres, but the average height it grows is about 1.5 metres.[1-2,5-6] The plant looks like a thistle. It has a strong single or branching stem. Its leaves are broadly elliptical or oblong-ovate, sinus-lobed, dark green in colour, shiny and speckled with white spots. Older leaves end with yellow spikes. During flowering, which lasts from late June to mid-August, *S. marianum* has pink-purple flowers gathered in basket-shaped inflorescences about five centimetres in size. Additionally, as a honey plant it attracts insects, especially bees and butterflies.[2,5-6] The fruit of milk thistle is achenes – a non-bursting, dry fruit with a leathery pericarp with a single seed in it.[1] The fruits obtained during the autumn harvest (September to October) are a source of valuable substances used in medicine, such as flavonolignans.[1,2] The largest amount (even 98%) is accumulated in the pericarp and the husk of the achenes.[1]

Substances contained in milk thistle

Milk thistle is used to obtain silymarin extract (fig. 1.), i.e. a complex of flavonolignans. It consists of silybin (fig. 2.) in the form of two diastereoisomers (silybin A and B), isosilybin (fig. 3.) (also in the form of two diastereoisomers of isosilybin A and B), silychristin (fig. 4.), silydianin (fig. 5.) and the precursor of the above flavonolignans – taxifolin (fig. 6.).[2,7,11-16] As the predominant part of the silymarin complex, silybin is the compound responsible for its biological activity.

Due to the high proportion of silybin (figure 2) in silymarin, it is mistakenly identified with the entire complex of this compound. Like other flavonolignans, silybin is an antioxidant. Its molecular formula is $C_{25}H_{22}O_{10}$ and its molecular weight is 482.441 u. It consists of two units – one based on taxifolin, the other – a phenylpropanoid unit, which are linked together by an orexan ring. It exists as two diastereoisomers (silybin A, silybin B) and is biotransformed in the liver. It is poorly soluble in water and therefore has low bioavailability.[3-4] Most of the silybin is excreted in the bile in conjugated form with sulphates and glucuronides, some is excreted unchanged in the urine and the rest is eliminated in the faeces.[17-18] This compound exhibits antioxidant, anti-inflammatory (related to antioxidant properties), anti-fibrotic (by reducing platelet-induced DNA synthesis and cell proliferation) and modulating (in some liver metabolic pathways activity).[3] In addition, its interaction with some cytochromes and interference with the regulation of the cell cycle was discovered, which is a factor in preventing cancer. Due to the above properties, silybin shows an action similar to silymarin, however, studies indicate significant differences in the interactions of these compounds with metabolizing enzymes. Nevertheless, silybin is also used as a completely separate component of silymarin and research is conducted to increase its bioavailability and absorption by the human body.[18]

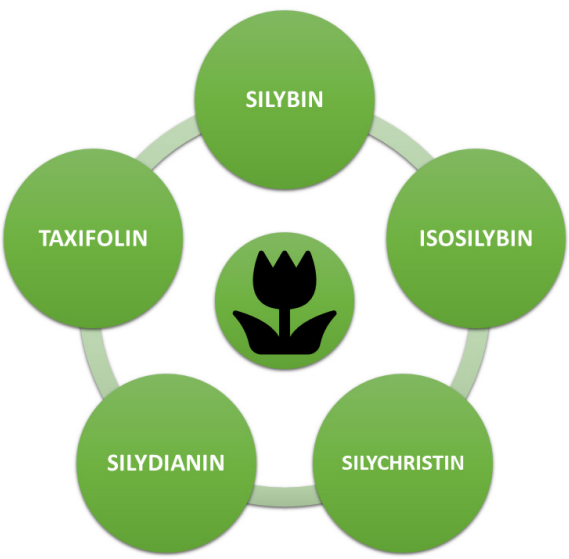

Fig. 1. Substances contained in Milk thistle

The composition of silymarin causes its low bioavailability when used orally due to poor water solubility, low absorption efficiency in the intestines, as well as the metabolism of the complex in the liver, which reduces the availability of this compound for the body's cells. To increase the efficiency of delivering silymarin to tissues and organs, it is administered in the form of a complex with the highly water-soluble antioxidant phosphatidylcholine and more soluble silymarin derivatives have been produced and administered to patients. Other substances increasing the bioavailability of this compound are the flavonoid found mainly in citrus fruits – tangeretin and piperine contained in black pepper. The increase in silymarin bioavailability allowed for more effective research on the health-promoting effect of this compound and its interaction with other medicinal substances.[3-4] Studies to date show that this compound does not interfere with the effects of medications used by patients and may alleviate

their side effects.[6,19] However, drug-drug interactions between silymarin and drugs have not been ruled out, and further detailed research is needed.

In therapeutic doses, the intake of silymarin does not show any toxic properties. It should also be mentioned that so far no serious side effects from the use of silymarin have been demonstrated. Cases associated with gastrointestinal disturbances such as diarrhoea and gastroenteritis have rarely occurred. Headaches or dermatological symptoms such as hives, itching or allergic dermatitis have also been reported.[3,20-21] Additionally, there were no documented contraindications to the use of silymarin preparations.[20]

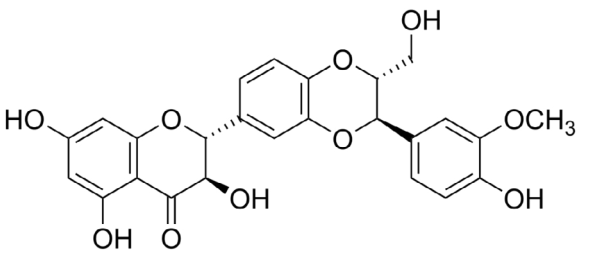

Fig. 2. Structural formula of silybin

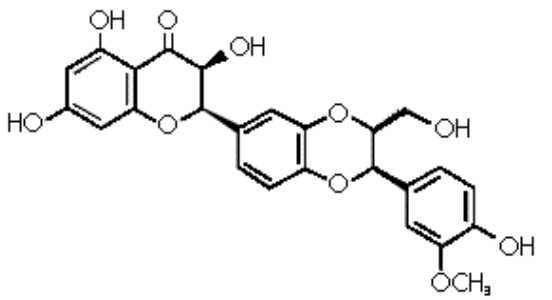

Fig. 3. Structural formula of isosilybin

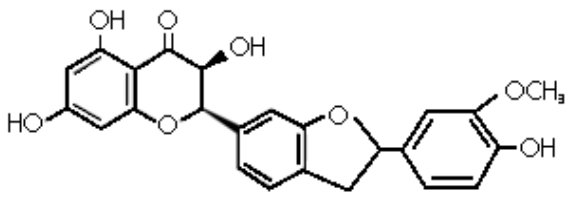

Fig. 4. Structural formula of silychristin

At this point, it is worth mentioning that milk thistle may be affected by some microfungi (eg. *Fusarium* or *Alternaria genera*) and their mycotoxins, contributing to the reduction of the beneficial effects of silymarin or even their complete elimination. The most important in this aspect are: alternariol methyl ether (AME), alternariol (AOH), beauvericin (BEA), deoxynivalenol (DON), enniatin A (ENNA), enniatin A_1 ($ENNA_1$), enniatin B (ENNB), enniatin B1 (ENNB1), HT-2 toxin (HT-2), T-2 toxin (T-2), tentoxin (TEN) and zearalenone (UAE). Their presence in supplements containing compounds isolated from milk thistle or in the plant itself may have a hepatotoxic, nephrotoxic, neurotoxic, teratogenic, immunosuppressive, genotoxic and even carcinogenic effect.[5]

At the same time, silymarin has been shown to be effective against the adverse effects of some mycotoxins. Many studies show its beneficial effect on weight gain and feed consumption by broilers treated with alphatoxin B1 (AFB1), as well as sensory and quality improvement of meat obtained from these animals.[5,22-23]

Research also indicates an improvement in the functioning of the immune system of Japanese quails after ingesting silymarin in their diet, as evidenced by a reduction in the relative weight of Fabrucius' capsule and spleen in these birds.[24] The effect of improving the immune response was also observed in broilers after feeding with milk thistle.[25]

Other compounds that, apart from silymarin are found in the seeds of milk thistle and its other parts include dietary fibre, proteins, sugars, polyunsaturated fatty acids (e.g. linoleic acid), monounsaturated fatty acids (e.g. oleic acid), phytosterols, alpha tocopherol, quercitin, histamine, tannins, minerals and vitamins.[2,7,18,26]

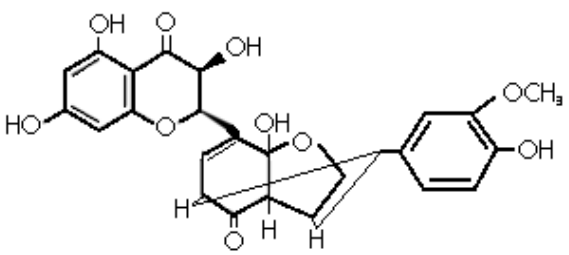

Fig. 5. Structural formula of silydianin

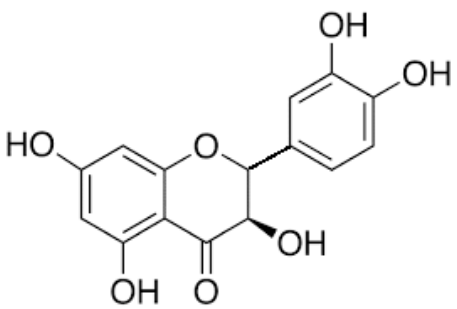

Fig. 6. Structural formula of taxifolin

The use of milk thistle in medicine

Thanks to the substances described above and their belonging to flavonoglycans, milk thistle shows valuable healing properties (Figure 7).

It interacts with some cytochromes and interferes with the regulation of the cell cycle. The effect of these substances is primarily hepatoprotective properties. They are based on the protection of liver cells against

harmful and toxic factors, such as alcohol or substances present in *Amanita phalloides* or *Amanita muscaria*.[6-7] Intravenous administration of silybin within 48 hours of poisoning at a dose of 2050 mg/kg per day, during 3-4 days completely inhibited liver damage. A study conducted on 250 people poisoned with *A. muscaria* indicated 46 deaths in the untreated group. However, among the 16 people who received silybin, there were no deaths.[9]

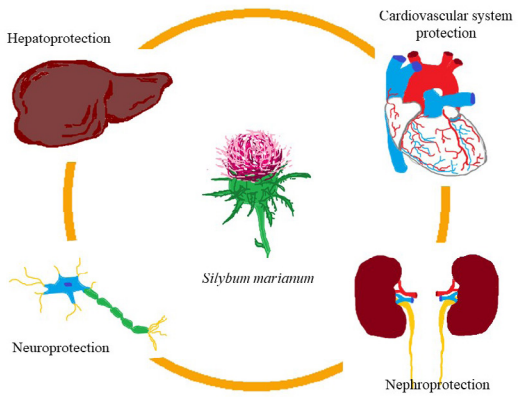

Fig. 7. Some healing properties of *Silybum marianum*

Another study involving 18 people poisoned with *A. muscaria* and treated with silybin resulted in only one death of a person who ingested the poison in order to commit suicide, with treatment not commenced until 60 hours after ingestion.[5] Protection of the liver against these factors mainly consists in improving the integrity of the hepatocyte membranes, which makes it difficult for the toxic factors to penetrate the liver cells.[10]

Other protective properties include antioxidant, antiviral and anti-inflammatory effects. In addition, the plant supports the regeneration of the liver damaged by both physical and chemical factors resulting from the metabolic function of this organ, while preventing the processes of fibrosis.[6-7] Accordingly, the hepatoprotective properties of milk thistle are used to alleviate and treat acute and chronic liver diseases such as non-alcoholic fatty liver disease, alcoholic cirrhosis, and viral hepatitis.[4,7,17]

Fatty liver is often associated with obesity, type 2 diabetes, insulin resistance and dyslipidemia. Nevertheless, the action of milk thistle also helps prevent these diseases by improving lipid metabolism. As a result of the use of preparations with silymarin, the excretion of low-density lipoproteins is increased, and the synthesis of cholesterol is reduced. The results of clinical trials also showed that the daily use of silymarin at a dose of 420 mg in 15 people with hypercholesterolemia led to lower cholesterol levels in the gallbladder compared to the control group. This suggests that silymarin may be introduced as a blood cholesterol reducer in patients with hypercholesterolemia.[6,27] It is of great importance for the proper functioning of the circulatory system, protecting against the formation of atherosclerotic plaques.[6,17]

The antioxidant properties of silymarin have a protective effect not only on the liver cells but also on the cells of other internal organs, such as the kidneys. This is because silymarin is concentrated in the cells of the kidneys, supporting their regeneration by increasing the synthesis of proteins and nucleic acids. On the other hand, the presence of silybin and silychristin in silymarin is attributed to an increase in cell replication by 30%.[5] It has been shown to prevent and reduce kidney damage resulting from cytostatics and to protect against radiation damage.[6-7,28-29] Additionally, the conducted studies indicate a beneficial therapeutic effect of silymarin in the development of diabetic nephropathy.[6]

Another effect of the substances contained in milk thistle is neuroprotection. As a result of the use of silymarin, the nerve conduction of nerve fibres improves, which suggests its use in the treatment of Alzheimer's disease.[5-6]

The use of milk thistle preparations also supports the lactation processes in breastfeeding women, has a positive effect on the bile ducts, spleen and pancreas. A beneficial effect of *S. marianum* seed oil on the skin was also found.[5,7,30]

An important aspect of the action of silymarin is the anti-cancer effect.[31] To date, its preventive activity has been demonstrated in the presence of prostate, breast, bladder, lung and ovarian cancer by inducing apoptosis of abnormal cancer cells. The substances contained in milk thistle also inhibit metastases accompanying neoplasms, including metastases to the brain.[6-8] It has also been proved that silymarin can reduce the undesirable effects of anti-cancer drugs such as paclitaxel, cisplatin, methotexate, and fluorouracil. However, there are concerns that the strong antioxidant activity of milk thistle may interfere in determining the lymphocytotoxicity function of chemotherapy drugs through biochemical peroxidative pathways.[5]

Conclusion

Milk thistle is a valuable medicinal plant due to the presence of numerous antioxidant and anti-inflammatory compounds. Although the mechanisms of their metabolism and the subsequent effects of the metabolized products on the cells of the human body are not fully understood, it cannot be denied that they ultimately exhibit hepatoprotective, antioxidant, immunomodulating and even anti-tumor effects. It is worth continuing research on the use of silymarin in diseases of the liver and biliary tract and introducing it into clinical practice as an adjunct to traditional therapy. However, additional research should be focused on the long-term effects of milk thistle and the interactions of its compounds with other drugs.

Nevertheless, the works carried out so far allow to draw a hypothesis that the plant described in the article may in the future be a good medicine and a preventive measure against many diseases affecting our society.

Declarations

Funding
This research received no external funding.

Author contributions
Conceptualization, M.S. and M.Sz.; Writing – Original Draft Preparation, M.S. and M.Sz; Writing – Review & Editing, M.S. and M.Sz.

Conflicts of interest
The authors declare no conflict of interest.

Data availability
Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

References

1. Andrzejewska J, Skinder Z. Ostropest plamisty-uwagi o nazewnictwie, substancjach czynnych i rozwoju rośliny. *Acta Sci Pol Agricultura*. 2006;5(1):5–10.
2. Grau J, Jung R, Münker B. *Leksykon Przyrodniczy: Zioła i Owoce Leśne,*Warszawa; Świat Książki; 1996:196.
3. Loguercio C, Festi D. Silybin and the liver: from basic research to clinical practice. *World J Gastroenterol.* 2011;17(18):2288–2301.
4. Federico A, Dallio M, Loguercio C. Silymarin/Silybin and Chronic Liver Disease: A Marriage of Many Years. *Molecules.* 2017;22(2):191.
5. Pickova D, Ostry V, Toman J, Malir F. Presence of Mycotoxins in Milk Thistle (Silybum marianum) Food Supplements: A Review. *Toxins (Basel).* 2020;12(12):782.
6. Bahmani M, Shirzad H, Rafieian S, Rafieian-Kopaei M. Silybummarianum: Beyond Hepatoprotection. *Evid Based Complement Alternat Med*. 2015;20(4):292–301.
7. Nurzyńska-Wierdak R, Dyduch J, Sawicka A, Łabuda H, Buczkowska H. Ostropest plamisty (Silybummarianum [L.] Gaertn.) – fitochemia i efekty terapeutyczne. *Ann Hortic*. 2018;*28*(4):15–32.
8. Verdura S, Cuyàs E, Ruiz-Torres V, et al. Lung Cancer Management with Silibinin: A Historical and Translational Perspective. *Pharmaceuticals (Basel).* 2021;14(6):559.
9. European Scientific Cooperative on Phytotherapy. ESCOP Monographs: *The Scientific Foundation for Herbal Medicinal Products*. 2nd editionsupplement. New York, NY: Thieme; 2009:222–248.
10. Salmi HA, Sarna S. Effects of silymarin on chemical, functional, and morphological alterations of the liver: a double-blind controlled study. *Scand J Gastroenterol.* 1982;17:517–521.
11. Gufford BT, Graf TN, Paguigan ND, Oberlies NH, Paine MF. Chemoenzymatic Synthesis, Characterization, and Scale-Up of Milk Thistle Flavonolignan Glucuronides. *Drug Metab Dispos.* 2015;43(11):1734–1743.
12. Kim NC, Graf TN, Sparacino CM, Wani MC, Wall ME. Complete isolation and characterization of silybins and isosilybins from milk thistle (Silybum marianum). *Org Biomol Chem.* 2003;1(10):1684–1689.
13. Kren V, Ulrichová J, Kosina P, et al. Chemoenzymatic preparation of silybin beta-glucuronides and their biological evaluation. *Drug Metab Dispos.* 2000;28(12):1513–1517.
14. Han YH, Lou HX, Ren DM, Sun LR, Ma B, Ji M. Stereoselective metabolism of silybin diastereoisomers in the glucuronidation process. *J Pharm Biomed Anal*. 2004;34(5):1071–1078.
15. Jančová P, Siller M, Anzenbacherová E, Křen V, Anzenbacher P, Simánek V. Evidence for differences in regioselective and stereoselective glucuronidation of silybin diastereomers from milk thistle (Silybum marianum) by human UDP-glucuronosyltransferases. *Xenobiotica*. 2011;41(9):743–751.
16. Křen V, Marhol P, Purchartová K, Gabrielová E, Modrianský M. Biotransformation of silybin and its congeners. *Curr Drug Metab.* 2013;14(10):1009–1021.
17. Gillessen A, Schmidt HH. Silymarin as Supportive Treatment in Liver Diseases: A Narrative Review. *Adv Ther.* 2020;37(4):1279–1301.
18. Bijak M. Silybin, a Major Bioactive Component of Milk Thistle (Silybum marianum L. Gaernt.)-Chemistry, Bioavailability, and Metabolism. *Molecules.* 2017;22(11):1942.
19. Xie Y, Zhang D, Zhang J, Yuan J. Metabolism, Transport and Drug-Drug Interactions of Silymarin. *Molecules*. 2019;24(20):3693.
20. Achufusi TGO, Patel RK. *Milk Thistle*. Treasure Island (FL): StatPearls Publishing; 2021.
21. Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. *Drugs*. 2001;61(14):2035–2063.
22. Alhidary IA, Rehman Z, Khan RU, Tahir M. Anti-aflatoxin activities of milk thistle (*Silybum marianum)* in broiler. *Worlds Poult Sci J*. 2017;73:559–566.
23. Zaker-Esteghamati H, Seidavi AR, Bouyeh M. A review on the effect of Silybum marianum and its derivatives on broilers under healthy and aflatoxicosis conditions: part 1: performance, carcass and meat characteristics, and intestinal microflora. *Worlds Poult Sci J*. 2020;76(2):318–327.
24. Khazaei R, Seidavi A, Bouyeh M. A review on the mechanisms of the effect of silymarin in milk thistle (Silybum marianum) on some laboratory animals. *Vet Med Sci.* 2022;8(1):289–301.
25. Zaker-Esteghamati H, Seidavi AR, Bouyeh M. The effects of Cynara scolymus and Silybum marianum on growth, carcass and organ characteristics, immunity, blood constitutes, liver enzymes, jejunum morphology, and fatty acid profile of breast meat in broilers. *Food Sci Nutr*. 2021;9(12):6692–6706.

26. Opyd PM, Jurgoński A. Intestinal, liver and lipid disorders in genetically obese rats are more efficiently reduced by dietary milk thistle seeds than their oil. *Sci Rep.* 2021;11(1):20895.
27. Nassuato G, Iemmolo RM, Strazzabosco M. Effect of silibinin on biliary lipid composition experimental and clinical study. *J Hepatol*. 1992;12:290–295.
28. Greenlee H, Abascal K, Yarnell E, Ladas E. Clinical applocations of Silybum marianum in oncology.*Integr Cancer Ther.* 2007;6(2):158–165.
29. Karimi G, Vahabzadeh M, Lari P, Rashedinia M, Moshiri M. Silymarin, a promising pharmacological agent for treatment of diseases. *Iran J Basic Med Sci.* 2011;14(4):308–317.
30. Bethesda MD. Drugs and Lactation Database (LactMed) National Library of Medicine (US); 2006. https://www.ncbi.nlm.nih.gov/books/NBK501922/
31. Sagar SM. Future directions for research on Silybum marianum for cancer patients. *Integr Cancer Ther*. 2007;6(2):166–173.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.4

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 272–283

REVIEW PAPER

Comparison of conventional syringe anesthesia and three computer-aided anesthesia systems (Quicksleeper, SleeperOne, and The Wand)

Nisha Singh [1], Sylvia Wyzga [1], Jessica Yune [1], Gauri Mathur [1], Katarzyna Błochowiak [1]

[1] Department of Oral Surgery and Periodontology, Poznan University of Medical Sciences, Poznan, Poland

ABSTRACT

Introduction and aim. The purpose of this study was to compare different Computerized-Controlled Local Anesthetic Delivery (CCLAD) systems to one another in addition to the conventional syringe.

Material and method. The CCLAD systems chosen for this study are the Quicksleeper, SleeperOne, and The Wand. These are discussed in categorical objectives, including the following: duration of anesthesia, analgesic effect, locality; anxiolytic effect; advantages and disadvantages; comfort and safety of use; limitations.

Analysis of the literature. The research found that many factors influence the effectiveness of dental injections, and the CCLAD systems are designed to reduce the ones that cause negative experiences. The injection systems are unique in their descriptions but show many similarities.

Conclusion. The research concluded that each device has its advantages and disadvantages and that its efficiency depends on outlying factors, independent of the injection system used.

Keywords. computerized-controlled local anesthetic delivery, conventional Syringe, local anesthesia, Quicksleeper, SleeperOne, The Wand

Introduction

Anesthesia plays a critical role in dentistry as it can provide a comfortable experience for the patient and allow the operator to perform procedures with ease. Much of the world's population is not keen on visiting the dental office due to previous unpleasant experiences or fear of the „needle". Among the pediatric population, various coping methods are implemented to ease the anxiety levels in children and adults; however, they are not always successful, and as a consequence, treatment is often deferred. Most of the time, their anxiousness is related to the fear of injection and the pain that is felt when the needle penetrates the oral mucosa and diffuses through the injected tissues. The distribution of anesthetics accompanying the conventional syringe technique causes painful swelling of the tissues and their administration at the site of anesthesia. Lack of control over the rate of anesthetic administration increases pain, swelling, and tissue administration, leading to discomfort and unpleasant sensations accompanying local anesthesia. In addition, an uncontrolled and shock increase of pressure in anesthetized tissues may lead to a short-term disturbance of their blood supply and local damage, reducing the effectiveness of anesthesia and increasing the risk of side effects. Some sources of an unpleasant and painful sensation in conventional syringe anesthesia are failure of inferior alveolar nerve block that may require additional attempts, lack of complete anesthe-

Corresponding author: Katarzyna Błochowiak, e-mail: kblochowiak@ump.edu.pl

Received: 4.07.2022 / Accepted: 29.07.2022 / Published: 30.09.2022

Singh N, Wyzga S, Yune J, Mathur G, Błochowiak K. *Comparison of conventional syringe anesthesia and three computer-aided anesthesia systems (Quicksleeper, SleeperOne, and The Wand)*. Eur J Clin Exp Med. 2022;20(3):272–283. doi: 10.15584/ejcem.2022.3.4.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

sia, and increased pain/anxiety on injection.[1-3] Repeated unpleasant and painful sensations related to the administration of local anesthesia in the past led to the memorized and permanent fear of anesthesia and even the development of dentophobia. The fear of conventional syringe anesthesia can be a reason for many people not to seek dental care or only visit a dentist for emergencies. Moreover, dental professionals often have difficulty performing mandibular blocks, which results in insufficient analgesia and requires a second injection to establish profound numbness leading to the accumulation of unpleasant sensations.[1,2,4,5] This added stress will contribute to the patients' pain and anxiety levels, which can place pressure on the clinician. Additionally, in pediatric patients, the use of conventional syringe anesthesia also poses pain/stress when puncturing the mucosa and can lead to injuries due to self-biting of soft tissues due to numbness.[3,6-8]

Therefore, all anesthetic delivery systems must be explored to determine the best option for our patients and eliminate traumatic experiences. To address the need to alleviate or eliminate the unpleasant effects of conventional syringe local anesthesia and improve its effectiveness, new computerized-controlled local anesthetic delivery (CCLAD) systems have been introduced. Among the well-known and widespread CCLAD systems are Quicksleeper, Sleeper One, and The Wand.

Aim

The aim of this study was to compare conventional syringe anesthesia with Quicksleeper, Sleeper One, and The Wand and discuss their analgesic and anxiolytic effect, the comfort of use, and limitations. It will allow practitioners to determine whether they will use intraosseous injection systems by providing cohesive information regarding the most popular computer-aided anesthesia systems currently used in dentistry. Therefore, our review was designed to answer "Can computer-aided anesthesia systems replace conventional syringe anesthesia?"

Material and methods

The following databases were used to research information regarding content discussed in this paper: Google Scholar, Pubmed, and Western's Online Database. The selected articles were used to summarize information about the selected objectives for the reader to compare the different computer-aided anesthesia systems and the conventional syringe.

Analysis of the literature

Duration of anesthesia, analgesia effect, and recommended type of anesthesia

One of the most desirable features of local anesthesia is to ensure a good and sufficiently long-lasting anesthetic effect that allows treatment or surgery procedure to be carried out. Although the duration of anesthesia depends on the type of anesthetic, the additional content of vasoconstrictor and the local anesthetic technique used, the method of distribution of the anesthetics may modify the final anesthetic effect. One of the most desirable properties of conventional syringe anesthesia is its versatility and the ability to perform all methods of local anesthesia. The duration of anesthesia for the conventional dental syringe is dependent on the type of anesthetic solution used, and the local anesthetic technique performed. In a study by Fernandez et al. directed specifically towards the inferior alveolar nerve block, the duration of lip anesthesia is between 192–411 minutes (3 hours to 6.8 hours) and the duration of pulpal anesthesia is between 127–258 minutes (2 hours and 24 minutes to 4 hours).[9] CCAL provides not so long the duration of anesthesia but on the other hand it is more targeted anesthesia of a specific area. The duration of anesthetic in the Quicksleeper system is between 30-60 minutes, however, various factors contribute to its effectiveness such as the use of vasoconstrictors, amount of anesthetic, and anatomical variations.[4] Beneito-Brotons et al. determined that the duration of anesthesia in soft tissue was 199.3 minutes using the conventional technique, and 1.6 minutes when using the intraosseous injection. This together concludes to a statistically significant difference between conventional syringe anesthesia and intraosseous injection.[2] According to Nieuwenhuizen et al. the injection time of the SleeperOne was averaged at 2.49 minutes.[10] Intraosseous injections specifically show high success rates, with easy administration and fast onset. Its numbing effects last long enough for endodontic treatment and limited treatment of 1–2 posterior teeth in the mandible.[11] When using the SleeperOne for intraosseous injections, the recommended doses are 1.5–1.8 mL of 4% articaine with 1:100,000 epinephrine for adults, and 0.6–0.8 mL of 4% articaine with 1:200,000 epinephrine for children.[11] In the prospective study between single tooth intraligamentary injections versus a conventional nerve block to extract posterior mandibular teeth by Adubae et al. the onset of action was immediate compared to a 10-minute wait time for the latter.[12] As the Single Tooth Anesthesia system allowed for treatment to begin simultaneously following the administration of local anesthesia, the duration of overall treatment was also faster. Furthermore, when the intraligamentary technique is used, the single tooth and its neighboring soft tissues are the only areas anesthetized. The duration of the anesthesia is shorter and the lips, cheek, and tongue are spared of any loss of sensation. In comparison, the conventional inferior alveolar nerve block had a significantly longer duration of action while numbing the surrounding anatomical structures. In addition, the numbness in these anesthe-

tized patients long surpassed the treatment time of the procedure. The onset of anesthesia using the Wand is quite immediate, resulting in a total duration of around 30 minutes. The analgesic effect of conventional syringe anesthesia is dependent on the injection technique used. Techniques commonly used in dentistry and as discussed in the mentioned studies of this paper are soft tissue infiltration, nerve block anesthesia, inferior alveolar nerve block, and anterior middle superior alveolar nerve block being the most studied. Ample knowledge of the selected technique and related intraoral anatomy is critical in increasing the success rate of the anesthetic, and as seen in the study by Thiem et al the success rate can be 100% if performed correctly.[13] In a study done by Cetkovic et al the overall success rate for pulpal anesthesia with the conventional syringe was between 68.4–94.7%.[14] One of the main factors focused on during this study was the importance of the quality of anesthesia being used. This was based on the relationship between the pharmacological profile of the anesthetic solutions and their capabilities of diffusion and penetration into the surrounding anatomical structures.[13] The onset of pulpal anesthesia also depends on the injection technique and solution used. Onset times are reported to be around 6 to 12 minutes.[13,14] Many authors have concluded through their research that the onset of Quicksleeper is immediate and faster than conventional syringe anesthesia. In a study conducted by Jensen et al. all volunteers reported that the effects of the anesthesia through intraosseous injection were instant.[15] Siwawut et al. examined the effects of Quicksleeper versus conventional anesthesia in 20 adult patients and yielded the following results: mean onset time for intraosseous injection was 1 minute and 3.56 minutes for buccal infiltration.[6] Bigby et al. have also confirmed that when the anesthetic solution is deposited into the spongy bone the onset of action is immediate.[16] The jaw and jawbones contain spongy bone that is highly vascularized, which allows for quick metabolism of anesthetic solution and explains why the analgesic effect is lower in Quicksleeper when compared to conventional syringe anesthesia.[15] Ozer et al. used 0.3 mL and 1.5 mL of 4% articaine solution with 1:100,000 adrenaline in the Quicksleeper system and used supraperiosteal and intraosseous injection methods in their study. They concluded that insufficient duration of anesthesia was due to variability in bone density rather than the amount of solution used.[4] The posterior mandibular region also shows a lower success rate of deep anesthesia with the use of Quicksleeper due to high density and low bone porosity.[17] The anatomy of the buccal and lingual cortical bone can also contribute to decreased success rates as the distance is reduced between these plates, which may cause later diffusion of the anesthetic.[18] In contrast, Siwawut et al. used 1.7 mL of 4% articaine with 1:100,000 epinephrine for intraosseous injection and 3.4 mL of 4% articaine with 1:100,000 epinephrine for buccal infiltration for mandibular first molars and obtained the following results of 95% and 80%, respectively.[6] Sovatdy et al. also confirmed the success and effectiveness when inferior alveolar nerve block was administered using Quicksleeper for mandibular third molars.[1] Sixou et al. conducted research among 181 children and adolescents aged 4 to 16, who underwent 215 sessions using intraosseous injections of 4% articaine with 1:200,000 epinephrine using the Quick Sleeper 2 system.[7] The analgesic effect among patients with primary dentition who had endodontically treated teeth, restorations, or needed extractions was 95%, while for permanent teeth with similar clinical presentations was 87.9%. This data suggests that the Quicksleeper is an effective aid that can be used in both dentitions while fulfilling routine dental procedures. Occasionally an inferior alveolar nerve block by intraosseous injection can be unsuccessful in controlling pain, and examples of such cases are molar incisor hypomineralization or severe pulpal inflammation. An inflamed pulp is often difficult to anesthetize and the additional solution is required to sedate the target tooth. Many authors have focused their research on the effectiveness of Quicksleeper in cases of irreversible pulpitis. A few papers have suggested that the success rate is between 82% and 95%, which can become 100% if a supplementary intraosseous injection is applied.[5,7,16-21] Smaïl-Faugeron et al conducted a single-blind, combined split-mouth and parallel-arm randomized controlled trial in the evaluation of Quicksleeper comparing conventional infiltration anesthesia in pediatric patients. They concluded that Quicksleeper had a profound analgesic effect in cases of molar incisor hypomineralization or severe pulpal infection.[22] Additional studies have also claimed a success rate of 71% to 98% in cases of irreversible pulpitis.[2,6,8,22,23] In the study done by Nieuwenhuizen dentists that planned on injecting 0.6mL of analgesia fluid to patients, tend to give more solution because SleeperOne ran quicker than other injection systems.[10] The SleeperOne is eligible for direct injection into cancellous bone in pediatric patients which prolongs anesthesia of the teeth and creates the fast onset of anesthesia.[11] In addition to reduced pain from the controlled delivery speed, the intraosseous injection earned a higher preference from patients 58.9–69.7%.[11] The Wand can perform multiple injection techniques. It can perform intraligamentary single tooth injections that are reported to be less toxic compared to the traditional inferior alveolar nerve block as less solution is needed to achieve the same depth of anesthesia. The Single Tooth Anesthesia system intraligamentary injection technique can provide a quick-acting localized numbness with the ability to regain normal levels of sensation.[12] The periodontal ligament injection can achieve the same efficacy level as

the conventional inferior alveolar nerve block intraosseous injection without having as many adverse effects. Its effects are limited to a single tooth and its surrounding structures, whereas the inferior alveolar nerve block intraosseous injection spans over a larger area, affecting the structures around the zone of treatment and the surrounding cheek and tongue.[24] Multiple studies have evaluated the efficacy of the Single Tooth Anesthesia system The Wand compared to conventional local anesthetic administration and its relation to different types of treatment performed such as restorative, pulpotomy, and extraction. They concluded that the effectiveness of the anesthesia was independent of the procedure being carried out.[25] Another assessed feature of local anesthesia techniques is their extent determined by the type of performed procedure. The locality of the conventional syringe technique depends on the anesthetic injection technique. Although conventional syringe anesthesia enables painless and reliable anesthesia of the tissues of the operating field, for some procedures it is too broad in relation to the real needs. Moreover, conventional intraligamentary anesthesia requires additional equipment. In this aspect, computer systems have an advantage over conventional syringe anesthesia because they enable the performance of all types of local anesthesia depending on the type of procedure performed. Many studies have concluded that with the use of Quicksleeper only the targeted tooth is anesthetized without affecting any accessory structures.[7] This advantageous feature is possible due to the deposition of the anesthetic solution directly into the cancellous bone of the target tooth.[2,7] Therefore, all patients but especially pediatric patients benefit from the use of Quicksleeper as these individuals reported lower incidents of self-biting injuries, which typically occur with the conventional inferior alveolar nerve block method.[3,4,7,23] Sixou et al. reported that only 6.5% of patients in their study experienced lower lip numbness but mildly as they could still feel their lips.[7] Additionally, a study conducted by Ozer et al. reported that Quicksleeper can anesthetize the palatal and lingual surfaces of a tooth with a single needle injection.[4] SleeperOne is eligible for most injection techniques and is designed for the intraseptal injections technique in pediatric patients as it can inject with minimal pressure due to the intercrestal bone being thinner in children and cancellous bone being more sparse.[11] Additionally, the local anesthetic can be injected directly into the cancellous bone adjacent to the tooth to be anesthetized. Similar to the Quicksleeper, this method prevents mucosal numbing and self-biting of soft tissue that would be the result of a traditional infiltration method such as buccal infiltration, or mandibular nerve block.[10] Dentists can also treat bilateral teeth in the same appointment with this technique without having to administer two infra-alveolar nerve blocks, which can be uncomfortable for the patient.[11] Upon the initial injection with the pen tip of the Single Tooth Anesthesia system wand, the anesthetic is deposited into the soft tissue at a very slow and controlled rate. The onset is immediate and localized to the area it is administered when used for intraligamentary local anesthesia (ILA) periodontal ligament single tooth injections. Although the Wand is designed primarily for anesthetizing a single tooth at a time with the ILA technique, its use is not limited to only these injections; infiltration and nerve blocks are also possible with this system.[26] The anesthetic is administered at a very slow and controlled rate that is below the patient pain threshold level. This allows for a painless experience as there is a significant decrease in the feeling of the pain due to its unperceivable injection.[27] Drops of anesthetic are released into the soft tissue and have an immediate onset. In response to pain sensation, a significant mean reduction of 1.09 point in Visual Analogue Scale (VAS) is recognized with the Wand when it was compared to a conventional needle.[26] A conventional needle and syringe used for buccal infiltration take 120 seconds at 0.01 mL/sec, while the Single Tooth Anesthesia The Wand administers the anesthetic at a much slower 'ControFlorate' of 0.005 mL/sec initially. When the computerized system recognizes that the needle is inserted at the correct location, cruise mode is activated and droplets of solution enter a RapidFlo rate of 0.03 mL/sec. Administration of the anesthetic takes a total of 100 seconds with The Wand. Painful injections are the effect of administration that is too rapid or with too much pressure which is not consistent due to the different elasticity of patients' soft tissues. Manual methods don't allow for a consistent parameter to be followed, whereas in computerized systems delivery is perceivable and consistent with indication/feedback that you are in the correct region.[28] Detailed data regarding duration of anesthesia, analgesic effect, and recommended type of anesthesia of conventional syringe anesthesia and its comparison with Quicksleeper, Sleeper One, and The Wand were presented in Table 1.

Anxiolytic effect

A patient's past experience and expectations at the dental office strongly influence anxiety levels, where the administration of local anesthetic is seen to be the most painful part of an uncomplicated visit. Pain and anxiety hold a strong relationship interchangeably. In addition, this fear of the pain-anxiety relationship also influences the patient's confidence in the dental professional.[29] Anxiety-inducement and pain during administration of local anesthesia with conventional syringe is reported to be greater compared to CCLAD systems.[30] In a study by Kuşcu et al., the influence of the physical appearance of dental injectors on children was assessed, and it was concluded that the physical appearance of the conven-

Table 1. The duration of anesthesia, analgesic effect, and locality of conventional syringe anesthesia, Quicksleeper, Sleeper One, and The Wand*

Local anesthetic equipment	Duration of anesthesia	Analgesic effect	Recommended type of local anesthesia
Conventional syringe	127–258 minutes.[9]	Dependent on injection technique	Dependent on injection technique
Quicksleeper	30–60 minutes.[4,8,15]	· Onset is immediate.[3,15,16] · Duration is lower than conventional anesthesia.[4,8,15] · Successful analgesic effect for endodontically treated teeth, extractions and teeth with MIH, irreversible pulpitis or severe pulpal inflammation.[2,6,8,22,23]	· Target tooth is only anesthetized.[3] · Can anesthetize the palatal and lingual surfaces with single needle penetration.[4]
Sleeper One	· Averaged at 2.49 minutes (SD=0.56) · Intraosseous injection have high success rates.[10,11] · Recommended dose: adults: 1.5–1.8mL of 4% articaine with 1:100,000, children: 0.6–0.8mL of 4% articaine with 1:200,000 epinephrine for children.[11]	· Quick onset.[11] · Pain free injection.[36] · Prolonged anesthesia.[11]	Most injection sites, and intraseptal injections technique for pediatric patients.[11]
The Wand	~ 30 min and can be used with immediate effect for all procedure types.[26]	· Immediate onset of action.[26] · Can achieve same depth of anesthesia as other techniques while often using less amounts of solution.[12,43] · Pain Free admin + procedure.[12,24]	Designed to be used to deliver single tooth anesthesia via PDL (intraligamentary) injection technique but can be used to deliver anesthesia of all techniques (infiltration – buccal/palatal/lingual, and blocks).[24,26,35,45]

*MIH – molar incisor hypomineralization, SD – standard deviation, PDL – periodontal ligament

tional syringe is more anxiety-inducing when compared to other CCALD systems.[31] Many authors through their studies have concluded that the Quicksleeper is painless or may produce mild discomfort and as result anxiety is also minimal or none. Sixou et al examined pain levels with the use of Quicksleeper in 50 children by using the VAS and concluded that most children felt no pain or only experienced slight pain.[3] They also reported that approximately 58.9% of children who had experience with traditional syringe anesthesia, preferred Quicksleeper as it was more comfortable and therefore patients had less anxiety regarding the injection.[3] Marques-Ferreira et al. selected 32 healthy individuals to compare periapical infiltration anesthesia with intraosseous Quicksleeper and established that most individuals did not feel any pain, but a small number of participants did have mild post-operative discomfort.[32] When performing dental injections, dental anxiety is the main co-variable in patient cooperation and emotion. This anxiety can cause the patient to experience more pain, even if the injection itself is less painful. An anxious patient can cause increased muscle tension and disruptive behaviours that ultimately lead to a less pleasant anesthetic experience. A study performed by Hembrecht et al. compared two types of computerized injection systems, SleeperOne and the Wand, to test the levels of pain-related behaviour in children between a first and second treatment appointment.[24] The results showed that even though SleeperOne had a significantly shorter injection time (2.49 min), compared to the Wand (3.2 min), the patient continued to show high levels of pain and distress during the sequential dental treatment session.[24] This study further concluded that the device used for injection did not have an impact on pain and stress related behaviour, and there was an overlying psychological factor involved in the patient's response to the injection systems.[33] Computerized systems aid in controlling pain while at the same time making the patient comfortable and cooperative.[34] Colares et al., found in a study that most fear and anxiety amongst children in a dental setting were in direct connection to injections.[26] Because of this, avoidance of treatment/checkups was prevalent. 41/67 cases reported a heart rate higher after delivery with the conventional method due to the fear and anxiety associated with the appearance needle. A method to make the injection less daunting is what brought about the design and function of the STA. Similar to the SleeperOne, the needle is camouflaged to fit into a pen-like holder attached to a central computerized docking station. San Martin-Lopez et al. concluded that the Wand

and other digital systems of local anesthesia are much better tolerated than conventional systems.[26] Visually induced anxiety that is instigated when a patient encounters a conventional syringe system is put to ease with the friendly-looking design of the Wand.[24] There is a direct correlation between pain/anxiety and the change in blood pressure and heart rate as they are directly proportional. The local anesthetic injection induces fear and anxiety amongst patients and consequently activates their sympathetic nervous system to elicit a heightened heart rate and blood pressure. When comparing heart rate and blood pressure, both are stable amongst patients treated with the STA Wand compared to those with the conventional local anesthesia needle. Previous study concluded that 86.11% of patients that received STA injection were less anxious.[28] With the STA Wand, pain upon administration encompassing insertion of the needle and flow of the anesthetic at the site was less than when compared to the traditional method reported by Jalevik et al. overall implying less anxious and more compliant patients.[28] Stress signals of patients associated with needles and injections were lower in the presence of the STA Wand compared to that of the conventional needle. The Wand is friendly to the eye and the needle can be hidden during administration. It mimics the look of a pen which puts patients, especially of a young age group, at ease as it does not provoke fear like the appearance of a conventional needle.[24] When the anesthetic onset is faster, patient stress is significantly less because there is no pain that is perceived. The height of anxiety is therefore reduced and treatment can therefore continue in a timely and efficient manner. When performing the intraligamentary method with the computerized systems, additional anesthesia to achieve the desired effect is not necessary. The result of this is a more comfortable and pain-free experience for the patient, resulting in higher patient satisfaction and cooperation for current and future treatments.[35]

Other postulated advantages and disadvantages

Aside from the local anesthetic conventional syringe being available in every dental office, and the practice and skill in using the conventional syringe comes in basic dental studies and training, there are some other advantages of the classic local anesthetic technique. Being the most affordable and universally known equipment in the dental office setting, the conventional syringe can anesthetize any location in and around the oral cavity, depending on the type of injection technique used. In a study by Özer et al., it is found that a longer duration of anesthesia is performed with the use of the conventional syringe compared to the computer-controlled system (Quicksleeper). This traditional technique is particularly more useful for longer surgical procedures.[4] Quicksleeper is recommended for minimally invasive procedures due to the short duration of anesthesia. Sixou et al. concluded that successful treatments such as pulpotomies and extractions could be completed in the primary dentition and 91.7–100% of restorative treatments, pulp capping, and scaling in the permanent dentition.[8] Although, this data must be viewed with caution as children who were non-compliant or had teeth that showed signs of physiological or pathological bone resorption were not included.[8] One main advantage of Quicksleeper is its ability to anesthetize the targeted tooth without affecting surrounding structures or adjacent teeth. In comparison to the IANB with traditional syringe anesthesia, Quicksleeper can achieve profound anesthesia without numbness of the cheek tissues or lip.[1] In comparison to traditional syringe anesthesia, the analgesic effect of Quicksleeper is rapid, owing to the direct injection of local anesthetic into the cancellous bone of the target tooth.[3] A study conducted by Beneito-Brotons et al. demonstrates that the onset of Quicksleeper is immediate with the following results of 7.1±2.23 minutes (range 3–14) for conventional technique and 0.48±0.32 minutes (range 0–4) with intraosseous anesthesia; the difference between both techniques are statistically significant.[2] Owing to the design of Quicksleeper it is possible to deposit the solution using similar injection methods as conventional syringe anesthesia such as transcortical, osteocentral, periodontal ligament injection (PLE), intraseptal injection, infiltration, and nerve block anesthesia.[2] Sovatdy et al reported that Quicksleeper requires less anesthetic solution when compared to the traditional IANBI technique.[1] Various studies have reported the Quicksleeper as being painless or less painful when compared to conventional anesthesia due to the asymmetric triple bevel of the needle tip which allows for easy perforation into bone.[2] The SleeperOne is a computer-controlled system that regulates the amount of analgesic fluid injected over a given period. It does so by running on a system called the permanent analysis of resistance (PAR), which controls the injection according to the density of the tissue. This allows the pressure within the tissue to remain low to not exceed the pain threshold.[36] It also allows for a quicker injection time at an average of 2.49 minutes.[10] The SleeperOne is also equipped with a double-beveled needle that makes it easier to penetrate bone when performing an intraosseous injection, specifically in children.[24] It is advantageous compared to its competitors as it has a pen grip for a more precise injection and has four injection speeds that can be controlled with a wireless foot pedal.[36] In a study by Garret-Bernardin et al. it was found that most fear and anxiety amongst children in a dental setting were in direct connection to injections.[26] With the less daunting appearance of the STA Wand, acceptance and cooperation with its use serve as a big advantage. The computerized anesthesia system can deliver

anesthetic solutions to administration sites at a slow and controlled flow rate much below the threshold level known to elicit pain.[27] The practitioner using the system is provided with perceivable and constant indication/feedback of being in the correct anatomical location allowing for efficient delivery and results obtained immediately.[28] This not only makes the deposit of anesthesia effective but also means that clinicians can begin their work immediately and waste no time; overall treatment duration is consequently shortened. The use of the STA Wand is for intraligamentary injections but can be used for all other methods as well inclusive of local infiltrations and nerve blocks. With the intraligamentary technique, it poses as a huge advantage in that inhibition of sensation is extremely localized without affecting neighboring structures such as the tongue and cheek. Less anesthetic solution is often observed to be needed to deliver the same profoundness of effect resulting in less toxicity risk, quick regain of sensations, and overall safety to use amongst healthy as well as medically compromised patients. With the ability to be such a localized form of anesthetic delivery, it is beneficial to both the patient and the dentist that they can have work done and operate on multiple regions of the mouth at the same visit. The success of conventional syringe anesthesia does not simply depend on a set of specific requirements. Hannan et al. studied needle placement for the inferior alveolar nerve block technique and concludes that accuracy of needle placement does not guarantee pulpal anesthesia.[37] Although operator skills such as excellent knowledge in intraoral anatomy, and variations in the location of important injection point landmarks are advantageous, there are many other factors influencing the success of the conventional syringe. Anatomical factors such as accessory innervation and soft and hard tissue barriers to diffusion influence the advancement of local anesthetic solution in which it can be found as unpredictable.[38] Equipment related factors, such as deflection of needle tip and needle gauge, pathological state factors such as pulpal pathologies, and patient's psychological state factors related to administration of local anesthetic and dental procedures, are influential, too.[5,39,40] Cetkovic et al. also mentions that having precise manual control over pressure gradients and flow rate by the operator during injection can be difficult to achieve and may negatively influence the success rate of the anesthetic solution diffusing through alveolar and palatal bone.[14] Overall, the advancement of local anesthetic solution is unpredictable due to a combination of all or some of these factors. In addition to the mentioned disadvantages, second injections and greater amount of anesthetic solution is needed compared to newer methods.[13] Quicksleeper is not recommended for procedures that are complicated or require longer than 60 minutes. Jensen et al conducted a study that showed the aaesthetic effect of Quicksleeper began to reduce at 30 minutes and was practically zero at 60 minutes.[15] Similarly, another study concluded that one-third of patients where CAIOI was used required supplementary anesthesia due to inadequacy.[1] Correspondingly the data shows the success rate of CAIOI was 68% and 72% for IANBI.[1] Therefore, with the use of Quicksleeper a greater concentration of epinephrine should be used to maintain profound anesthesia in bone for procedures that last longer than 30–45 minutes.[8] For surgical operations that required longer than 20 minutes such as removal of impacted third molars, Quicksleeper should not be used as the chances of hemorrhage are greater due to the shortening effect of anesthesia.[2,4,22] Many practitioners prefer the use of vasoconstrictors as it produces a long-lasting and confined anesthetic effect, however, a possible side effect can be increased heart and pulse rate in patients where Quicksleeper was used.[4] In contrast, two studies showed no increase in heart rate with 4% articaine with adrenaline, 1:100,000 with the use of Quicksleeper.[32] Hence, more data is required to sufficiently understand the effect of epinephrine on heart rate with the use of IO injections. Another drawback of Quicksleeper is the application itself takes longer than with conventional techniques, and when combined with the short analgesic effect it may not be the first choice for lengthier procedures.[4] Whether conventional syringe anesthesia or Quicksleeper is used, there is a possibility of unwanted lesions occurring. Siwawut et al. concluded that within their study 10% of patients had developed aphthous ulcers near the injection site a few days after the procedure.[6] Likewise, Graetz et al. established that osteonecrosis, external root resorptions, irreversible pulpal lesions, and/or periodontal lesions may occur in some circumstances.[41] The SleeperOne is aided by an automated anesthetic drop that is fast-acting. This sponsors as a disadvantage as there is a slight learning curve with dentists who are less practiced using this injection system as they might add more anesthetic than planned.[24] The SleeperOne is advertised as a painless injection system, studies mentioned previously prove this to be inaccurate as there are signs of distress and pain while injecting. Although the injection itself may not be as painful, according to the theory and study by Hembrecht, the consecutive deliverance of a local analgesic injection may condition a child's fear response.[24] In addition to this conclusion which was deemed true, it was also proven that even if the system was switched to a different device with which the patient has no experience, they still showed a degree of distress and pain-related behavior.[24] The SleeperOne's anesthetic cartridge is located within the handpiece causing it to be heavier and bulkier than its competitors.[33] It also does not feature a needle that rotates unlike its competitor, the Quicksleeper system, and therefore is unable to perform trans-

cortical and osteocentral injection techniques.[33] The STA Wand can be seen as an investment as it is an additional expense that needs to be considered. Its machinery, though simple, is bulky and requires extra space within an operating room.[28] In places with tighter space availability, the docking station and computerized screen, and the device's needle pen to which it connects to are quite bulky. Apart from purchasing the machine, device-specific needles also have to be purchased for its use, a cost that clinicians need to consider.[28] The length of the cable between the needle pen and the machine also can come in the way of administration and presents as additional clutter in an already compromised area. Lastly, there comes a learning curve associated with the use of the STA Wand system, and a time period before its use can be maximally beneficial.[25]

Comfort and safety of use

In addition to excellent knowledge of intraoral anatomy and variations of injection landmarks, manual control is essential for a comfortable dental procedure for both the operator and the patient. Velasco et al describe that decreasing the speed and pressure of the injection is an effective approach to decreasing pain levels for the patient, but it is a strenuous method for the operator.[42] Needle breakage during administration is a rare and serious complication, not only for the patient but for the operator as well because legal action may be acquired. Awareness and knowledge by the operator of the safety of use of the conventional syringe are important to avoid needlestick and sharp injuries. The use of Quicksleeper does require the clinician to properly examine the working field, take periapical radiographs to determine where the root is located, and determine if any protrusions of the cortical bone exist.[41] Therefore, having adequate knowledge regarding the use of this device as well as the patient's anatomy is extremely crucial.[41] The application time of Quicksleeper is longer when compared to conventional syringe anesthesia due to the components of the device and requires perfected insertion techniques to avoid causing pain or injury.[4] SleeperOne works on a dynamic feedback mechanism that regulates the injection according to the density of the tissue.[32] This leads to a faster average delivery time and because of this, is expected to have a higher comfort for the patient and the dentist. It provides the dentist with a handpiece that looks like a pen as opposed to a syringe grip to ensure a more accurate and precise injection.[36] At the same time, this grip is bulkier than competing computerized anesthetic systems and therefore might be less comfortable than other automated injection systems.[36] The pain caused by the administration of local anesthesia mainly arises due to the puncture of the needle. Computerized injection systems were created to address this.[28] The comfort and ability to use the STA Wand for methods of administration beyond just intraligamentary PDL injections implies convenience as devices do not have to be changed to establish the necessary level of effect desired.[26] During the initial administration of anesthesia, when compared to the conventional syringe, the STA Wand is reported to have a lower pain sensation. Furthermore, the efficacy or intended result in regards to depth of anesthesia results as adequate for both. The STA system can achieve local anesthesia to specified areas in the mouth, and as a result, multiple sites of the mouth can be treated simultaneously without compromising the safety of the patient. This serves as a great alternative to the conventional syringe nerve block method.[25] The administration of the anesthetic is very slow allowing for the perception of pain to be nullified. The numbing effect is extremely localized without extending to the cheek/tongue, therefore, eliminating common post-treatment cheek/lip biting trauma. Furthermore, the depth of necessary anesthesia is achieved more efficiently in regards to timing, location as well as overall toxicity as less solution is required.[26] The effects of anesthesia take longer to wear off with the block and can be very uncomfortable for the patient. No complaints or complications were reported with the ILA technique. With regards to the ILA technique, by the end of the treatment, the effect of the anesthetic is worn off and the patients' sensations are reported as normal.[43]

Limitations

Considering the previously mentioned factors that influence the success rate of anesthesia with the conventional syringe technique, following the methods of administration correctly with accurate knowledge and placement of injection, as well as clinically evaluating successful anesthesia such as lip numbness, there can be other factors that may limit the success and efficacy of the conventional syringe technique. Results may vary due to pharmacological properties, variations in innervations, and anxiety and psychological factors.[14,37] As discussed previously, the Quicksleeper has many advantages and can be a great accessory for practitioners, although it does have some limitations. Marques-Ferreira et al. discussed in their paper that the Quicksleeper does have the potential to overheat, thus resulting in irreversible damage to the targeted tooth and surrounding structures.[32] With traditional syringe anesthesia, only a syringe and needle are required to deliver the anesthetic solution while the Quicksleeper uses a handpiece. Woodmansey et al reported that osteonecrosis occurred in an HIV-positive individual with the use of diploe anesthesia because of the heat that was generated by the needle upon delivery of the solution.[44] Therefore, the rotation speed of the Quicksleeper should be monitored and kept to 11,000 rpm to avoid such situations.[44] Another limitation of Quicksleeper is reduced space in the operative field for the insertion of the needle. Graetz

Table 2. Advantages and disadvantages of conventional syringe and Quicksleeper, SleeperOne, and The Wand

Local anesthetic equipment	Advantages	Disadvantages
Conventional syringe	· Longer duration of anesthesia that is useful for longer surgical procedures.[4] · Can anesthetize any location depending on injection technique.[43] · Universally known and learned in dental university education.[4]	· The advancement of the local anesthetic solution is unpredictable, no matter the accuracy of needle placement.[38] · Operator skills which come with experience are helpful in the success of the anesthetic, therefore knowing the intraoral anatomy and variations of landmarks for different techniques is critical, but accuracy of needle placement does not guarantee pulpal anesthesia.[39] · Factors that are limited in controlling precisely, such as pressure gradients and flow rate by the operator, may negatively influence the success rate of the anesthetic solution diffusing through alveolar and palatal bone.[14] · Greater pain of injection experienced.[1] · Second injections usually needed.[13] · Greater amount of anesthetic solution needed compared to new methods.[13]
Quicksleeper	· Recommended for minimally invasive procedures.[6] · Can anesthetize the targeted tooth only.[3] · Lack of lip and cheek tissue numbness.[1,3] · Analgesic effect is rapid and immediate.[3,4,8] · Injection methods used in conventional syringe anesthesia can also be used with Quicksleeper.[1,3,4] · Less anesthetic solution required.[1,8] · Painless or less painful.[1,3,4,22,33]	· Not recommended for procedures longer than 60 minutes.[2,4,22] · Shortening of anesthetic effect due to haemorrhage.[4] · Can produce increased heart rate.[4] · Duration of its application is longer than traditional syringe anesthesia.[4] · Osteonecrosis, external root resorptions, irreversible pulp lesions, aphthous ulcers and/or periodontal lesions may develop.[45]
Sleeper One	· Regulated flow of fluid.[36] · Low pressure within tissue.[36] · Quick injection time.[10] · Double beveled needle.[10] · Multiple injection speeds.[36]	· Learning curve.[10] · Hand piece heavy and bulky.[33] · Non rotating needle.[33] · Not eligible for osteocentral and transcortical injections.[33]
The Wand	· Decreased anxiety levels.[26] · Increased cooperation.[26] · Painless and less daunting appearance.[26] · Controllable and predictable delivery of anesthetic.[26] · Immediate onset of action.[26] · Quicker overall treatment duration. · Less toxicity (less amount of anesthetic needed to achieve the same depth of anesthesia with conventional method).[26] · Ability to perform all anesthetic methods with the same device.[26] · Ability to work on multiple areas at the same visit.[43] · Inhibition of sensation localized to one area without extending to the tongue and cheek.[24] · Quick regain of sensations.[43] · Can be used across all procedure types.[26] · Avoiding postoperative self-inflicted injuries tongue and lip biting for example.[45] · Safe to use in patients with underlying medical conditions.[46]	· Additional expense machinery.[28] · Inconvenient to have in areas where a lot of space is not available.[28] · Device specific needles are needed.[28] · Learning curve associated with its use.[24]

et al analyzed situations where dentists accidentally perforated the root while administering the anesthetic solution, therefore compromising the pulp and peri-radicular tissues of the targeted tooth.[41] Traditional syringe anesthesia may be preferred in scenarios with reduced visibility or space. Despite the friendlier look of the SleeperOne compared to the traditional syringe, it is still a needle, and patients, specifically younger children that have anxiety with needles might still cause trouble for dentists during the anesthetic injection. As mentioned previously, a large factor that affects the pain of dental injections is patient anxiety. It seems that although the injection itself is designed to be less painful due to the C-CLAD's technology, the patient's anxiety levels cause there to be no significant difference in the experience of pain whether the dentist is using the SleeperOne or not.[10] The SleeperOne consists of a lead control box and a foot pedal that is connected to a handpiece, which is where the local anesthetic carpule is located. Because of this, the handpiece is bulkier than its competitors such as the Wand, and maybe more uncomfortable to hold for the dentist.[33] It also requires more equipment compared to the conventional syringe. The access and availability to the STA Wand device present a limitation of using the STA intraligamentary technique with the Wand. It is an extra piece of equipment that one would have to invest in for use at their clinic. Additional to the purchase of the machinery, further functional pieces with specific needle attachments need to be bought.[26] To be able to optimize its results, a practitioner must first and foremost also learn how to use it. Clinicians can pick up on this quite rapidly but it still poses a limitation compared to the conventionally taught ease of using a standard carpule syringe.[23] Table 2 summarizes the advantages and disadvantages of using conventional syringe anesthesia as compared to CCLAD.

Conclusion

Anxiety associated with dentistry, specifically due to the fear of needles, instigated curiosity to find out whether computer-assisted anesthetic devices including STA Wand, Quick Sleeper, and SleeperOne could potentially replace the use of the conventional daunting anesthesia syringe. Comparing factors associated with the anxiolytic effect, longevity, profoundness of anesthesia, accessibility, as well as overall practicality of use, positive outcomes have been able to be observed. Furthermore, it can be noted that all computerized methods can deliver anesthesia by conventional methods (nerve block and local infiltration) but additionally by intraligamentary and intraosseous techniques. The use of these devices encompasses all treatment/procedure types and is well tolerated by their recipients. Drawbacks inclusive of additional material and equipment expense, the training and learning curve, and availability of these devices are elements that indicate further research necessary to determine whether or not computer-assisted anesthetic devices can substitute the use of the conventional needle and syringe by dental clinicians.

Declarations

Funding

There are no external funding.

Author contributions

Conceptualization, N.S., J.Y. and S.W.,K.B; Resources, N.S.,S.W., J.Y.; Data Curation, N.S.,S.W., and J.Y.; Writing – Original Draft Preparation, N.S., S.W., J.Y.; Writing – Review & Editing, K.B.; Supervision, K.B.; Project Administration, K.B.

Conflicts of interest

None conflicts of interest are declared.

Data availability

Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

References

1. Sovatdy S, Vorakulpipat C, Kiattavorncharoen S, Saengsirinavin C, Wongsirichat N. Inferior alveolar nerve block by intraosseous injection with Quicksleeper® at the retromolar area in mandibular third molar surgery. *J Dent Anesth Pain Med*. 2018;18(6):339–347.
2. Beneito-Brotons R, Penarrocha-Oltra D, Ata-Ali J, Penarrocha MA. Intraosseous anesthesia with solution injection controlled by a computerized system versus conventional oral anesthesia: A preliminary study. *Med Oral Patol Oral Cir Bucal*. 2012;17(3):e426–429.
3. Sixou JL, Marie-Cousin A, Huet A, Hingant B, Robert JC. Pain assessment by children and adolescents during intraosseous anesthesia using a computerized system (QuicksleeperTM). *Int J Paediatr Dent*. 2009;19(5):360–336.
4. Özer S, Yaltirik M, Kirli I, Yargic I. A comparative evaluation of pain and anxiety levels in 2 different anesthesia techniques: Locoregional anesthesia using conventional syringe versus intraosseous anesthesia using a computer-controlled system (Quicksleeper). *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2012;114(5 Suppl):S132–139.
5. Zarei M, Ghoddusi J, Sharifi E, Forghani M, Afkhami F, Marouzi P. Comparison of the anaesthetic efficacy of and heart rate changes after periodontal ligament or intraosseous X-tip injection in mandibular molars: A randomized controlled clinical trial. *Int Endod J*. 2012;45(10):921–926.
6. Siwawut S, Panitvisai P. Anesthetic efficacy of intraosseous and buccal infiltration techniques: a randomized controlled crossover clinical trial. *CU Dent J*. 2018;41:1–12.
7. Sixou JL, Barbosa-Rogier ME. Efficacy of intraosseous injections of anesthetic in children and adolescents.

Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;106(2):173–178.
8. Sixou JL, Marie-Cousin A. Intraosseous anesthesia in children with 4 % articaine and epinephrine 1:400,000 using computer-assisted systems. *Eur Arch Paediatr Dent.* 2015;16(6):477–481.
9. Fernandez C, Reader A, Beck M, Nusstein J. A prospective, randomized, double-blind comparison of bupivacaine and lidocaine for inferior alveolar nerve blocks. *J Endod.* 2005;31(7):499–503.
10. Nieuwenhuizen J, Hembrecht EJ, Aartman IH, Krikken J, Veerkamp JS. Comparison of two computerised anesthesia delivery systems: Pain and pain-related behaviour in children during a dental injection. *Eur Arch Paediatr Dent.* 2013;14(1):9–13.
11. Tom K, Aps J. Intraosseous Anesthesia as a Primary Technique for Local Anesthesia in Dentistry. *Clin Res Infect Dis.* 2015;2(1):1012.
12. Kämmerer, PW, Adubae A, Buttchereit I, Thiem DGE, Daubländer M, Frerich B. Prospective clinical study comparing intraligamentary anesthesia and inferior alveolar nerve block for extraction of posterior mandibular teeth. *Clin Oral Investig.* 2018;22(3):1469–1475.
13. Thiem DG, Schnaith F, Van Aken CM, et al. Extraction of mandibular premolars and molars: Comparison between local infiltration via pressure syringe and inferior alveolar nerve block anesthesia. *Clin Oral Investig.* 2017;22(3):1523–1530.
14. Cetkovic D, Milic M, Biocanin V, et al. Efficacy and safety of 4% articaine with epinephrine for the anterior middle superior alveolar nerve block comparing to the computer-controlled and conventional anesthetic delivery: Prospective, randomized, cross-over clinical study. *Vojnosanit Pregl.* 2019;76(10):1045–1053.
15. Jensen J, Nusstein J, Drum M, Reader A, Beck M. Anesthetic efficacy of a repeated intraosseous injection following a primary intraosseous injection. *J Endod.* 2018;34(2):126–130.
16. Bigby J, Reader A, Nusstein J, Beck M, Weaver J. (2006). Articaine for supplemental intraosseous anesthesia in patients with irreversible pulpitis. *J Endod.* 2006;*32*(11):1044–1047.
17. Coggins R, Reader A, Nist R, Beck M, Meyers WJ. Anesthetic efficacy of the intraosseous injection in maxillary and mandibular teeth. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1996;81(6):634–641.
18. Gallatin J, Reader Al, Nusstein J, Beck M, Weaver J. A comparison of two intraosseous anesthetic techniques in mandibular posterior teeth. *J Am Dent Assoc.* 2003;134(11):1476–1484.
19. Nusstein J, Kennedy S, Reader A, Beck M, Weaver J. Anesthetic efficacy of the supplemental X-tip intraosseous injection in patients with irreversible pulpitis. *J Endod.* 2003;29(11):724–728.
20. Parente SA, Anderson RW, Herman WW, Kimbrough WF, Weller RN. Anesthetic efficacy of the supplemental intraosseous injection for teeth with irreversible pulpitis. *J Endod.* 1998;24(12):826–828.
21. Penarrocha-Oltra D, Ata-Ali J, Oltra-Moscardo MJ., Penarrocha-Diago M, Penarrocha M. Comparative study between manual injection intraosseous anesthesia and Conventional Oral Anesthesia. *Med Oral Patol Oral Cir Bucal.* 2012;17(2):e233–235.
22. Smaïl-Faugeron V, Muller-Bolla M, Sixou JL, Courson F. Evaluation of intraosseous computerized injection system (QuicksleeperTM) vs conventional infiltration anesthesia in paediatric oral health care: A Multicentre, single -blind, combined split-mouth and parallel-arm randomized controlled trial. *Int J Paediatr Dent.* 2019;29(5):573–584.
23. Collier T. Intraosseous anesthesia as a primary technique for mandibular posterior teeth with symptomatic irreversible pulpitis. *Acta Odontol Scand.* 2018;76(7):535–537.
24. Hembrecht EJ, Nieuwenhuizen J, Aartman IH, Krikken J, Veerkamp JS. Pain-related behaviour in children: A randomised study during two sequential dental visits. *Eur Arch Paediatr Dent.* 2013;14(1):3–8.
25. Campanella V, Libonati A, Nardi R, et al. Single tooth anesthesia versus conventional anesthesia: A cross-over study. *Clin Oral Investig.* 2018;22(9):3205–3213.
26. Garret-Bernardin A, Cantile T, D'Antò V, et al. Pain experience and Behavior Management in Pediatric Dentistry: A comparison between traditional local anesthesia and the wand computerized delivery system. *Pain Res Manag.* 2017;2017:1–6.
27. Hao Y, Zhang Z, Meng Y. Application effect of computer-assisted local anesthesia in patient operation. *Contrast Media Mol Imaging.* 2021;2021:1–6.
28. Hachem CE, Kaloustian MK, Cerutti F, Chedid NR. European journal of paediatric dentistry. Metallic syringe versus electronically assisted injection system: a comparative clinical study in children. *Eur J Paediatr Dent.* 2019;20(4):320–324.
29. Berrendero S, Hriptulova O, Salido MP, Martínez-Rus F, Pradíes G. Comparative study of conventional anesthesia technique versus computerized system anesthesia: A randomized clinical trial. *Clin Oral Investig.* 2020;25(4):2307–2315.
30. Flisfisch S, Woelber JP, Walther W. Patient evaluations after local anesthesia with a computer- assisted method and a conventional syringe before and after reflection time: A prospective randomized controlled trial. *Heliyon.* 2021;7(2):e06012.
31. Kuşcu ÖÖ, Akyuz S. Children's Preferences Concerning the Physical Appearance of Dental Injectors. *J Dent Child (Chic).* 2006;73(2):116–121.
32. Marques-Ferreira M, Carrilho E, Paulo S, Carrilho T, Figueiredo JP, Macedo R. Anesthesia in dental medicine with local infiltrative anaesthetic technique versus diploe anesthesia delivery systems: Efficacy and behaviour, an experimental study. *Acta Med Port.* 2017;30(12):848.
33. Chong BS, Miller JE, Sidhu SK. alternative local anaesthetic delivery systems, devices and aids designed to minimize

painful injections- a review. *ENDO - Endodontic Practice Today.* 2014;8(1):7–22.

34. Aggarwal K, Lamba AK, Faraz F, Tandon S, Makker K. Comparison of anxiety and pain perceived with conventional and computerized local anesthesia delivery systems for different stages of anesthesia delivery in maxillary and mandibular nerve blocks. *J Dent Anesth Pain Med*. 2018;18(6):367.
35. Saoji H, Nainan MT, Nanjappa N, Khairnar MR, Hishikar M, Jadhav V. Assessment of computer-controlled local anesthetic delivery system for pain control during restorative procedures: A randomized controlled trial. *J Dent Res Dent Clin Dent Prospects*. 2019;13(4):298–304.
36. June RM, Lewkowicz M, Milner PT, Rybak MA. Local anesthetics and advances in their administration – an overview. *J Pre-Clin Clin Res.* 2017;11(1):94–101.
37. Hannan L, Reader A, Nist R, Beck M, Meyers WJ. The use of ultrasound for guiding needle placement for inferior alveolar nerve blocks. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1999;87(6):658–665.
38. Wilson S, Johns P, Fuller PM. The inferior alveolar and mylohyoid nerves: An anatomic study and relationship to local anesthesia of the anterior mandibular teeth. *J Am Dent Assoc.* 1984;108(3):350–352.
39. Jeske AH, Boshart BF. Deflection of conventional versus nondeflect-ing dental needles in vitro. *Anesth Prog.* 1985;32(2):4–64.
40. Yesilyurt C, Bulut G, Taşdemir T. Pain perception during inferior alveolar injection administered with the wand or conventional syringe. *Br Dent J.* 2008;205(5):E10.
41. Graetz C, Fawzy-El-Sayed KM, Graetz N, Dorfer CE. Root damage induced by intraosseous anesthesia-an in vitro investigation. *Med Oral Patol Oral Cir Bucal.* 2013;18(1):e130–134.
42. Velasco I, Soto R. Anterior and middle superior alveolar nerve block for anesthesia of maxillary teeth using conventional syringe. *Dent Res J (Isfahan).* 2012;9(5):535.
43. Youssef BR, Söhnel A, Welk A, et al. RCT on the effectiveness of the intraligamentary anesthesia and inferior alveolar nerve block on pain during dental treatment. *Clin Oral Investig.* 2021;25(8):4825–4832.
44. Woodmansey KF, White RK, He J. Osteonecrosis related to intraosseous anesthesia: Report of A case. *J Endod.* 2009;35(2):288–291.
45. Giannetti, L et al. Single tooth anesthesia: a new approach to the paediatric patient. A clinical experimental study. *Eur J Paediatr Dent.* 2018;19(1):40–43.
46. Yuchen H, Zheqi Z, Yan M. Application Effect of Computer-Assisted Local Anesthesia in Patient Operation. *Contrast Media Mol Imaging.* 2021;2021:8643867.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.5

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 284–289

ORIGINAL PAPER

Gastroprotective effects of *Prunus laurocerasus* L. fruit extracts against the oxidative stress induced by indomethacin in rats

Ozlem Aydin Berktas [1], Arif Keskin [2], Emine Gulceri Gulec Peker [1]

[1] Departmenf of Nursing, Faculty of Healthy Science, Giresun University, Giresun, Turkey
[2] Department of Anatomy, Faculty of Meram Medicine, Necmettin Erbakan University, Konya, Turkey

ABSTRACT

Introduction and aim. *Prunus laurocerasus* L. is a perennial plant belonging to the Rosaceae family and is grown on the shores of the Black Sea Region. In the current study, the effect of *P. laurocerasus* fruits was investigated in the ulcer model created by the application of indomethacin to rats.

Material and methods. Rats divided into five groups: fruit water extract (200 mg/kg), fruit ethanol-water extract (200 mg/kg), lansoprazole agent (LAN, 25 mg/kg), and indomethacin (IND, 25 mg/kg). All administrations were given to animals by oral gavage. At the end of the experiment, macroscopic and biochemical measurements were made in rats.

Results. The lipid peroxidation was quite high in rat stomach tissues given IND. The applied LAN and extracts reduced this increase to almost a healthy rate. On the other hand, the amount of glutathione, catalase, and superoxide dismutase activities were found very low in IND applied tissues. The LAN and fruit extracts treatments tried to show their protective feature by increasing this decreased antioxidant level in their own groups.

Conclusion. The data obtained determined that both enzyme and non-enzyme antioxidant markers measured in fruit extracts had a protective effect almost as strong as lansoprazole.

Keywords. indomethacin, lipid peroxidation, *Prunus laurocerasus,* ulcer

Introduction

Gastrointestinal system disorders have become an important public health problem in terms of morbidity and mortality, which affects many people.[1] Among them, the most common are; peptic ulcer, gastric and duodenal ulcers. Peptic ulcers are characterized by deep necrotic lesions and destruction of epithelial and connective tissue components, including fibroblasts, smooth muscle cells, blood vessels, and nerves in the gastric mucosa.[2] Gastric and duodenal ulcers occur in the upper part of the gastrointestinal tract (GIT) as a result of an imbalance between protective (e.g. mucus production) and aggressive (e.g. HCl release) factors at the gastric mucosa level. Mainly stomach ulcer; It occurs when the balance between more than one harmful factor (such as hydrochloric acid and pepsin secretion) and several gastroprotection's (such as prostaglandins, bicarbonate and mucus production, mucosal barrier and adequate blood flow) is disturbed.[3] Especially acid secretion, disorders in the protective mucosal barrier, in addition to these, genetic predisposition (heredity), stress, cortisone-type drugs, NSAIDs (Non-steroidal anti-inflammatory drugs) such as aspirin and indomethacin (IND), *Helicobacter pylori*, *Herpes simplex* virus (Type I, HSV-1) and variables such as smoking and alcohol use cause ulcers. Among these variables, regularly used NSAIDs constitute a very large part of gastric mucosal ulceration. It is known that anti-inflammatory drugs such as aspirin

Corresponding author: Ozlem Aydin Berktas, e-mail: ozlem.berktas@giresun.edu.tr

Received: 28.04.2022 / Revised: 16.05.2022 / Accepted: 18.05.2022 / Published: 30.09.2022

Berktas OA, Keskin A, Peker EGG. *Gastroprotective effects of Prunus laurocerasus L. fruit extracts against the oxidative stress induced by indomethacin in rats*. Eur J Clin Exp Med. 2022;20(3):284–289. doi: 10.15584/ejcem.2022.3.5.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

and IND show anti-inflammatory activity by inhibiting the cyclooxygenase (COX) enzyme system. However, these drugs cause gastric damage by inhibiting the COX enzyme system, suppressing prostaglandin (PG) biosynthesis, and resulting in disruption of the gastric mucosal barrier. Suppressing PG biosynthesis means inhibiting mucus secretion, which is a defense factor against gastrointestinal damage, and is manifested by some events such as decreased local blood flow, topical irritation, and inhibition of tissue repair.[4]

The current pharmacological treatment of peptic ulcers is carried out using drugs that act by two main mechanisms depending on these variables: the first is the inhibition of gastric acid secretion, and the second is the inhibition of gastric acid secretion. Accordingly, proton pump inhibitors such as omeprazole, lansoprazole; histamine H_2-receptor antagonists such as ranitidine, cytoprotective agents such as sucralfate, and drugs that increase the natural mechanisms of gastric protection such as misoprostol. However, as these drugs often bring with them serious side effects, it justifies the search for new alternatives for ulcer treatment.[5,6] Especially recently, natural products have started to be promising new sources of therapeutic agents. Intensive studies are continuing to produce more practical treatment solutions that provide easier healing. These therapeutic agents are especially selected from products with known high antioxidant properties and which can provide strong antioxidant support. As in many tissues damage of ulcer, it has been determined in many studies that they affect each other depending on the interaction of antioxidant enzymes, which are protective enzymes against reactive oxygen species.

Prunus laurocerasus L. (*Laurocerasus officinalis* Roem.) is a perennial plant belonging to the Rosaceae family and is grown on the shores of the Black Sea Region.[7] It is locally called Taflan, Karayemiş and Laz cherry in Turkey. While it is consumed as fresh fruit, especially in August, it is also suitable to be consumed as dried fruit, such as molasses, marmalade or jam.[8] In Turkey, both its fruit and seeds have been used for many years as an anti-diabetic, analgesic and diuretic agent, as well as being used for stomach ulcers, digestive system, bronchitis, eczema, and hemorrhoid complaints.[9] Although there are studies on the functional content, antioxidant capacity and anti-diabetic activity of seeds, fruits, molasses and leaves of *P. laurocerasus*, they are very limited.[10]

Aim

In the present study, it was aimed to investigate the gastroprotective effects of water and ethanol-water extracts of *P. laurocerasus* L. fruits on indomethacin-induced gastric ulcer.

Material and methods

Chemicals

All chemical products used in the experiments were obtained from Sigma Chemicals Company (Germany). Ketamine (80 mg/kg) and xylazine (10 mg/kg) used as anesthetics were purchased from a legitimate retailer.

Plant material and extraction of plant material

P. laurocerasus fruits were used as study material in the research and were obtained from a vendor selling dried fruit. These fruits, which are grown in the eastern cities of the Black Sea Region, are collected and dried in August.

Extraction method

P. laurocerasus fruits were dried in the shade after being collected in Giresun Province. Then, the dried fruits separated from their seeds were ground and powdered, and then mixed in a water-ethanol mixture (4:4). It was then kept in a shaking water bath at room temperature for 7 days. After 7 days, it was filtered through filter paper and then the solvents were evaporated under reduced pressure in the evaporator at 50°C to obtain fruit extract. Extraction was prepared by the methods used in previous studies.[9]

Experimental procedure

Animals and treatments

The experimental animals were obtained from Saki experimental animals in Ankara, which is a legal seller. Giresun University experimental animals were included in the experiment after they were approved by the ethics committee (2019/13).

First of all, experimental animals were divided into 5 different groups. (I) Control group, (II) IND group 25 mg/kg, (III) LAN group 25mg/kg, (VI) Prunus L. water extract group 200mg/kg and (V) Prunus L. ethanol-water extract group 200 mg/kg.

After the groups were determined, the groups separated as 6 animals in each cage were starved for 24 hours. The next day, all groups were administered at the determined doses (except for the control). After 10 minutes, IND, the agent that will cause gastric damage, was applied to all groups. The rats were sacrificed by giving ketamine (80 mg/kg) and xylazine (10 mg/kg) 6 hours after all applications were completed. Macroscopic and biochemical examinations were made in gastric tissues obtained after sacrification.

Indomethacin-induced gastric damage

The protective effect of the experimental groups was determined by comparing with lansoprazole. In order for the IND to cause gastric damage, all animals were fasted for 24 hours and tested the next day. For administration, animals were administered orally by gavage with

200 mg/kg extracts and 25 mg/kg LAN. Then, IND was applied to all groups and the stomachs of the animals were removed at the end of 6 hours. The stomachs obtained were washed and counted macroscopically and ulcer areas were determined.

Biochemical investigation of stomach tissues
Stomach tissues extracted from animals were ground with liquid nitrogen for biochemical analysis. The ground stomach tissues were homogenized by treatment with appropriate homogenates and centrifuged at 4°C. The obtained supernatants were used for enzyme activities such as catalase (CAT), superoxide dismutase (SOD) and to determine the amount of glutathione (GSH) and lipid peroxidation level (LPO).

CAT activity
Decomposition of H_2O_2 in presence of catalase was at 240 nm.[11] Catalase activity was defined as the amount of enzyme required to decompose 1 nmol of H_2O_2 per minute, at 25°C and pH 7.8. Results were expressed as mmol/min/mg tissue.

SOD activity
SOD activity was measured according to the principle of superoxide radical formation of xanthine.[12] SOD activity was then measured at 560 nm by the degree of inhibition of this reaction.

GSH determination
The amount of GSH is determined according to the method of Sedlak et al. With homogenates compatible with the literature, glutathione in tissues is expressed as nmol/g.[13]

LPO determination
The level of LPO was determined with the homogenates prepared in accordance with Ohkawa's method. Data obtained were expressed as nmol/g tissue.[14]

Statistical analysis
The results were made using the appropriate SPSS program (IBM SPSS Statistics 20, Turkey). Statistical differences were determined by the ANOVA test. Multiple comparisons were expressed by Duncan. Significance was determined according to $p<0.05$.

Results

Gastroprotective effect of P. laurocerasus on indomethacin-induced gastric damage
The protective effects of the extracts on gastric damage caused by IND in rats are presented in Table 1 and Figure 1. While there was a very strong injury in the animals in the IND group, good protection was determined in water and ethanol-water extracts, as in the LAN group. When ulcer areas are compared; While the damage caused by IND was 32±0.6, it was seen that it was reduced by almost half in the treatment groups (16.5±2.6 and 9±1.6). We can even say that it was as effective as the LAN group, which is the positive control agent. In Figure 1, the damaged areas are clearly visible in the macroscopically counted tissues.

Table 1. Effects of different doses of species extracts and single dose of famotidine (FAM) on indomethacin (IND)-induced gastric damage in rats*

Treatment	n	Dose (mg/kg)	Ulcer Areas
Healthy	6	-	0 ± 0^a
IND	6	25	32 ± 0.6^d
LAN	6	25	6.5 ± 0.7^b
Prunus laurocerasus water extract	6	200	16.5 ± 2.6^c
Prunus laurocerasus ethanol-water extract	6	200	9 ± 1.6^b

*Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$), results are means ± SE of three measurements, n – the number of rats

The comparison of enzyme activities in rat stomach tissues
Biochemical enzymes in rat stomach tissues were measured to express how the antioxidant defense system works. The results are shown in Table 2. According to the table; CAT and SOD enzyme activity is very low in IND applied tissues. In the LAN group, which is the positive control drug, it was increased to a level as high as healthy. Likewise, *P. laurocerasus* extracts have increased this level almost as much as LAN. Similarly, the GSH level in applied IND tissues is also very low. Again, the extracts and LAN tried to increase this decrease and determined their protective effects. Although IND increased the tissue damage considerably in the tissues where lipid peroxidation was measured, the treatment groups and LAN reduced this damage. Thus, enzymes with high damage indicators and LPO and GSH levels clearly showed their protection thanks to the extracts.

Discussion

NSAIDs, which are widely used clinically as anti-inflammatory and analgesic agents, are drugs with strong side effects in many systems. It manifests itself with ulcerative lesions, especially in the gastrointestinal tract. There are restrictions on its use as an anti-inflammatory due to these side effects.[15] In eliminating this damage caused by NSAIDs; Inhibition of prostaglandin synthesis and inhibition of epithelial cell proliferation occurring around the ulcer is suggested.[16,17] IND is one of these drugs that are not corticosteroid and cause stomach damage. It contains lipids with good affinity for the lipophilic parts of cell membranes. With this advantage,

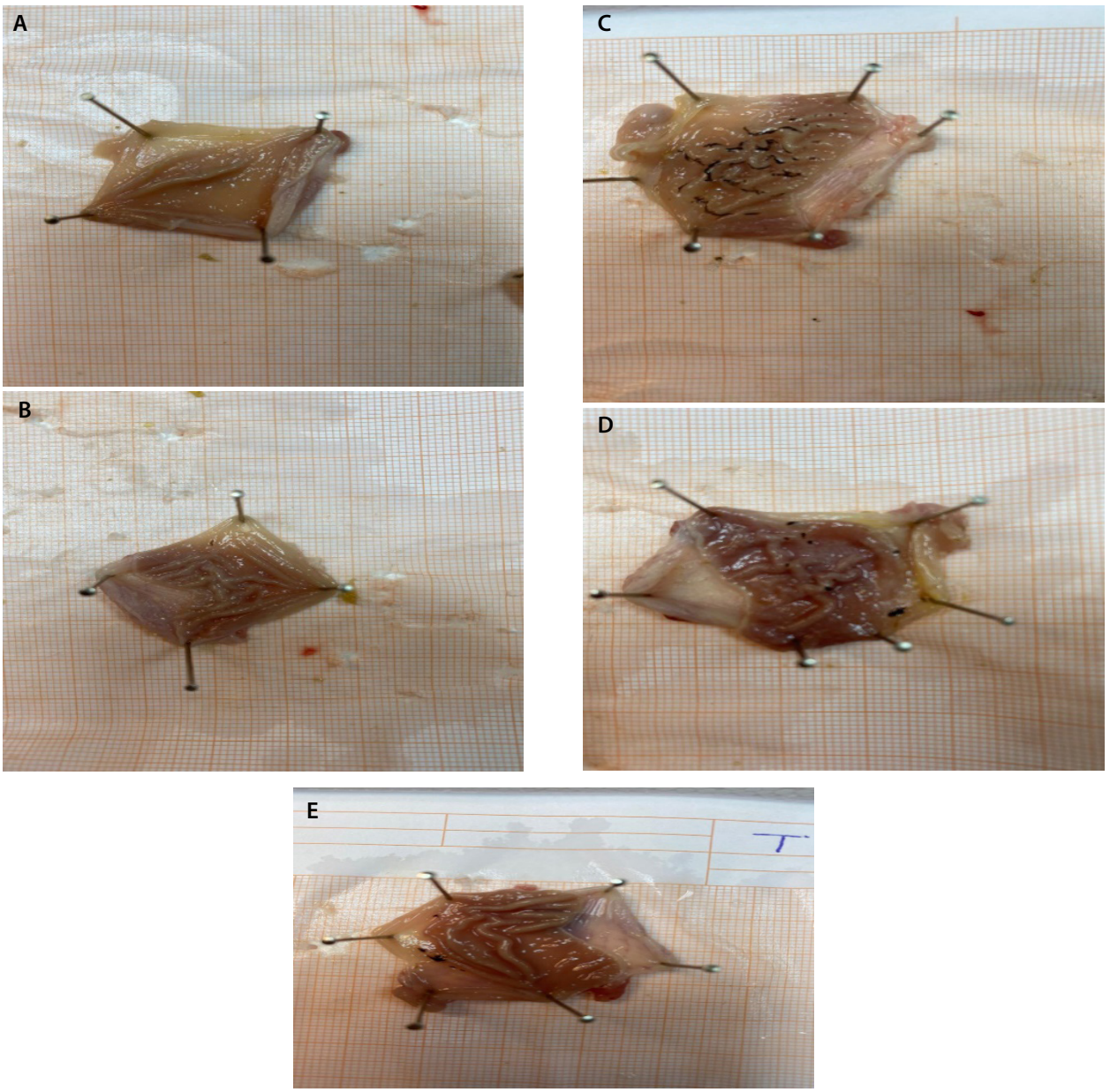

Fig. 1. The stomach samples taken from gastric damaged tissues induced by IND (25 mg/kg); (A) healthy, (B) LAN, (C) IND, (D) *P. laurocerasus* water extract, (E) *P. laurocerasus* ethanol-water extract

Table 2. Effects of *P. laurocerasus* extracts treatments on changes in activities of catalase (CAT), superoxide dismutase (SOD) and with levels of lipid peroxidation (LPO) and total glutathione (GSH) in rat's indomethacin (IND)-induced gastric tissue*

Treatment	n	Dose (mg/kg)	CAT activity (mmol/min/mg tissue)	LPO (nmol/g tissue)	SOD activity (mmol/min/mg tissue)	GSH (nmol/g tissue)
Healthy	6	-	19.11 ± 1^{b}	34.75 ± 1.4^{a}	4.13 ± 0.3^{c}	3.8 ± 0.2^{c}
IND	6	25	14.95 ± 1^{a}	90.24 ± 1^{d}	1.75 ± 0.2^{a}	0.7 ± 0.1^{a}
LAN	6	25	18.57 ± 0.4^{b}	34.5 ± 1.9^{a}	3.95 ± 0.1^{c}	3.1 ± 0.2^{b}
Prunus laurocerasus water extract	6	200	17.57 ± 0.5^{b}	62.25 ± 1.04^{c}	2.2 ± 0.1^{a}	3.3 ± 0.3^{b}
Prunus laurocerasus ethanol-water extract	6	200	19.32 ± 0.2^{b}	53.8 ± 1.7^{b}	3.03 ± 0.1^{b}	3.2 ± 0.4^{b}

*Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$), results are means ± SE of three measurements, n – the number of rats

it easily adheres to structural phospholipids and causes disruption of the cell membrane structure and hydrophobic structures in the mucosal structure. With this loss, it paves the way for lipid peroxidation and also allows the entry of water-soluble agents into the cell that will cause injury. Thus, damage occurs with the entry of substances such as pepsin, acid, bile salts, which easily pass through the cell membrane.[16,17]

The initiation of the ulcer process by IND administration is attributed to several processes, including production of reactive oxygen species, initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis, and inhibition of prostaglandin E2.[17] In the current study, when the macroscopic findings were examined, the degree of ulceration was found to be quite high in the stomach tissues of the IND applied rat, due to the combination of these reasons (32±0.6) This damage in the ulcer areas is also clearly seen in the macroscopic examination of the excised stomach tissues. The decrease in the ulcer areas extracted by the count made on the millimetric paper is expressed in figure 1. The findings in IND-induced ulcer experiments, which were carried out in some experimental studies before, are also parallel.[18-20] The applied treatment groups and the positive control ulcer drug showed their protective effects by significantly reducing this damage (*P. laurocerasus water extract;* 16.5±2.6, *P. laurocerasus ethanol-water extract;* 9±1.6, LAN; 6.5±0.7).

There are also in vivo experimental studies to eliminate the damage caused by the ulcer. These studies are particularly against IND-induced reactive oxygen species (ROS) at the tissue level.[21] These agents act as oxidants in cells and contribute to the production of ROS. Against this, organisms activate a series of enzymatic and non-enzymatic defense mechanisms. Antioxidant enzymes such as SOD, CAT, GPx, MPx, LPO and GSH play an important role in the elimination of free oxygen radicals and lipid hydroperoxides in gastric mucosal cells.[15,21] With the application of IND, a decrease in antioxidants in the rat gastric mucosa, deterioration in cell permeability and accordingly the oxidation of phospholipids in the cell membrane and their conversion to peroxide derivatives initiate lipid peroxidation. There is an increase in the level of malondialdehyde, the parent compound, indicating the onset of lipid peroxidation. This compound is a very harmful substance and affects the permeability negatively by causing ion exchange in the membrane.[22] LPO, which is found at a very high rate in the tissues together with the IND applied in the current study, is an indicator of these reasons. However, the extracts and LAN in the treatment groups decreased this increase positively and activated the antioxidant defense system. Also, in another study, Abdallah et al.[22], it has been observed that IND-induced gastric ulceration is accompanied by a severe oxidative stress in gastric tissue that damages essential biomolecules such as lipids. Again, in some studies, it has been shown that LPO causes an increase in reactive oxygen species.[23]

Another antioxidant molecule that tries to eliminate the oxidative damage caused by NSAIDs in tissues is GSH. It plays a role in neutralizing hydrogen peroxide, one of the reactive oxygen species, and stimulating prostaglandin synthesis. It shows the protective feature by transferring electrons to free radicals. As in the studies of Halici and Kaplan, the protective effects of fruit extracts were determined in this study.[24,25] Another enzyme that neutralizes hydrogen peroxide, one of the reactive oxygen species in the environment, is the SOD enzyme. It reduces superoxide, which is a highly reactive oxygen radical, to hydrogen peroxide. The enzyme catalase reagents, which convert this H_2O_2 into molecular water and oxygen, can destroy it. Therefore, since a high rate of reactive oxygen is produced in IND applied tissues, there will be no transformation in the environment and the antioxidant level will remain low. Likewise, the CAT activity that will convert the H_2O_2 in the environment to water will also be very low. The data obtained in the study also show this. The treatment groups, on the other hand, increased both SOD and CAT activities in parallel with the applied positive group, thereby removing superoxide radicals and hydrogen peroxide from the environment.[23,26-28] The enhancing antioxidant status could be one of the mechanisms behind *P. laurocerasus* gastroprotective effects.

Conclusion

In recent studies, gastric damage has occurred in IND-induced ulcer models, and antioxidant enzymes have been activated with some treatment groups. Some recent research is mostly on new natural therapeutic agents. It is to be used as antioxidant support due to positive reasons such as being easily available, inexpensive, easy healing and strong effect. We can say that *Prunus laurocerasus* fruit extracts used in the current research can be used easily by determining their protective effects. In addition, its protectiveness can be proven in previous studies. Its protection can be proven and its use can be expanded both in diseases such as diabetes, diuretic, bronchitis, eczema, and in digestive system disorders.

Declarations

Funding

Authors have no commercial interest and financial interest. The costs of the research were covered by the researchers.

Author contributions

Conceptualization, O.A.B.; Methodology, A.K and G.P; Software, A.K and G.P; Validation, O.A.B; Formal Analysis, O.A.B A.K and G.P; Investigation, A.K and G.P; Resources, A.K and G.P; Data Curation, A.K and G.P;

Writing – Original Draft Preparation, O.A.B; Writing – Review & Editing, O.A.B. and G.P; Visualization, O.A.B; Supervision, A.K; Project Administration, O.A.B; Funding Acquisition, G.P. and O.A.B.

Conflicts of interest

All authors declare that there are no conflicts of interest.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The ethical approval was obtained from Giresun University Animal Experiments Local Ethics Committee for the applications (2019/13).

References

1. Malfertheiner PF, Chan KL, McColl KEL. Peptic ulcer disease. *Lancet.* 2009;374:1449–1461.
2. Tarnawski AS, Ahluwalia A. Molecular mechanisms of epithelial regeneration and neovascularization during healing of gastric and esophageal ulcers. *Curr Med Chem*. 2012;19:16–27.
3. Calam J, Baron JH. Pathophysiology of duodenal and gastric ulcer and gastric cancer. *Br Med J*. 2001;323:980.
4. Hudson HA, Cole AT, Jones PD, et al. Mechanism of gastric and duodenal damage and protection. *Hepatogastroenterol,* 1992,39:31–36.
5. Gupta D, Bleakley BR, Gupta K. Dragon's blood: botany, chemistry and therapeutic uses. *J Ethnopharmacol*. 2008;115:361–380.
6. Issa AY, Volate SR, Wargovich MJ. The role of phytochemicals in inhibition of cancer and inflammation: new directions and perspectives. *J Food Compos Anal*. 2006;19:405–419.
7. Elmastas M, Genc N, Demirtas I, et al. Isolation and identification of functional components in seed of cherry laurel (*Laurocerasus officinalis* Roem.) and investigation of their antioxidant capacity. *TBAP.* 2013;3:115-120.
8. Alasalvar C, Al-Farsi M, Shahidi F. Compositional characteristics and antioxidant components of cherry laurel varieties and pekmez. *J Food Sci.* 2005;70:47–52.
9. Uslu H, Atila Uslu G, Özen H, et al. Effects of different doses of Prunus laurocerasus L. leaf extract on oxidative stress, hyperglycaemia and hyperlipidaemia induced by type I diabetes. *IJTK*. 2018;17:430-436.
10. Peker EGG. Antioxidant Phenolic Compounds From Molasses of Laurocerasus Officinalis Roem (Cherry Laurel) Ameliorate Carbon Tetrachloride CCl_4 Induced Liver Injury in Rats. *Fresenius Environmental Bulletin*. 2021;30(6 B):7780–7788.
11. Aebi H. Catalase Methods. *Enzymol.* 1984;105:121–126.
12. Sun Y, Larry WO, Ying L. A simple method for clinical assay of superoxide dismutase. *Clin Chem.* 1988;34:497–500.
13. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryls groups in tissue with Ellman's reagent. *Anal Biochem.* 1968;25:192–205.
14. Ohkawa H, Ohishi H, Yagi K. Assay for lipid peroxide in animal tissues by thiobarbutiric acid reaction, *Anal Biochem.* 1979;95:351–358.
15. Caldas GFR, do Amaral Costa IM, da Silva JBR, et al. Antiulcerogenic activity of the essential oil of Hyptis martiusii Benth. (Lamiaceae). *J Ethnopharmacol.* 2011;137:886–892.
16. Griffin MR, Scheiman JM. Prospects for changing the burden of nonsteroidal anti-inflammatory drug toxicity. *Am J Med*. 2001;110:33–37.
17. Robert A, Nezamis JE, Lancaster C, et al. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. *Gastroenterology*. 1979;77:433–443.
18. Mizui T, Doteuchi M. Effect of polyamines on acidified ethanol-induced gastric lesions in rats. *Jpn J Pharmacol.* 1983;33:939–945.
19. Djahanguiri B. The production of acute gastric ulceration by indomethacin in the rat. *Scand J Gastroenterol.* 1968;4:265–267.
20. Guslandi M. Effects of ethanol on the gastric mucosa. *Dig Dis*. 1987;5:21–32.
21. Zinkievich JM, George S, Jha S et al. Gastric acid is the key modulator in the pathogenesis of non-steroidal anti-inflammatory drug-induced ulceration in rats. *Clin Exp Pharmacol Physiol*. 2010;37:654–661.
22. Abdallah IZA, Khattab HAH, Heeba GH. Gastroprotective effect of Cordia myxa L. fruit extract against indomethacin-induced gastric ulceration in rats. *Life Sci J*. 2011;8(3):433–445.
23. Amr MA, Hussein FS. Effect of selenium and grape seed extract on indomethacin-induced gastric ulcers in rats. *J Physiol Biochem*. 2013;69:527–537.
24. Kaplan KA, Odabasoglu F, Halici Z, et al. Alpha-Lipoic Acid Protects against Indomethacin-Induced Gastric Oxidative Toxicity by Modulating Antioxidant System. *J Food Sci.* 2012;77(11):H224-H30.
25. Halici Z, Karaca M, Keles ON, et al. Protective effects of amlodipine on ischemia-reperfusion injury of rat ovary: biochemical and histopathologic evaluation. *Fertil Steril.* 2008;90(6):2408–2415.
26. Caldas GFR, Limeira MMF, Araújo AV, et al. Repeated-doses and reproductive toxicity studies of the monoterpene 1, 8-cineole (eucalyptol) in Wistar rats. *Food Chem Toxicol*. 2016;97:297–306.
27. Saadaoui N, Weslati A, Barkaoui T, et al. Gastroprotective effect of leaf extract of two varieties grapevine (Vitis vinifera L.) native wild and cultivar grown in North of Tunisia against the oxidative stress induced by ethanol in rats. *Biomarkers*. 2020;25(1):48–61.
28. Erol HS, C Ahmet, Koc M, Yildirim S, Halici M. Anti-ulcerogenic effect of osajin on indomethacin-induced gastric damage in rats. *Acta Vet Brno*. 2020;89:391–400.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.6

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 290–297

ORIGINAL PAPER

Predictors of blood pressure levels, knowledge and practices of adult hypertensives attending a Secondary Health Care Centre in South-Western Nigeria

Ismaheel Aderogba Azeez [1], Magbagbeola David Dairo [2], Joshua Odunayo Akinyemi [3], Olufemi Philippe Idowu [4]

[1] Consultant Family Physician, University College Hospital, Ibadan, Nigeria
[2] Consultant Community Physician, Department of Epidemiology and Medical Statistics, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Nigeria
[3] Department of Epidemiology and Medical Statistics, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Nigeria
[4] Department of Epidemiology and Medical Statistics, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Nigeria

ABSTRACT

Introduction and aim. The prevalence of hypertension is increasing with accompanying poor control. The aim of this study was to assess the predictors of knowledge and practices of adult hypertensives.

Material and method. The study was a prospective (before and after) study with health education as the intervention. A simple random sampling technique with computer-generated random numbers was used to recruit 386 patients. Data were analysed using SPSS version 23. Logistic regressions were used to determine the predictors of knowledge and practices.

Results. Those who had tertiary education were about four times more likely to have good knowledge of hypertension than those who had secondary education (OR=0.256; 95% CI=0.106–0.617). The females were about 1.73 times more likely to have good practices of hypertension than males (OR=1.729; 95% CI=1.008–2.966). For every 1 unit increase in the body mass index, there was a statistically significant increase in diastolic blood pressure by about 0.22 units (95% CI=0.046–0.394, p=0.013). The health education had a positive impact on the blood pressure reduction.

Conclusion. The predictors of knowledge of hypertension, practices of hypertensives and diastolic blood pressure were level of education, sex, and body mass index respectively.

Keywords. blood pressure, hypertensives, knowledge, practices, predictors

Introduction

Hypertension is defined as persistent elevated systolic blood pressure of 140 mmHg or above and/or diastolic blood pressure of 90 mmHg or above.[1,2] There are two main categories of hypertension. Primary hypertension which affects about 90–95% percent of the people suffering from hypertension.[3] The risk of developing primary hypertension is higher in those people with family history of hypertension according to a study conducted by Iloh et al. in Eastern Nigeria.[4] Hypertension occurring as a result of another illness or a side effect of medication is secondary hypertension.

Corresponding author: Ismaheel Aderogba Azeez, e-mail: kunleayilola14@gmail.com

Received: 29.01.2022 / Revised: 9.05.2022 / Accepted: 13.05.2022 / Published: 30.09.2022

Azeez IA, Dairo MD, Akinyemi JO, Idowu OP. *Predictors of blood pressure levels, knowledge and practices of adult hypertensives attending a Secondary Health Care Centre in South-Western Nigeria.* Eur J Clin Exp Med. 2022;20(3):290–297. doi: 10.15584/ejcem.2022.3.6.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

In Nigeria, it is the main risk factor for stroke, heart failure, ischemic heart disease and kidney failure.[5] Higher prevalence of hypertension and its complications have been found in people of African descent.[6] Essential hypertension is a public health problem due to its asymptomatic nature, its increasing prevalence, its chronicity, associated renal, cardiovascular and neuro-vascular complications.[7,8] The prevalence of hypertension was reported to be 44% in Western Europe and 28% in North America.[9] However, Azubuike and Kurmi reported 24.2% in their study conducted in Sanga, Kaduna, Northern Nigeria.[10] Despite the development of new anti-hypertensives, a lot of patients are still having poor blood pressure control. It was reported that in the United States of America, 29% had their blood pressure uncontrolled.[11] Hypertension has been reported to affect about one billion people worldwide.[12]

Diabetes mellitus and hyperlipidaemia were the most common comorbid conditions of hypertension.[13] Controlled blood pressure is a blood pressure of less than 140/90 mmHg in hypertensives and less than 130/80 in hypertensives with diabetes mellitus and patients with chronic renal failure. The prevalence of hypertension was higher in urban areas than rural areas and this was attributable to obesity.[14] There has been increased burden on the healthcare system, loss to productivity and economic loss. In a review of literature, knowledge, awareness, treatment and control of hypertension were generally low with associated high load complications.[9] Majority of the patients were reported to have poor knowledge of hypertension and its management in another study. This would have negative impact on the treatment and control of hypertension.[15] The results of a study carried out in Owerri, Nigeria, revealed that adults had high level of knowledge of the theory and risk factors of hypertension. However, the level of education was a very important factor that determined the knowledge of hypertension.[16] According to the report of a study conducted in Ghana, hypertensive patients were said to have average knowledge of hypertension and life style modifications[17] Ebid et al. reported that hypertensive patients who were educated about the nature of hypertension, its complications and adherence to medications showed better control of blood pressure. And also, those informed about lifestyle modifications that included diet and physical activities had better control of hypertension.[18]

Aim

The aim of this study was to assess the knowledge, attitude, practices of hypertensives and also to assess factors associated with blood pressure levels among adult hypertensives presenting to a secondary health care centre in Nigeria. This was the first study in this particular area in Nigeria.

Material and methods

The study was conducted at the State Hospital, Oyo, Nigeria, to assess the knowledge, attitude and practices of hypertensive patients. Oyo is a town located in Oyo central senatorial zone of Oyo State in the South-Western zone of Nigeria. The study was carried out at the Medical outpatient clinic of the Hospital. It has about 170 beds capacities with various speciality units, paramedical and outpatient services. Patients are referred to the Hospital from other Hospitals around Oyo town.

The study was a prospective (before and after) study of patients with uncontrolled hypertension. Respondents were recruited from April 2015 to July 2015. A simple random sampling technique with computer-generated random numbers was used to recruit the patients. The study population was composed of adults 18 years to 70 years with an established diagnosis of hypertension and already on treatment and follow up for a year. Ethical approval was granted by the Ethical Committee of the Oyo State Ministry of Health, Ibadan, Nigeria. Written informed consents were obtained from eligible patients before administration of the questionnaires and examinations. Privacy and confidentiality of the respondents were guaranteed by anonymity of respondents. The Committee's reference number is AD 13/479/.

Definition of hypertensive patients

Hypertensive patients were those with systolic blood pressure ≥ 140 mmHg and diastolic ≥ 90 mmHg diagnosed a year previously or patients on drugs for hypertension for at least a year.

Inclusion criteria included patients who were 18–70 years with uncontrolled hypertension. Exclusion criteria included patients with severe hypertension, systolic >180 mmHg, diastolic > 110 mmHg, who would need immediate adjustment of treatment; hypertensives with renal insufficiency, hyperkalemia, pregnant women, lactating women and patients with diabetes mellitus. This group of patients were excluded so that they would not pass through the rigours of the study and they were not part of the study targets.

Sample size estimation

The Sample size was estimated using the formula:[19]

$$n = (Z^2pq)/d^2$$

Quoting n = minimum sample size

Z_α=the standard normal deviate, usually set at 1.96, which corresponds to the 95% confidence level. The prevalence of controlled hypertension was 34.5% for Nigeria. [13]

From the same study, the prevalence of uncontrolled hypertension was 65.5%.
P = 0.655
q = 1.0 – p= 0.345
d = degree of accuracy desired usually set at 0.05.
$n = (1.96)^2(0.655)(1 – 0.655) / (0.05)^2 =$
$= (1.96)^2(0.655)(0.345) / (0.05)^2 =$
$= (3.84)(0.226) / (0.05)^2 = 347$
q = 1/1-f
q is the adjustment factor
f = non response rate, if f =10%
q = 1/0.9 =1.11
n = 1.11 x 347 = 385.5 = 386
For the purpose of this study, a minimum 386 patients were recruited.

The intervention
During recruitment of the patients at first visit, they were counselled about the nature of hypertension, associated morbidities, drugs and compliance with management. They were told about lifestyle modifications that included diet and physical exercises. Attaining blood pressure targets of less than 140/90 mmHg was stressed to the respondents.[20] The counselling was repeated before the assessment of blood pressure during the periods of follow-up. The blood pressures were taken at four-week intervals for two months.

Measurement of blood pressure
A standard mercury sphygmomanometer (Accosson, London) was used, and systolic blood pressure and diastolic blood pressure were taken as Korotkoff sound phases I and V respectively. The measurements were taken with the patient in a seated position with their arms supported at heart level, after five minutes of rest, after abstinence from food, nutritional supplements, caffeinated beverages and smoking for a minimum of two hours before the appointment at approximately the same time and day of the week. The cuff was applied to the exposed upper arms and was rapidly inflated to 30 mmHg above the level at which the pulse disappeared and then deflated gradually. The mean of two measurements was calculated for systolic blood pressure and diastolic blood pressure separately.

Data collection and analysis
A structured interviewer administered questionnaire was administered to consenting patients. This is a questionnaire that assesses Knowledge, Attitudes and Practices of hypertensives. It is a validated instrument used in a previous study in Nigeria.[21] The Cronbach alpha coefficient is 0.82.[22]

Knowledge score
The knowledge of hypertension was assessed on a scale of 0-29. The responses were scored 0-2 irrespective of the responses been true or false. Also, for the last two questions, correct answers were scored one irrespective of the response been yes or no, while incorrect answers were scored zero. Analysis showed the mean score to be 19. Scores were totalled and categorised into two groups. Patients with knowledge scores less than 19 were classified as having poor knowledge while those with knowledge scores of 19 and above were classified as having good knowledge.

Attitude score
Attitudes were assessed with total scores ranging from 0-18. The responses to the statements were scored 0-2 depending on the answers. Statistical analysis showed the mean score to be 16. Scores were totalled and categorised into two groups. Respondents with attitude scores less than 16 were classified as having poor attitude while those with attitude score of 16 and above were classified as having good attitude.

Practice score
The practices were assessed with total scores ranging from eight to 35. Statistical analysis showed the mean score to be 23. Scores were totalled and categorised into two groups. Respondents with scores of less than 23 were classified as having poor practices while those with scores of 23 and above were classified as having good practices.

A pre-test of the questionnaire was done on 40 patients to identify potential problems, and amendments were done where necessarily.

Statistical analysis
The dependent variables were knowledge, attitudes, practices and blood pressure. The independent variables included socio-demographic factors and socio-economic factors. Data were analysed using SPSS software version 23 from IBM Corporation, New York, United States. Frequency tables and diagrams in form of charts were used for relevant variables. Chi-square test was used to analyse the association between categorical variables and logistic regressions were used to determine the predictors of knowledge and practices of hypertensives. A p-value of ≤ 0.05 was considered to indicate statistical significance.

Results
Table 1 shows the distribution of knowledge, attitude and practice scores of the respondents. More than half, 205 (53.1%) of the respondents had poor knowledge scores. Majority of the respondents 274 (71%) had good attitude towards management of hypertension while majority of the respondents 236 (61.1%) also had good practice scores.

Table 1. Distribution of respondents' knowledge, attitude and practice scores (n=386)

	Frequency (n)	Percentage (%)
Knowledge scores		
Poor knowledge	205	53.1
Good knowledge	181	46.9
Attitude scores		
Poor attitude	112	29
Good attitude	274	71
Practice scores		
Poor practice	150	38.9
Good practice	236	61.1

Effect of health education on blood pressure over time

Figure 1 shows the distribution of mean values of three clinic blood pressure measurements at intervals of four weeks. A reduction in blood pressure was observed over time.

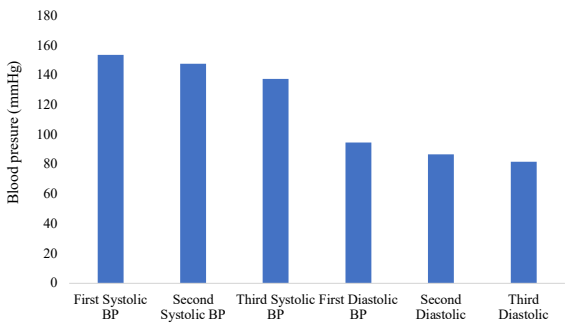

Fig. 1. Distribution of mean values of Blood pressure over 2 months at intervals of 4 weeks

Table 2. Association of knowledge of hypertensives with selected variables*

Association of knowledge of hypertension with selected variables				
Variable	**Poor (n)**	**Good (n)**	**χ^2**	**p**
Education				
No formal education	114 (61%)	73 (39%)	36.55	0.0001*
Primary	55 (63.2%)	32 (36.8%)		
Secondary	27 (45%)	33 (54.2%)		
Tertiary	9 (17.3%)	43 (82.7%)		
Age				
< 45 years	19 (50%)	19 (50%)	2.267	0.324
45-54 years	64 (48.5%)	68 (51.5%)		
55 years above	122 (56.5%)	94 (43.5%)		
Sex				
Male	28 (43.8%)	36 (56.2%)	1.257	0.553
Female	177 (55%)	145 (45%)		

*Significant at 5% level of significance

Table 2 shows association of levels of education with knowledge of hypertensives. A higher proportion of patients with no formal education (61.0%) and a higher proportion of patients with primary education (63.2%) had poor knowledge of hypertension. However, a little bit above average of those with secondary school education (54.2%) and higher proportion of respondents with tertiary education (82.7%) had good knowledge of hypertension. The association was statistically significant (χ^2=36.254, p=0.0001).

Logistic regression analysis of good knowledge of hypertension on selected variables

Table 3 shows the Logistic regression analysis of good knowledge of hypertension on selected variables. After adjusting for other variables, the predictors of good knowledge of hypertension was the level of education. Those who had tertiary education were about four times more likely to have good knowledge of hypertension compared with those who had secondary education (OR=0.256; 95% CI=0.106–0.617).

Table 3. Logistic regression analysis of good knowledge of hypertension on selected variables*

Variable	Odd ratio	95% CI	p
Educational level			
No formal education	0.134	0.062–0.291	0.0001*
Primary	0.122	0.053–0.282	0.0001*
Secondary	0.256	0.106–0.617	0.002*
Tertiary	1		

*Significant at 5% level of significance, predictors: level of education, dependent variable: knowledge of hypertension

Table 4. Association of attitudes of hypertensives with selected variables

Association of attitudes of patients with hypertension with selected variables				
Variable	**Poor (n)**	**Good (n)**	**χ^2**	**p-value**
Education				
No formal education	61 (32.6%)	126 (67.4%)	2.512	0.476
Primary	23 (26.4%)	64 (73.6%)		
Secondary	16 (26.7%)	44 (73.3%)		
Tertiary	12 (23.1%)	40 (76.9%)		
Age				
< 45 years	13 (34.2%)	25 (65.8%)	1.257	0.553
45-54 years	41 (31.1%)	91 (68.9%)		
55 years above	58 (26.9)	158 (73.1%)		
Sex				
Male	15 (23.4%)	49 (76.6%)	1.159	0.297
Female	97 (30.1%)	225 (69.9%)		

Association of attitudes of hypertensives with selected variables

Table 4 shows association of attitudes of patients with hypertension with selected variables. A higher proportion of patients who were less than 45 years (65.8%)

had good attitude toward hypertension. Also, a higher proportion of those with secondary school education (73.3%) and higher proportion of respondents with tertiary education (76.9%) had good attitudes toward hypertension. The association was not statistically significant (χ^2=2.512, p=0.476).

Association of practices of hypertensives with selected variables

Table 5 shows association of practices of patients with hypertension with selected variables. A higher proportion of patients who were less than 45 years (73.7%) had good practices toward hypertension. Also, a higher proportion of those who were females (63.4%) had good practices toward hypertension compared with a lower proportion who were males (50%). The association was statistically significant (χ^2=4.007, p=0.045).

Table 5. Association of practices of patients with hypertension with selected variables

Association of practices of patients with hypertension with selected variables				
Variable	**Poor (n)**	**Good (n)**	**χ^2**	**p-value**
Education				
No formal education	74 (60.4%)	113 (39.6%)	4.659	0.201
Primary	26 (29.9%)	61 (70.1%)		
Secondary	26 (43.3%)	34 (56.7%)		
Tertiary	24 (46.2%)	28 (53.8%)		
Age				
< 45 years	10 (26.3%)	28 (73.7%)	2.792	0.257
45-54 years	53 (40.2%)	79 (59.8%)		
55 years above	87 (40.3%)	129 (59.7%)		
Sex				
Male	32 (50%)	32 (50%)	4.007	0.045*
Female	118 (36.6%)	204 (63.4%)		

Logistic regression analysis of good practices of hypertensives on selected variables

Table 6 shows the logistic regression analysis of good practices of patients with hypertension on selected variables. After adjusting for other variables, the predictor of good practices of hypertensives was sex. The females were about 1.7 times more likely to have good practices of hypertension compared with males (OR=1.73; 95% CI=1.008–2.966).

Table 6. Logistic regression analysis of good practices of patients with hypertension on selected variables*

Variable	Odd Ratio	95% CI	p-value
Female	1.729	1.008–2.966	0.047*
Male	1		

*Significant at 5% level of significance, predictor: sex, dependent variable: practices of hypertensives

Relationship of blood pressure levels, body mass index and age

The association of body mass index with first diastolic blood pressure was positive, weak in strength and statistically significant (Tab. 7, p=0.013).

Table 7. Relationship of blood pressure levels, body mass index and age*

Relationship between blood pressure levels and body mass index		
	First diastolic blood pressure	**First Systolic blood pressure**
Body mass index		
Pearson correlation	0.126	0.071
p-value	0.013*	0.163
Age		
Pearson correlation	0.106	0.064
p-value	0.037*	0.213

*Significant at 5% level of significance

Linear regression for the first diastolic blood pressure on significant variables

As shown in table 8, for every 1 unit increase in body mass index, there was a statistically significant increase in Diastolic blood pressure by about 0.249 units (95% CI=0.072–425, p=0.006).

Table 8. Linear regression for the first diastolic blood pressure on significant variables*

ANOVA TABLE					
Model	Sum of squares	Degree of freedom	Mean square	F	Significant
1 Regression	1214.066	2	607.33	6.065	0.003*
Residual	38336.65	383	100.096		
Total	39550.72	385			

Linear regression for the first diastolic blood pressure on significant variables					
Variable	Regression coefficient (B)	Standard Error for B	95% CI for B	p-value	T
Age	0.120	0.062	0.091– 0.82	0.054	0.93
BMI	0.249	0.090	0.072– 0.425	0.006*	2.77

*Significant at 5% level of significance, predictor: body mass index, dependent variable: diastolic blood pressure

Discussion

The prevalence of hypertension is increasing especially in developing countries with accompanying poor control and increasing burden on the healthcare system.[23] Visco and colleagues reported that older age, family history of hypertension, female sex and high blood pressure levels were predictors of difficult to control hypertension.[24] Another study showed that blacks had poorer control of blood pressure than whites and Hispanics.[25] The results of this study showed that majority of the respondents had poor knowledge of hypertension and its management. Health education given to this co-

hort of hypertensives was in the right direction to improve their knowledge of hypertension. This was similar to what, was described by Busari et al. and Iyalomhe in their studies where they reported that the majority of hypertensive patients had poor knowledge of their disease with an important negative impact on adherence to medications.[15,21] Shaikh et al. and Oladapo et al. also reported that a significant number of hypertensive patients had poor knowledge of hypertension.[26,27] Besides, Chiazor and Oparah reported that most patients were using thiazide diuretic for the treatment of hypertension. Patients' knowledge of hypertension was below average hence they should be educated on hypertension and its current management using drugs and lifestyle changes.[28] However, in a study conducted in Ghana, Marfo et al. reported that patients with hypertension had average knowledge of hypertension and life style modifications.[17] In a work done in Lagos, it was found that knowledge of antihypertensive therapy was good, however, adherence to therapy was poor and forgetfulness was the major reason for poor adherence. Good knowledge of hypertension and its management does not necessarily translate to good adherence to anti-hypertensive therapy. Therefore, hypertension can be best controlled when patients are adherent to their treatment regimen, involve themselves in physical activity regularly and adherent to other lifestyle modifications.[29]

The association between the level of education and knowledge of hypertension was investigated in this study. The results showed that the higher the level of education the higher the knowledge of hypertension. The predictor of good knowledge of hypertension was the level of education. It shows that those who had higher levels of Western education were more likely to have better knowledge of hypertension. This was corroborated by a study conducted in Owerri, Nigeria, by Kate et al. which revealed that higher level of education was associated with good knowledge of hypertension. Adults with tertiary education had higher important difference in the level of knowledge of concept and the risk factors of hypertension compared with adults in other levels of education.[16] Another study conducted in Ibadan revealed poor overall knowledge of cardiovascular diseases and its risk factors but respondents who had more years of formal education had good knowledge of cardiovascular diseases and their risk factors.[27]

The attitudes of patients with hypertension were assessed in this study and it was found that majority of the patients had good attitudes towards hypertension and its management. This should assist patients in the control of their blood pressure. The practices of patients with hypertension were also assessed in this study, and it was shown that majority of the respondents had good practices towards hypertension and its management. However, some aspects of the practices were not satisfactory. Most of the respondents consult their doctors at their convenience not according to appointments and most of them check their blood pressures every three months. Besides, majority of the respondents did not do adequate physical exercises and were not doing enough to reduce their salt intake. All these would lead to poor control of hypertension. The predictor of good practices of the hypertensives was female sex. The females were more likely to have good practices than males. In a study conducted in Baghdad, Iraq, health education on physical exercise, diet, other lifestyle modifications and adherence to pharmacotherapy had impact on blood pressure control.[30] This was similar to the findings of this study which showed that health education had impact on blood pressure reduction over time. The results of a study conducted in Shagamu, Nigeria showed that the determinants of knowledge of hypertension included family history, co-morbidities and educational level. Also, majority of the respondents had good knowledge of hypertension.[31] This was similar to the findings of this study which showed that educational level was a predictor of knowledge of hypertension. Age, obesity, self-perception of good health and low level of education were reported as predictors of isolated systolic hypertension, isolated diastolic hypertension and hypertension in a study conducted among apparently healthy individuals in South-Eastern Nigeria.[32] This was similar to the findings of this study which showed that Body mass index was a predictor of diastolic blood pressure.

Implication of the study to research and clinical practice

Health care workers would have to update themselves on hypertension and health educate the patients especially those with lower levels of education and men on hypertension and its management. Also, patients have to be counselled on lifestyle modifications as a form of therapy for secondary prevention in the management of hypertension.

Limitations of the study and future research

This study was an intervention (before and after) study, there was no comparison group and no randomisation into groups, therefore, large scale randomised controlled trials would be necessary. The study was hospital based making secondary generalisation difficult, although the hospital serves a very big population. Also, the study was an experimental study with health education as the intervention, so causal relationships could be established to some extent.

Conclusion

The predictor of good knowledge of hypertension was the level of education, and the predictor of good practices was female sex. In addition, the predictor of diastolic blood pressure levels was the body mass index.

The health education had a positive impact on the blood pressure reduction over time. Health care workers would have to educate patients on the management of hypertension including lifestyle modifications. This study was able to determine the predictors of knowledge and practices of hypertensives which were rare from the previous studies.

Declarations

Funding
The project was self-funded.

Author contributions
Conceptualization, I.A.A., M.D.D. and J.O.A.; Methodology, I.A.A., M.D.D., J.O.A. and O.P.I.; Formal Analysis, I.A.A., M.D.D., J.O.A. and O.P.I.; Data Curation, I.A.A., M.D.D., J.O.A. and O.P.I.; Writing – Original Draft Preparation, I.A.A., M.D.D., J.O.A. and O.P.I.; Writing – Review & Editing, I.A.A., M.D.D., J.O.A. and O.P.I.; Project Administration, I.A.A., M.D.D. and J.O.A.

Conflict of interests
The authors declare no competing interests.

Data availability
The datasets generated during and/or analysed during the current study are not publicly available due to the caveat for ethical approval that the responses of the respondents would be kept confidential. However, they would be available from the corresponding author on reasonable request.

Ethics approval
Ethical approval was granted by the Ethical Committee of the Oyo State Ministry of Health, Ibadan, Nigeria. Written Informed consents were obtained from eligible patients before administration of the questionnaires and examinations. Privacy and confidentiality of the respondents were guaranteed by anonymity of respondents. The Committee's reference number is AD 13/479/.

References

1. Ramli A, Ahmad NS, Paraidathathu T. Medication adherence among hypertensive patients of primary health clinics in Malaysia. *Patient Prefer and Adher*. 2015;6:613–622.
2. Unger T, Borghi C, Charchar F, et al. ISH, Global Hypertension Practice Guidelines. *Hypertension*. 2020;75:1334–1357.
3. Ibekwe RU. Modifiable risk factors of hypertension and socio-demographic profile in Oghara, Delta State; prevalence and correlates. *Ann Med Health Sci Res*. 2015;5:71–77.
4. Iloh G, Amadi AN, Njoku PU, Ofoedu JN, Awa-Madu J. The magnitude of abdominal adiposity and atherogenic dyslipidemia among geriatric Nigerians with arterial hypertension in a rural hospital in South-eastern Nigeria. *Niger J Clin Pract*. 2012;15:462–468.
5. Odusola AO, Hendriks M, Schultsz C, et al. Perceptions of inhibitors and facilitators for adhering to hypertension treatment among insured patients in rural Nigeria: a qualitative study. *BMC Health Services Research*. 2014;14:624.
6. Ugwuja EI, Ezenkwa US, Nwibo AN, Ogbanshi M, Idoko O, Nnabu R. Prevalence and determinants of hypertension in an agrarian rural community in Southeast Nigeria. *Ann Med Health Sci Res.* 2015;5:45–49.
7. Potchoo Y, Goe-Akue E, Damorou F, Massoka B, Redah D, Guissou IP. Effect of Antihypertensive Drug Therapy on the Blood Pressure Control among Hypertensive Patients Attending Campus' Teaching Hospital of Lome, Togo, West Africa. *Pharm Pharmacol.*. 2012;3:214–223.
8. Ogah OS, Rayner BL. Recent advances in hypertension in sub-Saharan Africa. *Heart*. 2013;99(19):1390–1397.
9. Ogah OS, Okpechi I, Chukwuonye I, et al. Blood pressure, prevalence of hypertension and hypertension related complications in Nigerian Africans: A review. *World J Cardiol*. 2012;4(12):327–340.
10. Azubuike SO, Kurmi R. Awareness, practices, and prevalence of hypertension among rural Nigerian women. *Arch Med Health Sci.* 2014;2(1):23–28.
11. Maruf FA, Akinpelu AO, Salako BL. Effects of aerobic exercise and drug therapy on blood pressure and antihypertensive drugs: a randomized controlled trial. *Afr Health Sci*. 2013;13(1): 1–9.
12. Adeoye AM, Adebiyi A, Bamidele O T, Salako BL, Ogunniyi A, Cooper RS. Hypertension Subtypes among Hypertensive Patients in Ibadan. *Int J Hypertens.* 2014;10:1–6.
13. Tamuno I, Fadare J. Drug treatment for hypertension in a tertiary health care facility in northern Nigerian. *Int J Pharm Biomed Res.* 2011;2(2):104-109.
14. Adediran OS, Okpara IC, Adeniyi OS, Jimoh AK. Hypertension prevalence in an Urban and Rural area of Nigeria. *J Med Med Sci*. 2013;4(4):149–154.
15. Busari OA, Olanrewaju TO, Desalu OO, et al. Impact of Patients' Knowledge, Attitude and Practices on Hypertension on Compliance with Antihypertensive Drugs in a Resource-poor Setting. *TAF Prev Med Bull*. 2010;9(2):87–92.
16. Kate OO, Okwuchi EV, Samuel ES. Knowledge of Hypertension Among Adults in Owerri Senatorial Zone of Imo State, Nigeria. *Mediter J Soc Scie*. 2013;4(5):1–12.
17. Marfo AFA, Owusu-daaku FT, Addo MO, et al. Ghanaian Hypertensive patients understanding of their medicines and life style modification for managing Hypertension. *Int J Pharmacy Pharmaceutical Sci*. 2014;6(4):1–6.
18. Ebid AIM, Ali Z T, Ghobary MAF. Blood pressure control in hypertensive patients: impact of an Egyptian pharmaceutical care model. *J App Pharm Sci.* 2014;4(09):093–101.
19. Goyal RC. *Research Methodology for Health Professionals.* Daryaganj, New Delhi, India: Jaypee Brothers Medical Publishers(P) LTD; 2013.
20. Hypertension in adults: diagnosis and management. NICE guideline [NG136] Published:28th, August,2019.https://

www.nice.org.uk/guidance/ng136/chapter/recommendations. Accessed March 15, 2022.

21. Iyalomhe GBS, Iyalomhe SI**.** Hypertension-related knowledge, attitudes and life-style practices among hypertensive patients in a sub-urban Nigerian community. *J Public Health Epid.* 2014;2(4):71–77.
22. Erkoc S B , Isikli B, Metintas S, Kalyoncu C. Hypertension Knowledge-Level Scale (HK-LS): A Study on Development, Validity and Reliability. *Int J Environ Res Public Health.* 2012;9:1018–1029.
23. Safah B, Onyame A, Ankrah C, et al. Knowledge, attitude and lifestyle practices pertaining to hypertension among the people of Ahoe-Ho. *J Hypertens Manag*. 2021;7(1):061.
24. Visco V, Finelli R, Pascale AV, et al. Difficult-to-control hypertension: identification of clinical predictors and use of ICT-based integrated care to facilitate blood pressure control. *J Hum Hypertens*. 2018;32(7):467–476.
25. Shelley D, Tseng T, Andrews H. Predictors of blood pressure control among hypertensives in Community health centres. *Am J Hypertens*. 2011:24(12):1318–1323.
26. Shaikh MA, Yakta D, Sadia, Kumar R. Hypertension Knowledge, Attitude and Practice in Adult Hypertensive Patients. *JLUMHS*. 2012;11(02):113–116.
27. Oladapo O O, Salako BL, Sadiq L, Soyinka K, Falase AO. Knowledge of Hypertension and other Risk Factors for Heart Disease among Yoruba Rural Southwestern Nigerian Population. *JAMMR*. 2013;3(4):993–1003.
28. Chiazor IE, Oparah AC**.** Assessment of Hypertension Care in a Nigerian Hospital. *Tropical J Pharm Research*. 2012;11(1):137–145.
29. Campbell PC, Oladeyi OO. Compliance to and Knowledge of Anti-Hypertensive Therapy amongst Hypertensive Patients Attending Lagos University Teaching Hospital (Luth), Idi-Araba, Lagos, Nigeria. *IOSR-JDMS*. 2014;13(5):108–115.
30. Al-Shatari SAE, Al-Joboori YBH, Salih K, et al. Effect of health education on blood pressure control and life modification in hypertensive patients: sample from primary health care centers, Al-Resafah sector/Baghdad. *Iraq J Com Med*. 2021;32(2):24–51.
31. Odelola OI, Akinpelu A, Idowu AO. Hypertension: the predictor of knowledge among market women in the Sub--Urban town of Shagamu. South West Nigeria. *Afr J Health Sci*. 2021;34(4): 526–536.
32. Ayogu RNB, Ezeh MG, Okafor AM. Prevalence and predictors of different patterns of hypertension among adults aged 20 to 60 years in rural communities of Southeast Nigeria: a cross-sectional study. *Arch Pub Health*. 2021;79:210.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.7

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 298–305

ORIGINAL PAPER

Mentat® ameliorates hypoxia-induced attention deficit hyperactivity disorder like behavior in rats

Sai Sindhura [1], Gollapalle Lakshminarayanashastry Viswanatha [2], Chirag Jain [1], Onkaramurthy Mallappa [3], Mohammed Mukhram Azeemuddin [3], Mohamed Rafiq [3], S.N. Manjula [1]

[1] JSS College of Pharmacy, Mysore, Karnataka, India
[2] Toxicology Division, Clinchoice Private Limited, Bangalore, Karnataka, India
[3] Research and Development, Himalaya Wellness Company, Makali, Bengaluru, Karnataka, India

ABSTRACT

Introduction and aim. The objective of the study was to evaluate the effect of Mentat® an herbal formulation in experimental models of hypoxia-induced attention deficit hyperactive disorder (ADHD) like behavior in rats.

Material and methods. Mentat® was evaluated at the dose of 100 and 200 mg/kg body weight. per oral., in two experimental models of hypoxia in Wistar rats. In the first model, after parturition, on a postnatal day 2 (PND-2), the pups were subjected to hypoxic exposure for 10 minutes to induce neonatal hypoxia. Pups were weaned from dams on PND-21 and subjected to drug treatments for 10 days. In the second model, phenytoin 150 mg/kg. b.wt. p.o. was administered orally to all pregnant animals throughout gestation to induce intrauterine hypoxia. Pups were subjected to assigned treatments after weaning. Behavioral and biochemical parameters relevant to ADHD were assessed.

Results. In the positive control group, hypoxic exposure resulted in significant changes in cognitive and neurologic skills compared to normal control. Open field test, elevated plus maze test, and Acetylcholine esterase levels showed a significant increase in positive control compared to normal control. In treatment groups, there was a dose-dependent decrease in all the above parameters compared to positive control. Dopamine and Nor-epinephrine levels in brain homogenate were decreased in positive control which subsequently increased with Mentat® treatment.

Conclusion. Mentat® showed a neuroprotective effect in different experimental models of ADHD. It may be recommended for the effective/preventive management of ADHD, especially associated with memory impairment and neurologic conditions.

Keyword. ADHD, herbal, Mentat®, neonatal hypoxia, neuroprotective

Introduction

Attention-Deficit Hyperactivity Condition (ADHD) is a neurobehavioral and developmental disorder that is chronic and clinically diverse. Prefrontal dopamine insufficiency and insufficient central dopaminergic activity are the main causes of ADHD. ADHD is one of the most common childhood psychiatric diseases, affecting 3 to 5% of all children in the United States and the Netherlands. Majority of youngsters are diagnosed with ADHD when they start school for the first time. 2 to 16 percent of all school-aged children have been diagnosed with ADHD, with roughly 75 percent of these children being male.[1,2]

CNS stimulants such as methylphenidate and amphetamine, non-stimulants such as Atomoxetine, Cat-

Corresponding author: S.N. Manjula, e-mail: snmanjula@jssuni.edu.in

Received: 22.01.2022 / Revised: 16.04.2022 / Accepted: 8.05.2022 / Published: 30.09.2022

Sindhura S, Viswanatha GL, Jain C, Mallappa O, Azeemuddin MM, Rafiq M, Manjula SN. *Mentat® ameliorates hypoxia-induced attention deficit hyperactivity disorder like behavior in rats*. Eur J Clin Exp Med. 2022;20(3):298–305. doi: 10.15584/ejcem.2022.3.7.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

echolaminergic antidepressants, alpha-agonists, and more recently reported drugs such as Mondafinil and nicotinic agonists are the most often used pharmaceuticals for treating ADHD. The most common side effects with these medicines include nausea, dry mouth, lack of appetite, sleep difficulties, dizziness, irritability and mood swings, and headache.[3] The current study is meant to evaluate Mentat® a well-known neuroprotective proprietary herbal formulation of the Himalaya Wellness Company, Bangalore, India, in experimental models of hypoxia-induced ADHD-like behaviour in rats, according to the safety profile.

There are a variety of environmental and genetic factors that may play a role in ADHD such as Cigarette smoking, alcohol use, infection and stress during pregnancy, brain injury, and heavy metal (lead).[4] Variants of the dopamine D4 receptor gene, the dopamine D5 receptor gene, the dopamine transporter gene (DAT), have all been found to be strongly related with ADHD in molecular genetics investigations. Nor epinephrine transporter (NET), monoamine oxidase-A (MAO-A) and catechol-o-methyltransferase (COMT), serotonin receptor (HTR1B), serotonin transporter (5-HTT), are some of the other genes linked to an elevated risk of ADHD (SNAP-25).[5] Dopamine is a key neurotransmitter in neuropharmacology because it has a role in a variety of brain illnesses, including Parkinson's disease, schizophrenia, and attention deficit disorder, as well as drug addiction and endocrine disorders. According to new studies, raising dopamine levels in youngsters with ADHD improves their conduct.[6] Mentat® was clinically evaluated in children for the improvement in cognition, learning capacity, mental fatigue and neuroprotective effect. As Mentat® was found to be promising in these conditions, a preclinical study was performed on rodents to evaluate its effect in ADHD condition.[7-9] By modulating multiple neurotransmitters, Mentat® shown neuroprotective efficacy in diverse experimental paradigms.

Aim

The study's goal was to see how Mentat® affected different psychological markers of ADHD, such as dopamine, acetylcholine esterase, and norepinephrine, as well as behavioral parameters associated with ADHD.

Material and methods

Phenytoin (Eptoin) manufactured by Abbott India Ltd. India, was procured form retail pharmacy store of Makali, Bengaluru, India. Mentat® tablets were obtained from Himalaya Wellness Company, Bengaluru, India, Atomoxetine (Attentrol) manufactured by Sun Pharmaceutical Industries, Ltd., India. was procured form retail pharmacy store of Makali, Bengaluru, India all other chemicals were of analytical grade and were procured from HiMedia Laboratories GmbH, Germany. The rat feed was procured from VRK Nutritional Solutions, MIDC Miraj, Pune, India. The diet which was provided to the rats was the standard pellet diet which contains protein (19%), Total fat (4.6%), total carbohydrate (60%), total minerals (6.8%), crude fiber (2.5%) with all essential amino acids and minerals.

Experimental animals

Inbreed Wistar rats, weighing 200–250 g were procured from central animal facility, R&D Center, Himalaya Wellness Company, Makali, Bangalore-562162, India. and housed at the temperature of 25 ± 1° C, relative humidity of 45 to 55% and 12:12 h light–dark cycle. The Himalaya Wellness Company's Institutional Animal Ethics Committee (IAEC) approved the experimental protocol (protocol no 127/13) and the experiments were carried out in accordance with the principles and guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India.

Procedure

Monoamine oxidase inhibitor (MAO-B) inhibitory activity (in-vitro)

Brain homogenate was prepared in 0.25 M sucrose-0.1 M Tris-0.02 M EDTA buffer (pH 7.4). Centrifugation was carried out in three steps; first step homogenate was centrifuged at 3000rpm for 20 mins. Supernatant was collected and centrifuged at 10000 rpm for 20 min, pellets were collected and washed twice with 0.25 M sucrose-0.1 M Tris-0.02 M EDTA buffer and pellets were resuspended in 10 mM sodium phosphate buffer (pH 7.4) containing 320 mM sucrose and centrifuged at 12000 rpm for 30 mins. The whole procedure was carried out at 4°C. Pellets collected and resuspended in ice cold 100 mM sodium phosphate buffers (pH 7.4). 2.5 ml 100 mM sodium phosphate buffer (pH 7.4) was taken in test tube, to which 100 µl test drug (Mentat®) and 150 µl brain homogenate was added and incubated at 37°C for 10 min, followed by the addition of 100 µl Benzylamine (substrate), absorbance was recorded in spectrophotometer at 249 nm. Blank (without substrate and test drug) and control (without test drug) readings were recorded.[10]

Neonatal hypoxia induced ADHD in rats

Sixteen female Wistar rats of 180–200 g were kept for cohabitation with male rats at 2:1 ratio. To confirm the mating vaginal smear was examined from the day–1 to day–5, once the mating is confirmed females were separated from the males and checked for abdominal enlargement on day-10. The pregnant dams will be isolated and housed individually. After the parturition, on PND2 male pups were divided into 5 groups consists of 8 animals each and they were subjected to hypoxic ex-

posure on PND2, PND3, PND4 and PND8 for 10 minutes. In brief, the pups were placed in an air tight glass chamber and made deprived of oxygen, depletion of oxygen within the chamber was confirmed based on the combustion principle i.e., by placing a burning candle in an air tight chamber and covering it with the lid, candle turns off after few seconds indicating that the oxygen within the chamber has been completely used, Animals show cyanosis which indicates that there was oxygen insufficiency. After the hypoxic exposure all pups were shifted to respective dams, after periodic hypoxia exposure the pups were weaned from the dams on PND 21 and subjected to following drug treatments for 10 days. Group-I and group-II served as control (normal control) and positive control (ADHD control), respectively and received only vehicle (demineralized water, 10 mL/kg, p.o.). Group-III served as reference standard and received Atomoxetine 50 mg/kg, p. o, Group-IV and group-V animals received Mentat® at the dose of 100 and 200 mg/kg, b. wt, respectively. All the group of animals were treated for a period of 10 days. At the end of assigned drug treatments, the animals were evaluated for hyperactivity and learning impairment and subjected to Actophotometer test, open field test and elevated plus maze test.[11-14] These tests were performed to evaluate the locomotor activity,

Following euthanasia with excess of 5% Isoflurane inhalation anesthesia, a brain homogenate was produced with ice cold 0.15 M KCl (10%) and centrifuged at 10,000 g for 10 minutes at 4°C, with the post-mitochondrial supernatant (PMS) utilised for total protein estimate and lipid peroxidation assay. The amount of protein in the brain homogenate was determined using the Bradford technique and a standard of bovine serum albumin.[15] The amount of malondialdehyde in the brain homogenate was determined quantitatively using the Wills method to determine the degree of lipid peroxidation.[16] Sedlak and Lindsay's method was used to calculate reduced glutathione in the brain homogenate.[17] Ellman's approach was used to calculate acetyl cholinesterase in brain homogenate.[18]

Phenytoin induced intrauterine hypoxia model

Phenytoin when administered to pregnant rats, phenytoin reduces uteroplacental blood flow, resulting in foetal hypoxia, leading to neurological abnormalities such as hyperactivity, learning difficulties, mental retardation, epilepsy, cerebral palsy, dystonia, and more. Phenytoin was produced in deionized water (pH 11.5) and given orally to pregnant rats from the 7th to the 20th day of pregnancy in this study. Following weaning, the pups of treated mothers were evaluated for behavioral measures. Fourteen female Wistar rats (200-250 g) were kept for cohabitation with seven male Wistar rats (200-250 g) at 2:1 ratio for five days. To confirm the mating vaginal smear was examined from the day-1 to day-5, once the mating is confirmed females were separated from the males and checked for abdominal enlargement on day-10. The pregnant dams were isolated and housed individually and they were further divided in to 5 groups and consisting of two mothers.[19] Group-I and group-II served as control (normal control) and positive control (ADHD control), respectively and received only vehicle (demineralized water, 10 mL/kg, p.o.). Group-III served as reference standard and received Atomoxetine 50 mg/kg, p. o. Group-IV and group-V animals received Mentat® at the dose of 100 and 200 mg/kg, b. wt, respectively. All the groups of animals were treated for a period of 10 days. Phenytoin (150 mg/kg, p.o.) was administered orally to all pregnant animals throughout the gestation except group-I. At the end of assigned drug treatments, the animals were subjected to the evaluation of hyperactivity and learning impairment by Actophotometer test, open field test and Elevated Plus maze test. The treatments and evaluation were completed between PND30-PND36.

The behavioral parameters pertaining to ADHD like hyperactivity and learning impairment were evaluated by similar procedures as mentioned earlier through Actophotometer test, open field test and elevated plus maze test.

Dopamine and nor-epinephrine was estimated using high performance liquid chromatography (HPLC) and based on the standard calibration curve of dopamine and nor-epinephrine: Briefly the method is explained as, the composition of mobile phase was prepared from filtered and degassed mixture of Acetonitrile and 1-Octane sulfonic acid in the ratio of 70:30. Pharmaceutical grade of dopamine and nor-epinephrine were procured from Sigma Aldrich. Stock solution of DA and NE were prepared by dissolving 1 mg in 1 mL of methanol in 10 mL volumetric flask separately and volume was made up to 10 mL using the diluents to get a standard stock solution of concentration 0.1 mg/mL (1000 ng/mL). the solutions were filtered using 0.2 µ syringe filter and this solution was used for analysis. Brain tissue samples (1 mm thick slice of brain) were homogenized using individual 1.5 mL centrifuge tubes which contain 400 µL of tissue homogenizing solution (0.1 M perchloric acid, 1x10-7 M ascorbic acid; chilled on ice), the samples were centrifuged at 10,000 rpm for fifteen minutes. The supernatant samples were filtered using 0.2 µm nylon disposable syringe filters and used for the analysis.

Statistical analysis

The values were expressed as mean ± SEM. The results were analyzed statistically using one-way ANOVA followed by Dunnett's multiple comparison test using Prism GraphPad 6.07 (GraphPad Software Inc, San Diego, CA, USA) software. A p value < 0.05 was considered as statistically significant.

Results

Mentat® was evaluated in experimental models of neonatal hypoxia and phenytoin induced intrauterine hypoxia-brain injury in rats. Neurologic, biochemical, morphologic, and histopathologic data were used to examine Mentat's protective® impact. Mentat's® potential free radical scavenging and antioxidant activities were further investigated using in vitro antioxidant tests. In the present study IC50 values of MOA-B inhibitory activity of Mentat® and Amitriptyline was found to be 9.70 mg/mL and 0.45 mg/mL respectively. In the experimental model of neonatal hypoxia. Mentat® at 200 mg/kg showed a significant improvement in locomotor activity (Figure 1, Table 1) and associated parameters when compared to positive control, in elevated plus maze Mentat® at 200 mg/kg showed significant improvement in exploratory score ($p<0.05$) and transfer latency at dose 100 mg ($p<0.001$), 200 mg ($p<0.05$) (Table 2)

Table 1. Behavioural parameters of the rats treated with Mentat® in open field test (neonatal hypoxia)

Groups	Time spent with movement (seconds)	No. of squares crossed	Time spent in periphery (seconds)	No. of rearing
Control	8.25 ± 1.95	2.57 ± 0.37	15 ± 3.45	4.429 ± 0.57
Positive control	16.8± 1.79$^{\#}$	4.75 ± 0.8$^{\#}$	18.22 ± 3.37$^{\#\#}$	10 ± 1.59$^{\#\#}$
Atomoxetine (50 mg/kg)	9.83 ± 3.72	2.83 ± 0.54*	10.60 ± 2.68**	3.5 ± 1.18***
Mentat® (100 mg/kg)	2.83 ± 0.66***	2.25 ± 0.25**	1.50 ± 0.34****	1.125 ± 0.64****
Mentat® (200 mg/kg)	3 ± 1.38***	2.43 ± 0.2**	3.6 ± 1.6**	1.857 ± 0.8****

[a] All the values are expressed as mean ± SEM, mean of all the groups were compared by one way ANOVA followed by Dunnett's test using Graph pad Prism software version 6.07 for windows, "p" value less than 0.05 was considered to be statistically significant. #p<0.05, ##p<0.01 compare to control, ****p<0.0001, ***p<0.001 and **p<0.01 compare to positive control

when compared to positive control. In ADHD induced group there was a free radical generation which is indicated by increased MDA and decreased GSH levels with respect to normal control. Mentat® at 200 mg/kg significantly ($p<0.05$) decreased the malonaldehyde (MDA) in lipid peroxidation assay and significantly increased the GSH levels at dose 100 and 200 mg/kg ($p<0.05$) which showed its protective effect against free radical generation and offered significant protection against hypoxia induced lipid peroxidation. The findings are provided in (Table 3), where the concentration of MDA was expressed as Nmoles MDA/mg of protein in the brain homogenate AChE activity in positive control was more than normal control and in the treatment groups there was a significant decrease at dose 100mg/kg ($p<0.05$), 200 mg/kg ($p<0.01$) (Figure 2) with respect to positive control. Dopamine (Figure 3) and nor-epinephrine (Figure 4) levels which are the hallmarks in ADHD were increased with the treatment of Mentat®.

Table 2. Behaviorial parameters of rats treated with Mentat® in elevated plus maze test (neonatal hypoxia) [a]

Groups	Exploratory score	Transfer Latency
Control	2.25 ± 0.25	73.63 ± 6.77
Positive control	4.25 ± 0.45$^{\#}$	35.71 ± 2.37$^{\#\#}$
Atomoxetine (50 mg/kg)	2 ± 0.03***	90 ± 0.01**
Mentat® (100 mg/kg)	2 ± 0.04	57.25 ± 13.15***
Mentat® (200 mg/kg)	2.571 ± 0.36*	78 ± 12.01*

[a] All the values are expressed as mean±SEM, mean of all the groups were compared by one way ANOVA followed by Dunnett's test using Graph pad Prism software version 6.07 for windows, "p" value less than 0.05 was considered to be statistically significant. #p<0.05, ##p<0.01 compared to control, ***p<0.001, **p<0.01 and *p<0.05 compared to positive control

In the experimental model of phenytoin induced hypoxia. Mentat® at 100 mg/kg showed a significant improvement in locomotor activity ($p<0.05$) (Figure 5, Table 4) and associated parameters when compared to positive control, in elevated plus maze Mentat® showed significant improvement in exploratory score and transfer latency at dose 100 and 200mg/kg ($p<0.01$) (Table 5) when compared to positive control.

Table 3. Effect of Mentat® on glutathione, lipid peroxidation, dopamine and norepinephrine levels [a]

Groups	Parameters			
	GSH µmol/L	LPO nmol MDA/ µg proteins	Dopamine mg/g protein	Norepinephrine mg/g protein
Control	0.7	350	0.0025	0.0048
Positive	0.25$^{\#}$	450	0.0017	0.0036
Atomoxetine	0.7**	375	0.0022	0.0047
Mentat® (100 mg/kg)	0.4*	325	0.0025	0.0039
Mentat® (200 mg/kg)	0.3*	300*	0.0024	0.0047

[a] The mean values of all the groups were compared by one way ANOVA followed by Dunnett's test using Graph pad Prism software version 6.07 for windows, "p" value less than 0.05 was considered to be statistically significant. #p<0.05 compare to control, *p<0.05 and **p<0.01 compare to positive control

Table 4. Locomotor activity of animals treated with Mentat® (phenytoin induced hypoxia) [a]

Group	Time spent with movement (seconds)	No of squares in centre	No of squares crossed in periphery (seconds)	No of rearing
Control	32.83 ± 4.191	2.8 ± 0.374	25 ± 2.076	4.667 ± 0.989
Positive control	59.33 ± 9.656$^{\#}$	7 ± 0.7303$^{\#}$	46 ± 5.468*	18.33 ± 2.155$^{\#\#\#}$
Atomoxetine 50mg/kg	22.6 ± 5.115**	2.5 ± 0.289*	20 ± 3.467**	7.2 ± 2.709
Mentat® 100mg/kg	36.8 ± 4.954	5 ± 1.225	23 ± 6.807	22.4 ± 2.293
Mentat® 200mg/kg	36.5 ± 10.53	4.333 ± 2.333	29 ± 9.452	20.25 ± 2.016

[a] All the values are expressed as mean±SEM, mean of all the groups were compared by one way ANOVA followed by Dunnett's test using Graph pad Prism software version 6.07 for windows, "p" value less than 0.05 was considered to be statistically significant #p<0.05 compared to control, *p<0.05 and **P<0.01 compared to positive control

Table 5. Effect of Mentat® on the behaviour of the rats in EPM (Phenytoin induced hypoxia)[a]

Group	Transfer latency	Exploratory score
Control	74.2 ± 7.14	3.667 ± 0.615
Positive control	27 ± 3.67	5.667 ± 0.615$^{\#}$
Atomoxetine 50mg/kg	66 ± 15.68	2.4 ± 0.4**
Mentat® 100mg/kg	59.4 ± 17.37	2.4 ± 0.4**
Mentat® 200mg/kg	47 ± 24.83	2.5 ± 0.5**

[a] All the values are expressed as mean±SEM, mean of all the groups were compared by one way ANOVA followed by Dunnett's test using Graph pad Prism software version 6.07 for windows, "p" value less than 0.05 was considered to be statistically significant #p<0.05 compare to control, **P<0.01 compare to positive control

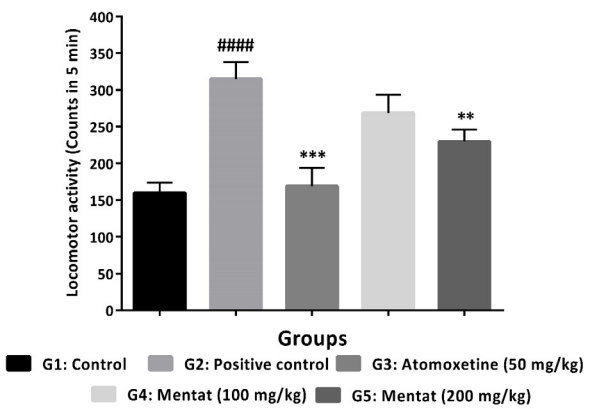

Fig. 1. Locomotor activity of the animals treated with Mentat® in Actophotometer (Neonatal hypoxia), all the values are expressed as mean ± SEM, mean of all the groups were compared by One-way ANOVA followed by Dunnett's test, "p" value less than 0.05 was considered to be statistically significant. ####p<0.0001compared to control, **p<0.01 and ***p<0.001 compared to positive control

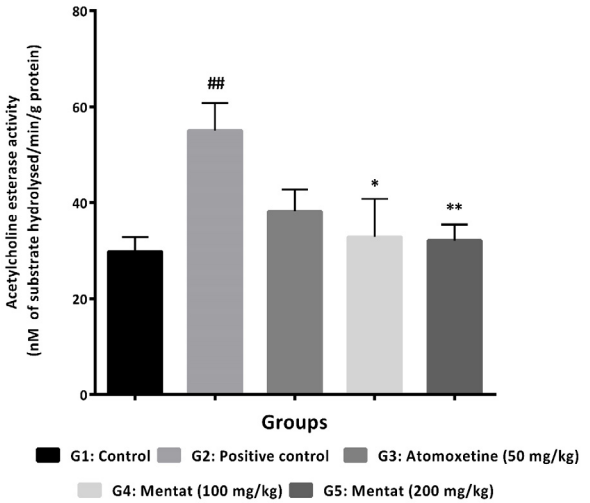

Fig. 2. Effect of Mentat® on acetylcholine esterase inhibitory activity, all the values are expressed as mean±SEM, mean of all the groups were compared by one way ANOVA followed by Dunnett's test, "p" value less than 0.05 was considered to be statistically significant #p<0.05 compare to control, *p<0.05 and **p<0.01 compare to positive control

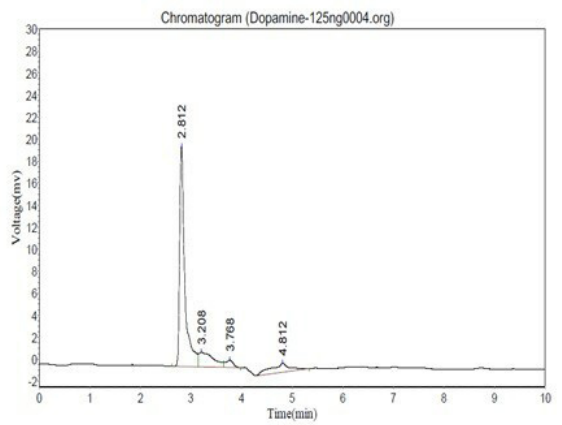

Fig. 3. HPLC Chromatogram of standard Dopamine

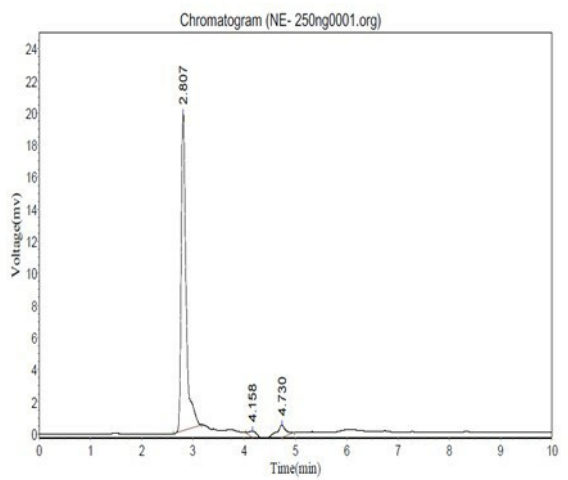

Fig. 4. HPLC Chromatogram of standard Nor-epinephrine

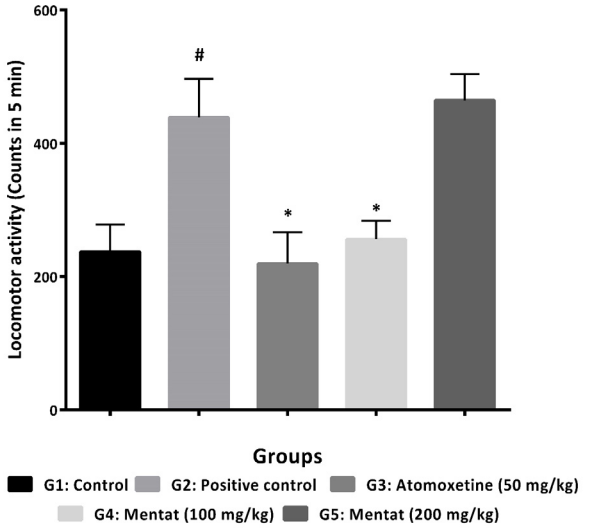

Fig. 5. Locomotor activity of animals treated with Mentat® (Phenytoin induced hypoxia), All the values are expressed as mean±SEM, mean of all the groups were compared by one Way ANOVA followed by Dunnett's test, "p" value less than 0.05 was considered to be statistically significant #p<0.05 Compare to control, *p<0.05 compare to positive control

Discussion

ADHD is one of the most common childhood disorders, and it can last far into adulthood." Treatment for ADHD includes variety of stimulant drugs as well as more natural therapies. Many neurons are packed into distinct regions of brain when it comes to brain function. Each region performs a certain function in our body and is responsible for it. Neurotransmitters are created in minute amounts by neurons. Their job is to deliver messages. They excite the relevant cell in the brain, ensuring that the message is delivered to the correct brain region.[20]

ADHD is caused by a shortage in certain neurotransmitters such as adrenaline and dopamine, according to brain scientists. Brain uses neurotransmitters to activate or inhibit activation in brain cells. The brain must be sufficiently aroused in order to pay attention. Areas of the brain must be effectively controlled, repressed, or slowed down in order to have proper control over our impulses. Both the stimulation and repression mechanisms are malfunctioning in ADHD children.[21] The diagnosis of ADHD is a multi-step process that takes a lot of time. Parents, teachers, and other caregivers should all be involved in the child's evaluation.[22]

Stimulant drugs work by inducing the brain to generate more nor epinephrine; non-stimulant medications work by reducing the pace at which nor epinephrine is broken down. The brain operates normally once the level has been corrected.[23] Dietary supplements are becoming the preferred choice as they are easily accessible and with relatively no side effects. Essential fatty acids are among the most popular. Essential fatty acids are needed for proper cerebral functioning and may aid in the transmission of nerve impulses. Many children with ADHD cannot absorb essential fatty acids normally. There are evidences that herbal medications ameliorate the behavior of ADHD patients. Ginkgo Biloba is effective for neuronal disorders such as memory impairment. Lemon balm is known to help restore the balance and function of the brain and nerve cells

Mentat® which is a proprietary polyherbal formulation of Himalaya Wellness Company is used for neuroprotective activity. It has been found to be beneficial in rat models of transient global ischemia and reperfusion–induced brain injuries.[24] Mentat's® protective impact was assessed by evaluating its ability to alleviate cognitive, motor, and behavioral impairments caused by I/R-induced brain injury.[25] It improves memory and learning abilities. Mentat® contains natural substances that boost mental quotient, memory span, and attention, as well as treating neurological illnesses. Mentat® lowers tribulin levels, an endogenous monoamine oxidase inhibitor that rises during anxiety. Mentat® has relaxing properties that help with insomnia and seizures. Mentat® is useful as an adjuvant in the treatment of epilepsy and enuresis because of its anticholinesterase, dopaminergic-neuroprotective (important neurotransmitter in the brain), adaptogenic, and antioxidant qualities.[26,27] Because there is no evidence of Mentat® effects on ADHD, experimental models of neonatal hypoxia and Phenytoin-induced hypoxia were chosen to assess the drug's efficacy. Rats subjected to neonatal hypoxia presented global brain atrophy ipsilateral to arterial occlusion in the regions analyzed: the total hemisphere, cerebral cortex, white matter, hippocampus and striatum. In addition, contralateral white matter was also affected by the hypoxia procedure.[28] Hypoxia in perinatal rats causes a malfunctioning nigrostriatal dopaminergic system, which is thought to produce ADHD behavior by increasing the expression of vesicular monoamine transporter 2 (VMAT2) and D1 receptor in the striatum. When phenytoin, a powerful anticonvulsant, is given to pregnant women, it reduces utero-placental blood flow, resulting in foetal hypoxia. Hypoxia during pregnancy might result in temporary or permanent brain damage. Through overstimulation of excitatory amino acid receptors, cellular calcium influx, and the production of free radicals and nitric oxide, the hypoxia/ischemia cascade causes neuronal cell death. Reactive oxygen species cause embryonic mortality or teratogenicity by oxidizing molecular targets such as DNA, protein, and lipid.[29]

Bacopa monnieri (Brahmi), *Withania somnifera* (Ashwagandha), *Centella asiatica* (Mandookaparni), *Valeriana wallichi* (Tagar), *Evolvulus alsinoides* (Shankhapuspi) are important herbal extracts present in Mentat® which in combination are responsible for showing the desired efficacy. *Bacopa monnieri* is most

studied nootropic plant for ADHD patients' Clinical trials have shown that it improves memory. *B. monnieri* revitalizes the nervous system, strengthens the mind, and promotes both energy and sleep; it is frequently used to treat insomnia. It is also used to aid in the recovery from fatigue and stress. It is prescribed for conditions such as Parkinson's disease, Alzheimer's disease, dementia, and ADHD, and *B. monnieri* demonstrated a 66% reduction in total ADHD score.[30] *Withania somnifera* roots contain active phytoconstituents, primarily withanolides; alkaloids used in treatment of a variety of brain disorders, and have a wide range of neuroprotective properties. Clinical research suggests that *W. somnifera* may help children with ADHD improve their attention and behavioral control by enhancing normal brain development. According to research, *W. somnifera* accomplishes this by inhibiting the activity of the enzyme that degrades acetylcholine, a neurotransmitter associated with cognition and memory, as well as stimulating neuronal growth.[31] *Centella asiatica* is a brain tonic that improves memory and brain strength. It improves the ability to speak and the poetic imagination. It is an effective treatment for children who are mentally retarded or emotionally disturbed. It aids in the treatment of stress, insomnia, ADHD, depression, mental fatigue, and anxiety.[32] *Valeriana wallichi* has primarily antioxidant properties and is a choline esterase inhibitor. *V. wallichi* roots aid in the reduction of anxiety and the improvement of sleep by relaxing the central nervous system due to their sedative and anxiolytic properties.[33] *Evolvulus alsinoides* balances neurotransmitter levels of noradrenaline, glutamate, and acetylcholine in children and provides neuroprotection against free oxidative radicals and amyloid-induced neurotoxicity with its high antioxidant compounds. *E. alsinoides* lowers cortisol levels and helps to combat stress. It relaxes the nervous system and is extremely effective against insomnia.[34] When these herbal extracts are combined, they may have a synergistic effect that will be beneficial in treating ADHD.

Conclusion

Mentat® showed improvement in the behavior of ADHD rats, which exerts its effect by increasing the dopaminergic and norepinephrine response, decreasing the acetylcholine esterase and inhibiting the monoaminoxidase activity. As Mentat® showed a neuroprotective effect in different experimental models of ADHD, it may be recommended for the effective/preventive management of ADHD, especially associated with memory impairment and neurologic conditions. It can also be recommended as an adjuvant along with modern medicine in the management of ADHD. However clinical trials are required to further support the claim.

Acknowledgements

The authors acknowledge Himalaya Wellness Company for providing Mentat® samples and support for conducting the study and Dr. Pramod, Professor and Principal, JSS College of pharmacy, Mysore, for providing technical insights and support during this research.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, M.R., and S.N.M.; Methodology, S.S., G.L.V., C.J., and M.M.A.; Software, O.M.; Validation, S.S., G.L.V., C.J., and M.M.A.; Formal Analysis, G.L.V., O.M.; Investigation, G.L.V., O.M., and M.M.A.; Resources, S.S., C.J., and M.M.A.; Data Curation, G.L.V., O.M., C.J., and M.M.A.; Writing – Original Draft Preparation, S.S., G.L.V., C.J.; Writing – Review & Editing, O.M., and M.M.A.; Visualization, M.R., and S.N.M.; Supervision, M.R., and S.N.M.; Project Administration, M.R., and S.N.M.; Funding Acquisition, M.R.

Conflicts of interest

Some of the authors were the employees of Himalaya Wellness Company during the course of research work; authors declare no other conflict of interest.

Data availability

The data have not been made public, but are kept with the authors and will be provided, if necessary.

Ethics approval

The study was approved by Himalaya Wellness Company's Institutional Animal Ethics Committee (IAEC) approved the experimental protocol (protocol no 127/13) and the experiments were carried out in accordance with the principles and guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India.

References

1. van der Kooij MA, Glennon JC. Animal models concerning the role of dopamine in attention-deficit hyperactivity disorder. *Neurosci Biobehav Rev*. 2007;31(4):597–618.
2. Faraone SV, Banaschewski T, Coghill D, et al. The world federation of ADHD international consensus statement: 208 evidence-based conclusions about the disorder. *Neurosci Biobehav Rev*. 2021;128:789–818.
3. van Hienen FJ. Organophosphate based pesticides and ADHD 2010. https://studenttheses.uu.nl/. Accessed June 21, 2022.
4. Rigan S. RANG & DALES Pharmacology 9th edition (TRUE PDF). Elsevier; 2012.

5. Kim HJ, Joo SH, Choi IH, et al. Effects of Red Ginseng on neonatal hypoxia-induced hyperacitivity phenotype in rats. *J. Ginseng Re*. 2010;34(1):8–16.
6. Dogra P, Lata S. ADHD: Pathophysiology, Management, and Assessment Scales. *JMSCR*. 2018;6:341–347.
7. Saha A, Bhatia BD, Kulkarni KS. Evaluation of the efficacy of Mentat in children with learning disability: a placebo--controlled double-blind clinical trial. *Neurosci Today*. 2002;6(3):184–188.
8. Sharma AK, Agrawal A, Agrawal U, et al. Influence of mentat on memory and mental fatigue in cases of anxiety neurosis and depression. *Ind J Cancer Bio Res*. 1990;68(7.58):90–95.
9. Dave UP, Chauvan V, Dalvi J. Evaluation of br-16 a (mentat) in cognitive and behavioural dysfunction of mentally retarded children—a placebo-controlled study. *Ind J Paed*. 1993;60(3):423–428.
10. Dhingra D, Goyal PK. Inhibition of MAO and GABA: probable mechanisms for antidepressant-like activity of *Nardostachys jatamansi* DC. in mice. *Indian J Exp Biol*. 2008;46:212–218.
11. Pitchaiah G, Viswanatha GL, Srinath R, et al. Anxiolytic and anticonvulsant activity of aqueous extract of stem bark of Erythrina variegata in rodents. *Int J Parm Tech Res*. 2010;2(1):40–48.
12. Naewla S, Sirichoat A, Pannangrong W, et al. Hesperidin alleviates methotrexate-induced memory deficits via hippocampal neurogenesis in adult rats. *Nutrients*. 2019;11(4):936.
13. National institute of mental health. U.S. https://www.nih.gov/. Accessed July 21, 2022.
14. Kulkarni SK, Singh K, Bishnoi M. Comparative behavioral profile of newer antianxiety drugs on different mazes. *Indian J Exp Biol*. 2008;46:633–638.
15. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72(1-2):248–254.
16. Wills E. Mechanisms of lipid peroxide formation in animal tissues. *Biochemic J*. 1966;99(3):667.
17. Kharbangar A, Khynriam D, Prasad SB. Effect of cisplatin on mitochondrial protein, glutathione, and succinate dehydrogenase in Dalton lymphoma-bearing mice. *Cell Biol Toxicol.* 2000;16(6):363–373.
18. Ellman GL, Courtney KD, Andres Jr V, et al. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7(2):88–95.
19. Mach M, Dubovický M, Navarová J, et al. Experimental modeling of hypoxia in pregnancy and early postnatal life. Interdiscip Toxicol. 2009;2(1):28.
20. Romm AJ, *Romm T. ADHD alternatives: A natural approach to treating Attention-Deficit Hyperactivity Disorder*. Storey Publishing; 2000.
21. Feldman R. *Child development, 4th edition. Upper Saddle River, NJ: Pearson Prentice Hall. An overview of child development. Middle Childhood Years and Cognitive Development*. MD, Gelfand; 2008.
22. Allen KE, Allen CG. *The Exceptional Child*. Albany: Delmar Publishers; 2010.
23. ADDitude - ADD & ADHD Symptom Tests, Signs, Treatment, Support n.d. https://www.additudemag.com. Accessed December 21, 2021.
24. Viswanatha GL, Kumar LM, Rafiq M, et al. LC-MS/MS profiling and neuroprotective effects of Mentat® against transient global ischemia and reperfusion–induced brain injury in rats. *Nutrition*. 2015;1,31(7-8):1008–1017.
25. Hillard CJ. Role of cannabinoids and endocannabinoids in cerebral ischemia. *Curr Pharm Des*. 2008;14(23):2347–2361.
26. Himalaya Mentat Tablets - Enhances Memory – Himalaya Wellness (India) n.d. https://himalayawellness.in/products/mentat. Accessed December 21, 2021.
27. Scheuer C, Boot E, Carse N, et al. *Disentangling inclusion in physical education lessons: Developing a resource toolkit for teachers*. In: Physical Education and Sport for Children and Youth With Special Needs Researches–Best Practices–Situation. Slovakia; Slovak Scientific Society for Physical Education and Sport and FIEP: 2021;343–354.
28. Miguel PM, Schuch CP, Rojas JJ, et al. Neonatal hypoxia--ischemia induces attention-deficit hyperactivity disorder--like behavior in rats. *Behav Neurosci*. 2015;129(3):309.
29. Prince J. Catecholamine dysfunction in attention-deficit/hyperactivity disorder: an update. *J Clin Psychopharmaco*l. 2008;28(3):39–45.
30. Katz M, Adar Levine A, Kol-Degani H, et al. A compound herbal preparation (CHP) in the treatment of children with ADHD: a randomized controlled trial. *J Atten Disord*. 2010;14(3):281–291.
31. Zahiruddin S, Basist P, Parveen A, et al. Ashwagandha in brain disorders: A review of recent developments. *J Ethnopharmacol*. 2020;257:112876.
32. Negi J. The ayurvedic pharmacopoeia of india the ayurvedic pharmacopoeia of India. Part - II. Government of India Ministry of Health and Family welfare department of Ayush. http://www.ayurveda.hu/. Accessed July 21, 2022.
33. Suhani S, Soubhik K. Effects and safety profile of commonly used herbal anti-diabetic preparations. *GSC Biol Pharm Sci.*2018;4(2):66–79.
34. Sethiya NK, Nahata A, Mishra SH, et al. An update on Shankhpushpi, a cognition-boosting Ayurvedic medicine. *Chin J Integr Med*. 2009;7(11):1001–1022.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.8

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 306–315

ORIGINAL PAPER

The effects of acute high intensity interval training on hematological parameters and neutrophils to lymphocytes ratio in elite taekwondo athletes according to gender

Bülent Işık [1], Serkan Küççüktürk [2], Mehmet Fatih Yüksel [3], EkremBoyalı [4], Mehmet Ali Karaselek [5], Kenan Erdağı [3]

[1] Department of Physiology, Faculty of Medicine, Karamanoğlu Mehmetbey University, Karaman, Turkey
[2] Department of Medical Biology, Faculty of Medicine, Karamanoğlu Mehmetbey University, Karaman, Turkey
[3] Department of Physical Education and Sports, Faculty of Education, Necmettin Erbakan University, Konya, Turkey
[4] Department of Recreation, Faculty of Sports Science, University of Selçuk, Konya, Turkey
[5] Department of Internal Medicine, Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey

ABSTRACT

Introduction and aim. Intense taekwondo (TKD) training, it is important to know the exercise-induced hematological and inflammatory conditions and to develop conditions suitable for physiological needs. The aim of study is to investigate the effects of TKD-specific training containing a high-intensity interval training (HIIT) component hematological parameters and on systemic inflammatory biomarkers between gender.

Material and methods. The research was carried out with twenty-four elite TKD athletes (12 female, 12 male). 90 minutes of TKD-specific unit training, including 50 minutes of HIIT component was applied to the athletes. Hematological parameters included erythrocytes, platelets, leukocytes and their subgroups and inflammatory biomarkers.

Results. With the effect of TKD-specific HIIT, erythrocytes and hematocrit values decreased regardless of gender ($p=0.003$, $p<0.001$, respectively). Platelet values decreased in male and increased in female ($p=0.637$). White blood cells and neutrophil ($p<0.001$) and inflammatory biomarkers neutrophils-to-lymphocytes ratio (NLR) and platelet-to-lymphocytes ratio PLR ($p<0.001$, $p=0.022$, respectively) increased regardless of gender. Lymphocyte decreased marginally significantly ($p=0.059$).

Conclusion. This study showed that TKD-specific HIIT increased systemic inflammatory conditions and decreased oxygen-carrying blood parameters. These fundamental findings can contribute to training science in arranging a specific taekwondo training program and sports medicine in protecting the health of athletes.

Keywords. hematological parameter, high intensity interval training, taekwondo

Introduction

Taekwondo (TKD), a traditional martial art, originated in Korea. According to the World Taekwondo Federation (WTF) and International Taekwondo Federation (ITF) reports, there are approximately 80 million individuals worldwide interested in TKD sports. TKD training includes systematic, chronic and progressive activities. Many combat sports including TKD involve extremely high-intensity and interval exercise patterns for short periods, and the possibility of frequent contact, exposure, and injury during competition.[1] TKD training requires high aerobic capacity and anaerobic power, fast

Corresponding author: Bülent Işık, e-mail: drbulent42@gmail.com

Received: 10.05.2022 / Revised: 17.05.2022 / Accepted: 18.05.2022 / Published: 30.09.2022

Işık B, Küççüktürk S, Yüksel MF, Boyalı E, Karaselek MA, Erdağı K. *The effects of acute high intensity interval training on hematological parameters and neutrophils to lymphocytes ratio in elite taekwondo athletes according to gender*. Eur J Clin Exp Med. 2022;20(3):306–315. doi: 10.15584/ejcem.2022.3.8.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

movement skills, high muscle strength, excellent body composition and practical agility.[2,3] Also, TKD training includes a high-intensity static exercise and a low-intensity dynamic exercise pattern. Therefore, high pressure and low volume load occur in the hearts of TKD athletes.[4] From this point of view, in combat sports like TKD, athletes have to perform training sessions that vary according to the mode, effort, pause, intensity, and rate of exercise.to cope with the physiological demands of these sports and high-intensity intermittent exertion situations.[5,6] High-Intensity Interval Training (HIIT), which has been applied for a long time in many combat sports, including taekwondo, has gained popularity in the last two decades as it has resulted in the special classification of different types of variable combinations to develop particular adaptations.[7,8] Combat-sport-specific combat simulation training like TKD is intermittent in nature.[9]

Recently, many researchers have focused on investigating the effects of martial arts specific standard with complementary HIIT on morphological, physiological and performance adaptations. Therefore, understanding the benefits of HIIT on physiological adaptation and performance can provide valuable insights into the field, in order to improve the training programs and performance of TKD athletes, especially by trainers and sports scientists.TKD athletes are physiologically very tired during competitions and they are at risk of injury. Because, according to Olympic rules and World Taekwondo (WT) regulations, TKD competitions consist of at least 3 rounds with 2 minutes of fighting and 1 minute of rest in between. Athletes may have to fight 4-5 consecutive fights a day for the championship.[10]

Like all professional athletes, TKD athletes and coaches are also concerned for the combat sports training program they apply to achieve the best results in the competition. However, the effects of exercise are of great importance because some hematological disorders seen in athletes can negatively affect training intensity and exercise success.[11] Hematological parameters are affected by both the frequency, intensity, type and duration of the exercise, as well as the age of the person, eating habits and the environment.[12] In many studies, it has been shown that regular training makes changes the hematological patterns of many athletes, including TKD, but in some studies it does not.[13-16]

Due to the close contact between the athletes during the TKD sport, the risk of injury that causes various levels of tissue damage has always been inherent in this sport.[17] In addition, it is well known that inflammation plays an important role in the formation of metabolic and hormonal responses in the body against sequential exercise-induced physiological stress.[15-18] In particular, it has been shown that the duration and intensity of exercise have a positive effect on the regulation of leukocyte response.[19] In addition, neutrophils-to-lymphocytes ratio (NLR) and platelet-to-lymphocytes ratio (PLR) are the most important biomarkers reflecting systemic inflammatory status in athletes including taekwondo.[20]

Most of the studies conducted in TKD athletes to date focused on performance training, field testing methods, physiological responses during competition, and sports injuries.[1-3,21] To our knowledge, these studies did not examine the effects of training on hematological parameters and inflammatory biomarkers such as NLR and PLR in elite TKD athletes. Keeping this in mind, it is critical to develop conditions suitable for the physiological needs specific to TKD sport, in order to achieve high sportive performance, to develop an appropriate periodic training program and to prevent and eliminate possible health problems related to hematological parameters. We hypothesized that the TKD-specific training containing a high-intensity interval training component might affect the hematological profiles of taekwondo athletes and NLR.

Aim

In line with the available information, in this study, it was aimed to investigate the effect of 90-minute TKD-specific unit training, including 50 minutes of HIIT component on systemic inflammatory biomarkers and hematological parameters in elite TKD athletes between gender.

Material and methods

Participants

The information and consent interviews were conducted with the participants, their trainers, and club managers. The study was conducted in accordance with the Declaration of Helsinki and under the approved protocol by Meram Medical Faculty, Ethics Committee of non-Pharmaceuticals and non-Medical Device Researches of Necmettin Erbakan University with the number 2018/1312. Participants were informed about the study's method and potential risks, and informed consent was obtained.The subjects of this research were selected among the volunteer athletes of the Selcuk University Taekwondo Team.The study was conducted on 24 elite level taekwondo athletes (12 males and 12 females) from the Selçuk University Taekwondo Team, who regularly continued Taekwando training and participated in tournaments. All of the subjects were black belt holders and classified in the national Division I category. Furthermore, in order to form the elite level athlete group, inclusion criteria were formed. According to inclusion criteria, the athletes to be included in the study must have at least a 5-year exercise background (min; 6, max; 14 years), must be participated in national and international level Taekwando competitions, and must have continued taekwando training for 5 days and

over in a week. Additionally, based on the inclusion criteria the athletes must be between 17-23 years old and have voluntary to participate in the study. Moreover, they must beno smoking, no use of notorious, alcohol or nutritionalsupplements. All of them had to be physically and mentally healthy. Those with diseases that may impair their hematological terms, such as anemia, thyroid diseases, musculoskeletal injuries, infectious and inflamatuar diseases, were excluded. Moreover, female athletes who are in menstrual period were excluded from the study. Detailed medical examinations of the athletes were made by B.I. who is a medical expert doctor. Hence, all of the athletes who were included this study were healthy in hematological terms. Training experience, demographic values and hematological parameters of the subjects are available in Table 1.

Procedures and hematological analysis

The study was performed in December 2019. Measurements were made during the regular training hours in the afternoon (03:00 PM-05:00 PM) in a gym the athletes were familiar with. The athletes were given a directive not to exhaust alcohol and caffeinated foods/drinks at least 2 days before the measurements. Furthermore, subjects were instructed to refrain from doing any training or vigorous physical activity for at least 2 days before the study to control possible confounding factors influencing blood inflammatory biomarker assessments. An easily digestible diet had been suggested for them, so as not to aggravate their digestive system. Subjects also were restricted from consuming any form of anti-inflammatory drug or antioxidants to minimize the individual variability in detecting inflammatory state before and during the study period.

Baseline anthropometric data were measured in a room at room temperature in the morning the day before the study and were measured after overnight fasting (12 h). All instruments were calibrated before the test. The body mass was measured with light clothes using an electronic scale, and height was measured in an upright position without shoes in the morning. Blood samples were taken from the forearm vein of athletes 10-min before and after the training, per the rules of disinfection by the specialist medical staff. Blood samples were collected in 4 mL of hemogram tubes with K3 EDTA. According to the instructions, haematological parameters were examined at the Konya Application Center of Özel Sistem Laboratories through a Cell-Dyn 1800 (Abbott Diagnostics, Abbott Park, IL, USA) hematological analyzer. The leukocyte subparameters as the white blood cells (WBC 10^3/μL) count, neutrophil (NEU 10^3/μL) count, lymphocytes (LYM 10^3/μL) count and MID cells (10^3/μL) count, NEU %, LYM % and MID cells %; erythrocyte subparameters as the red blood cells (RBC 10^6/μL) count, hemoglobin (HBG g/dL), mean corpuscular volume (MCV fL), mean corpuscular hemoglobin (MCH pg), mean corpuscular hemoglobin content (MCHC g/dL) counts, red blood cells distribution width (RDWC %) and hematocrit (HCT %); platelet subparameters as platelets (PLT 10^3/μL) count, mean platelet volume (MPV fL) count, plateletcrit (PCT %) and platelet distribution width (PDW %) values of the haematological parameters were analyzed from each blood sample. Then, systemic inflammatory biomarkers were calculated such as NLR and PLR from these values.[20] To calculate changes in plasma volume, Dill and Costill formula were used:[22] $\%\Delta PV = [(HBG_1/HBG_2) \cdot (100 - HCT_2/100 - HCT_1) - 1] \cdot 100$.

All blood samples and raw data were subsequently analyzed and statistical analysis applied. The experimental design is summarised in Figure 1.

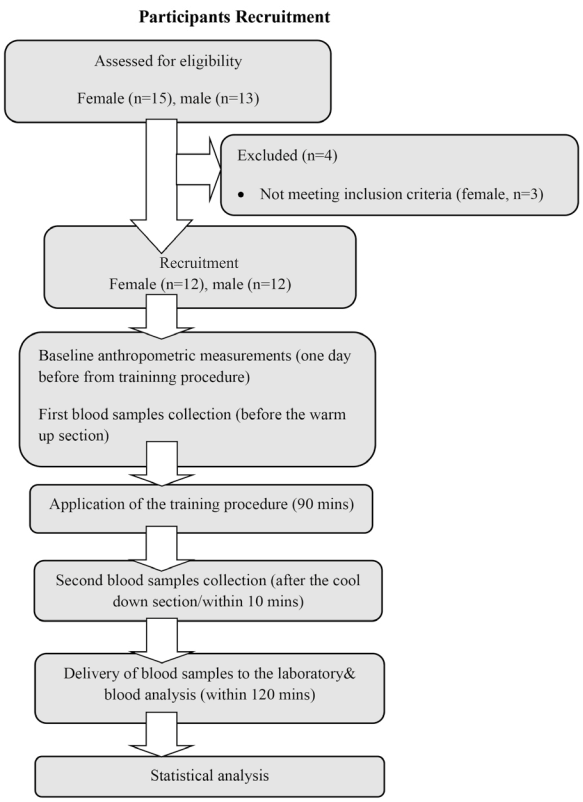

Fig. 1. Schematic of the experimental design

Training procedure

Athletes trained for 90 minutes on the test day according to the content stated below. The high-intensity interval Taekwondo-specific unit training period consisted of three parts:

a. Warm-up part (30 mins); Athletes started training with 10 minutes of light jogging. Afterwards, applied dynamic and static flexibility, joint mobility, coordination and balance exercises to the athletes.
b. Main part (50 mins); During the technical exercises and training competition applied in the main phase of the training, Work, one of the high-inten-

sity interval training (HIIT) protocols reported by Franchini et al.; rest ratios were applied as 1:2 minute.[23] In addition, training intensity ranged from 80–90%, as reported in many previous studies.[24-26] Athletes performed single and combined technical exercises on gloves (with all the techniques used in competitions) for 15 minutes. In addition, single and combined technical studies were carried out on the safeguard for a period of 15 minutes. Work: Rest ratios were applied in the form of 1:2 minutes and 5 repetitions in single and combined technical studies on both gloves and safeguards. Finally, a training competition called Sparring was held for 20 min (the athletes participated in the competition with the "pull system" method). In the meantime, tactical indications were given by the coach regarding the offensive and defensive stance, technical demonstration and protection, by intervening when necessary.

c. Cool-down part (10 mins): Light jogging and static flexibility exercises were applied to the athletes for 5 minutes each.

During the training, the heart rates of the athletes were recorded with an electronic polar watch. The average heart rate of both male and female athletes was between 57-70 beats/min before the warm-up period and around 100 beats/min after the warm-up period. In the main circuit, the heart rates of male athletes were between 158-172 beats/min and between 160-174 beats/min for female athletes. During the collection of second blood samples within 10 minutes after the cooling section, the mean heart rates of female and male athletes were 79 and 73 beats/min, respectively. The heart rates of the athletes were checked every three minutes from the warm-up to the cool-down.

Statistical analysis

These analyses were performed using open-source jamovi statistical platform [The jamovi project 2021, Sydney, Australia, *Jamovi* (Version 1.2.1.1)] [Computer software]. Retrieved from https://www.jamovi.org.

The data were presented as mean ± standard error of mean (mean ± SEM.) Normal distribution was analysed by Kolmogorov-Smirnov test and age, training experience, BMI by independent t-test.The direction and strength of the relationship between training experience, hematological parameters and some these ratio such as NLR, PLR were evaluated with the Pearson correlation. A two-way repeated measure analysis of variance (ANOVA) was performed to test for the main effects corresponding to groups (male, female) and time (pre-post), as well as the interaction between the two (groups and time: the effect of gender on the pre-post change). Results were considered statistically significant at the level of $p < 0.05$.

Table 1. Training experiences, demographic values, plasma volume changes, hematological parameters and inflammatory biomarkers of Taekwondo athletes (mean ± SEM)[a]

	Female athletes (n= 12)	Male athletes (n= 12)	p
Age (years)	20.33 ± 0.57	20.67 ± 0.43	0.645
BMI (kg/m²)	20.91 ± 0.66	21.33 ± 0.34	0.579
Training experience (years)	8.83 ± 0.56	9.67 ± 0.7	0.363
Plasma Volume Change (%)	1.48 ± 0.52	2.55 ± 0.51	0.155
	Female athletes (n= 12)		**Male athletes (n= 12)**	
	Pre	**Post**	**Pre**	**Post**
RBC*,† (10⁶/µL)	4.53 ± 0.08	4.38 ± 0,07	5.27 ± 0.12	5.19 ± 0.1
HBG*,¶,† (g/dL)	13.1 ± 0.2	12.64 ± 0.24	15.24 ± 0.31	15.17 ± 0.42
HCT*,† (%)	39.71 ± 0,61	38.88 ± 0.64	46.45 ± 0.88	45.11 ± 0.94
MCV¶ (fL)	87.9 ± 1.68	88.85 ± 1.48	88.49 ± 2.3	87.04 ± 1.9
MCH¶ (pg)	28.99 ± 0.54	28.91 ± 0.53	29.01 ± 0.8	29.27 ± 0.82
MCHC¶,† (g/dL)	33 ± 0.18	32.5 ± 0.15	33.12 ± 0.21	33.94 ± 0.29
PLT¶ (10³/µL)	244.42 ± 15.7	264 ± 16.87	253.5 ± 10.8	241.83 ± 9.83
MPV*,¶,† (fL)	11.29 ± 0.3	10.86 ± 0.36	10.26 ± 0,25	10.2 ± 0.24
MPV/PLT ratio¶	0.05 ± 0	0.04 ± 0	0.04 ± 0	0.04 ± 0
WBC*,† (10³/µL)	7.07 ± 0.55	8.81 ± 0.7	6.19 ± 0.34	7.06 ± 0.44
NEU*,† (10³/µL)	4.65 ± 0.53	6.38 ± 0.74	3.42 ± 0.28	4.52 ± 0.37
NEU*,† %	63.78 ± 3.34	70.64 ± 3.78	54.93 ± 1.5	64.86 ± 1.99
LYM* (10³/µL)	1.82 ± 0.1	1.74 ± 0.19	2.12 ± 0.08	1.78 ± 0.12
LYM*,† %	26.73 ± 2.79	21.67 ± 3.23	34.77 ± 1.59	25.91 ± 1.89
MID (10³/µL)	0.67 ± 0.06	0.71 ± 0.06	0.66 ± 0.03	0.66 ± 0.05
MID*,† %	9.48 ± 0.75	8.13 ± 0.64	10.74 ± 0.27	9.55 ± 0.34
NLR*,†	2.68 ± 0.39	4.29 ± 0.71	1.62 ± 0.11	2.7 ± 0.28
PLR*	138.46 ± 10.8	171.69 ± 21.46	121.7 ± 7.11	143.09 ± 11.31

[a](*: within subject <0.05, ¶: interaction <0.05 †: between subject <0.05)

Results

This study was conducted on 24 elite level taekwondo athletes (12 males and 12 females), who regularly continued TKD training and participated in tournaments. The TKD-specific training containing a high-intensity interval training component was applied to athletes. Blood samples were taken from the athletes 10-min before and after the training (Figure 1). Subjects' characteristics are display in Table 1.Statistical results of hematological parameters are presented in the text and Figure 2 and 3.

The main effect for time training-induced RBC count was significant [$F(1, 22)=11.615$, $p=0.003$, $\eta^2=0.346$]. Its trend was downward. However, time and group interaction was not significant [$F(1, 22)=0.868$, $p=0.362$, $\eta^2=0.038$]. The mean of RBC count was significantly different between groups, with males being higher [$F(1, 22)=37.669$, $p<0.001$, $\eta^2=0.631$], (Figure 2).

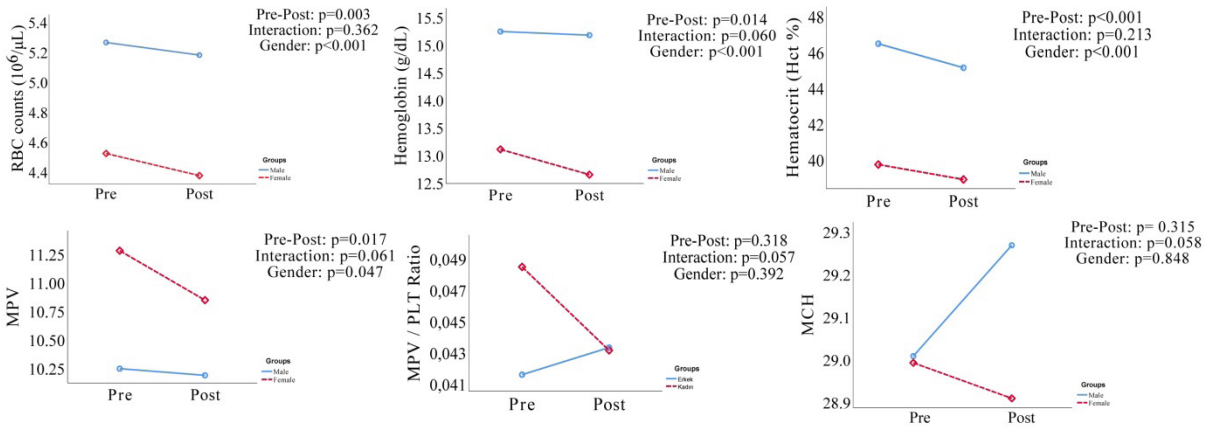

Fig. 2. Changes in hematological parameters and MPV/PLT ratio in response to TKD-specific training containing a high-intensity interval training component. The changes of RBC count, HBG, HTC, MPV, MPV/PLT ratio and MCH were measured pre and post TKD-specific training. There were statistically differences in pre and post (RBC, HBG, HTC, MPV), interaction (marginal significant HBG, MPV, MPV/PLT, MCH) and gender (RBC, HBG, HTC, MPV), (p<0.05)

The main effect for timetraining-induced HBGvalue was significant [F(1, 22)=7.084, p=0.014, η^2 =0.244]. Also, time and group interaction was marginally significant [F(1, 22)=3.943, p=0.060, η^2 =0.0152]. The change in HBG was more in female gender. The mean HBG was significantly different between the groups, with males being higher [F(1, 22)=31.267, p<0.001, η^2 =0.587], (Fig. 2).

The main effect for time training-induced HCT % was significant [F(1, 22)=30.395, p<0.001, η^2 =0.580]. However, the time and group interaction was not significant [F(1, 22)=1.648, p=0.213, η^2 =0.070]. The change in HCT % was similar in both genders. The mean HCT % was significantly different between the groups, with it being higher in males [F(1, 22)=35.735, p<0.001, η^2 =0.619], (Figure 2).

The main effect for time training-induced MCV value was not significant. [F(1, 22)=0.383, p=0.542, η^2 =0.017]. However, time and group interaction was significant [F(1, 22)=8.962, p=0.007, η^2 =0.289. A change in different ways was observed in the MCV in the form of an increase in females and a decrease in males. Mean MCV was similar in groups [F(1, 22)=0.555, p=0.816, η^2 =0.003].

The main effect for time training-induced MCH value was not significant [F(1, 22)=1.055, p=0.315, η^2 =0.046]. However, time and group interaction was marginally significant [F(1, 22)=3.986, p=0.058, η^2 =0.153]. A decrease in MCH in females and an increase in males were observed in different ways.MCH mean was similar in groups [F(1, 22)=0.038, p=0.848, η^2 =0.002], (Figure. 2).

The main effect for time training-induced MCHC value was not significant [F(1, 22)=0.677, p=0.419, η^2 =0.030]. However, time and group interaction was significant [F(1, 22)=11.436, p=0.003, η^2 =0.342]. A decrease in the MCHC in females and an increase in males were observed in different ways.The mean of MCHC was significantly different between groups [F(1, 22)=11.349, p=0.003, η^2 =0.340].

The main effect for timetraining-induced PLT count was not significant [F(1, 22)=0.229, p=0.637, η^2 =0.010]. However, time and group interaction was marginally significant [F(1, 22)=3.573, p=0.072, η^2 =0.140]. The PLT count trend was downward in males and upward in females. The mean of PLT count was similar between groups [F(1, 22)=0.141, p=0.711, η^2 =0.006], (Figure.3).

The main effect for time training-induced MPV value was significant [F(1, 22)=6.709, p=0.017, η^2 =0.234]. Time and group interaction was marginally significant [F(1,22)=3.903, p=0.061, η^2 =0.151]. The MPV downward trend was more in females. The mean MPV value was significantly different between the groups, with it being higher in females [F(1, 22)=4.431, p=0.047, η^2 =0.168], (Figure 2).

The main effect for time training-induced MPV/PLT was not significant [F(1,22)=1.044, p=0.318, η^2 =0.045]. However, time and group interaction was marginally significant [F(1,22)=4.028, p=0.057, η^2 =0.155]. The MPV/PLT trendwas downward in females and upward in males. The rate of change in the mean MPV/PLT with the effect of training was 11.1 % in females and 4.1 % in males. MPV/PLT mean was not significantly different between groups [F(1,22)=0.761, p=0.392, η^2 =0.033], (Figure 2).

The main effect for time training-induced WBC count was significant [F(1, 22)=17.352, p<0.001, η^2 =0.441]. Its trend was upward. However, time and group interaction was not significant [F(1, 22)=1.953, p=0.176, η^2 =0.082]. Although the increase in WBC count was slightly higher in females, there was marginally significant difference between the groups [F(1, 22)=3.798, p=0.064, η^2 =0.147), (Figure 3).

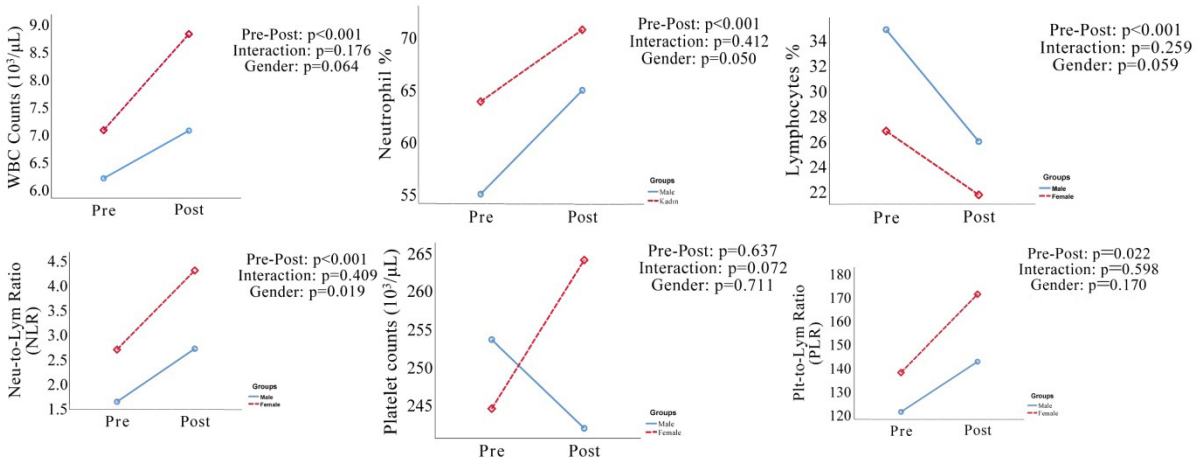

Fig. 3. Changes in WBC subparameters, PLT and systemic inflammation biomarkers in response to TKD-specific training containing a high-intensity interval training component. The changes in total WBC and PLT counts and leukocytes subparameters % (NEU, LYM) and systemic inflammation markers NEU-to-LYM Ratio and PLT-to-LYM Ratio were measured pre and post TKD-specific training, ($p<0.05$)

The main effect for time training-induced NEU count and NEU % were significant [$F(1, 22)=19.709$, $p<0.001$, $\eta^2=0.473$, $F(1, 22)=20.987$, $p<0.001$, $\eta^2=0.488$, respectively]. Both trendswere upward. However, the time and group interaction for both parameters were not significant [$F(1, 22)=0.985$, $p=0.332$, $\eta^2=0.043$, $F(1, 22)=0.701$, $p=0.412$, $\eta^2=0.03$, respectively]. While the mean of NEU count was significant [$F(1, 22)=5.712$, $p=0.026$, $\eta^2=0.206$), the NEU % value was marginally significant, between the groups($F(1, 22)=4.293$, $p=0.050$, $\eta^2=0.163$]. While the average increase in NEU count in females was significantly higher than that of males, the increase in NEU % in the proportional distribution of leukocyte subgroups such as NEU and LYM within the total leukocyte count was more pronounced in males, (Figure 3).

While, the main effect for time training-induced LYM countwas marginally significant, the main effect of Lym % was significant [$F(1, 22)=3.957$, $p=0.059$, $\eta^2=0.152$, $F(1, 22)=18.137$, $p<0.001$, $\eta^2=0.452$, respectively]. Both trends were downward. However, time and group interaction in Lym count and LYM % value was not significant [$F(1, 22)=1.621$, $p=0.216$, $\eta^2=0.069$, $F(1, 22)=1.345$, $p=0.259$, $\eta^2=0.058$, respectively]. While the mean of LYM count was not significant between groups, the mean of LYM % was marginally significant [$F(1, 22)=1.271$, $p=0.272$, $\eta^2=0.055$, $F(1, 22)=3.977$, $p=0.059$, $\eta^2=0.153$, respectively]. In other words, in the proportional distribution of leukocytes within themselves, the LYM % decreased more significantly in males. In males, this supported the marginally significantly higher distribution of NEU % in leukocytes, (Figure 3).

While the main effect for time training-induced MID count was not significant, the main effect of MID % change was significant [$F(1, 22)=0.353$, $p=0.558$, $\eta^2=0.016$, $F(1, 22)=16.693$, $p<0.001$, $\eta^2=0.431$, respectively]. While the MID count trend was upward in females, it was linear in males. MID % trend was downward in both groups. However, time and group interaction in MID count and MID % value was not significant [$F(1, 22)=0.353$, $p=0.558$, $\eta^2=0.016$, $F(1, 22)=0.071$, $p=0.792$, $\eta^2=0.003$, respectively]. Also, the MID count mean was similar between groups, while the MID % mean was marginally significant [$F(1, 22)=0.183$, $p=0.673$, $\eta^2=0.008$, $F(1, 22)=3.733$, $p=0.066$, $\eta^2=0.145$, respectively]. In other words, although the decrease in the mean % of the MID value, which includes the sum of the small group leukocytes, eosinophils, basophils and monocytes, with the effect of training, was more pronounced in females than in males, the mean MID % was found higher in males.

The main effect for time training-induced NLR was significant [$F(1,22)=18.332$, $p<0.001$, $\eta^2=0.455$]. Its trend was upward. However, time and group interaction in NLR value was not significant [$F(1,22)=0.707$, $p=0.409$, $\eta^2=0.031$]. Athletes' training-related NLR change was not affected by gender. The NLR change rate with the effect of training was 60.1% in females and 66.3% in males. The mean NLR was significantly different between the groups, with the females being higher [$F(1, 22)=6.353$, $p=0.019$, $\eta^2=0.224$], (Figure 3).

The main effect for time training-induced PLR was significant [$F(1,22)=6.096$, $p=0.022$, $\eta^2=0.217$]. Its trend was upward. However, time and group interaction in the PLR value was not significant [$F(1,22)=0.287$, $p=0.598$, $\eta^2=0.013$]. Athletes' training-related PLR change was not affected by gender. The rate of change in the mean PLR with the effect of training was 24% in females and 17.57% in males. Although the mean PLR was higher in females, there was no significant difference between the groups [$F(1,22)=2.013$, $p=0.170$, $\eta^2=0.084$], (Figure 3F).

Although there was a weak negative correlation (r = -0.303, p=0.150) between training experience and NLR in taekwondo athletes, no relationship could be found between training experience and PLR (r=-0.17, p=0.938). Surprisingly, a negative correlation (r = -0.259, p=0.221) was shown between the mean of MPV and PLT values, while a positive correlation (r=0.145, p=0.498) was shown between the mean of MPV/PLT and the training experience. However, statistical significance was not found in these relationships.

Discussion

In this study, adaptation responses of hematological parameters and systemic inflammatory biomarkers including NLR and PLR to 90-minute TKD-specific training containing HIIT component were investigated in elite TKD athletes. In the current study, the main effect of training-induced change in RBC and its subparameters HBG and HCT was shown to be significant. It was observed that the changes in RBC and HCT values in the direction of decrease were not affected by the gender factor, but the change in HBG was marginally significantly affected. It was determined that the mean of the aforementioned values was significantly higher in men. In any study, investigating the effects of chronic exercise on hematological parameters in TKD athletes, it was interpreted that significant changes in oxygen-carrying blood parameters were caused by intravascular hemolysis due to exercise-induced trauma.[27] But, in another study, increases in hematological parameters have been associated with exercise-induced plasma losses.[28] Plasma change was not remarkable in our study; therefore, we think that other factors rather than plasma change are effective in the decrease of hematological parameters.Erdağı et al. showed that RBC, HBG and HCT decreased with the effect of acute exercise, similar to the findings of our study, in a study they conducted in elite female weightlifters in 2018.[29] It is known that TKD and weightlifting sports trainings include high-intensity static and low-intensity dynamic exercise patterns.[4] Surprisingly, a decrease in RBC, HBG and HCT values was detected in TKD athletes in our study, as well as in weightlifters, due to acute exercise. It has been shown that the type, duration and intensity of exercise, as well as individual factors such as gender and age, have important effects on the hematological changes that occur in the body with the effect of exercise.[30] For this reason, it can be said that intravascular hemolysis in the vessel wall as a result of high blood pressure, stress and trauma due to training with acute HIIT component are effective in the decrease in RBC, HGB and HCT values as well as the type, duration, intensity of exercise and sports branch. On the other hand, Özen et al. showed that there was a significant increase in RBC subparameters MCH and MCHC values in professional male football players with the effect of 6-week preparation training.[12] Similarly, in the findings of our study, the increase in MCH and MCHC values with the effect of TKD-specific training in males can be considered as a adaptation response to the increased metabolic and oxygen demand during exercise.

In this study, PLT counts decreased in males and increased in females with the effect of training. The change in MPV values, on the other hand, decreased more in female than in males. The change in both coagulative parameters was marginally significant. The present findings are in full agreement with the findings of Boyalı et al.'s study on TKDathletes.[27] Similar studies have shown increasesin PLT and its sub-parameters, and decreases in some others,with the effect of acute and chronic exercise.[28,31,32] Consistent with the literature, in our study, PLT increase in female taekwondo athletes; It may be due to activation of the sympathetic nervous system, which occurs to meet the increased metabolic needs in the tissues with the effect of acute exercise, release of platelets into the circulation from blood cell stores such as the spleen and bone marrow, and hemoconcentration due to plasma loss. The decrease in PLT in males may be due to intravascular trauma, cellular damage in the body under stress and pressure due to acute exercise and platelet fragmentation. Decrease in MPV value, which is an indicator of platelet hyperactivity in both genders, on the other hand, may indicate that the platelet hyperactivity caused by the effect of exercise in athletes can be adequately compensated. However, more extensive research is needed to elucidate its cellular mechanisms.

The duration and intensity of exercise have an important effect on the regulation of leukocyte response.[19] Belviranlı et al. showed that WBC, NEU, and LYM, which are associated with all aspects of the immune system, increase immediately after acute HIIT exercise.[33] In some studies, LYM was shown to be decreased in the early period after exercise and recovered over time.[15,34] In the current study, which has similar findings with the literature, it was shown that the main effect of the change in WBC and NEU due to training was significant, but the gender group interaction was not significant. The change was in the direction of increase. While NEU mean was found to be significantly higher in females than in males, it was observed that the increase in NEU % within the leukocyte subparameters was marginally significantly more pronounced in males than in females. Exercise is a factor that causes systemic inflammatory response and cell damage by causing the secretion of stress hormones such as cortisol and catecholamines from the adrenal gland under the influence of stress in both the normal population and martial arts athletes including taekwondo players.[34] Hemodynamic shear stress, which is caused by stress hormones and exercise, cause an increase in WBC and NEU in the early

phase of exercise with a series of immunological reactions and demargination.[35] In the current study, while the main effect of training-related LYMcount and LYM % change was significant, gender-group interaction was not. LYM change lines are trending in the decreasing direction. Similar to our findings, Chuang et al. showed a significant decrease in the number of circulating LYM after TKD competition in 2019. The authors speculated that LYM reduction may be related to cytotoxic agents released from injured muscles and altered immune cell balance profiles during recovery after combat exercise.[36] In our study, it was also observed that, in the proportional distribution of leukocyte subparameters, it was observed that the % of LYM decreased significantly more in males. This was a finding supporting the proportional distribution of NEU % within the leukocytes subparameters in males than in females. The mean % of the MID value, which is the sum of the subparameters of leukocytes, eosinophils, basophils and monocytes decreased more significantly in females than in males under the training effect. In the current study, it was shown that the mean % of MID was higher in males, as demonstrated by Erdoğdu et al.[15] This may be due to the inflammatory process triggered by stress hormones such as cortisol and catecholamines that increase in order to tolerate the increased meatbolic needs during exercise.[29,35] Inflammation is considered as a basic physiological process inducing exercise adaptation mechanisms, as well as facilitating the repair process by collecting the internal resources of the organism to the damaged area.[18] NLR and PLR are accepted as indicators of systemic inflammatory status in athletes, including TKD.[20,37] It was thought that NLR could be a safe and appropriate biomarker to determine the systemic inflammatory state during acute HIIT in TKD athletes.But there was no study on this field in the literature review. Therefore, in this study, these biomarkers were selected to determine the systemic inflammatory state caused by acute HIIT in TKD athletes. In a study by Chen et al. in TKD athletes in 2017, they showed that chronic exercise increases NLR. They associated the increased systemic inflammatory state with weakening the adaptive response to training, resulting in decreased aerobic capacity, low anabolic hormone (testosterone, DHEA-S), and increased catabolic hormone (cortisol) levels. Furthermore, they argued that increased systemic inflammation after training may impair the development of aerobic capacity and local muscle repair mechanisms.[20] Interestingly, a negative correlation between training experience and NLR was detected in our study. Therefore, we think that intense TKD-specific training for a long time can reduce inflammation due to training. Also in this study, although the main effect of the change in NLR and PLR, which occurred in the direction of increase with the training effect, was found to be significant, it was observed that this change was not affected by gender. In this study, while the mean NLR was found to be significant in females and the PLR was non-significantly high, the rates of change were observed to be 60.1%, 24% in females, 66.3% and 17.57% in males, respectively. Similar to our findings, in their study, Chuang et al. showed that NLR and PLR increased with the effect of real TKD competition including acute TKD training, and the increase after the competition was more than the increase after simulated combat training. The authors declared that this increased systemic immune response is associated with many endocrine, metabolic and immune processes that occur with the effect of exercise.[36] On the other hand, in a study conducted in 2016 among elite Taekwondo athletes, it has been claimed that the increase of approximately 48% in the NLR value measured after 10 weeks of competition preparation training after an 8-week period of no training may be related to the anti-inflammatory effect of regular exercise.[37]

Our study had particularly limitations, as well. First, it comprises a small sample of elite TKD athletes. The research findings may not apply to other TKD populations, such as disabled and amateur athletes in this field. Second, the markers of muscle damage, such as myoglobin, creatine kinase, caused by exercise, and the blood parameters of the recovery period after exercise could not be measured in the current study. Thus, eliminating these limitations in future studies and refining these findings may contribute significantly to the literature.

Conclusion

Finally, it can be concluded that the TKD specific training containing a HIIT component caused some changes in hematological parameters and systemic inflamatuar status of both male and female TKD athletes. It can increase systemic inflammatory biomarkers such as NLR and PLR or decrease oxygen transporter hematological parameters such as RBC and HGB in both males and females. This limited effect, which does not lead to a surprising change, can be attributed to the fact that the athletes in the research group were elite-level competitors, and to their ability to develop physiological adaptation to exercise stress thanks to their training background.From this point of view, the hematological and inflammatory conditions of TKD athletes should be evaluated periodically at different times. The basic data provided by the present study may help coaches, athletes, and sport scientists to develop a more realistic TKD-specific training program and for sports medicine to prevent or rehabilitate possible health problems in the future. However, the precise physiological mechanisms underlying the inflammatory response to the consecutive full-contact TKD combat warrant further investigations.

Acknowledgments

We thank instructor Erkan Özbay for his support in the preparation of the manuscript and sincerely the volunteers who participated in this study.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, B.I. and M.F.Y.; Methodology, M.F.Y., E.B, B.I.; Software, B.I, K.E.; Validation, B.I., M.A.K. and S.K..; Formal Analysis, S.K..; Investigation, M.F.Y., B.I., E.B.; Resources, B.I.; Data Curation, B.I., M.F.Y.; Writing – Original Draft Preparation, B.I., M.F.Y., S.K..; Writing – Review & Editing, B.I., M.F.Y., S.K., M.A.K.; Visualization, B.I., S.K., M.F.Y.; Supervision, K.E., E.B.; Project Administration, B.I., M.F.Y.; Funding Acquisition, B.I., M.F.Y., K.E.

Conflicts of interest

No conflict of interest was declared by the authors.

Data availability

The data that support the findings of this study are available from corresponding author, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. However, data are available from the authors upon reasonable request.

Ethics approval

The information and consent interviews were conducted with the participants, their trainers, and club managers. The study was conducted in accordance with the Declaration of Helsinki and under the approved protocol by Meram Medical Faculty, Ethics Committee of non-Pharmaceuticals and non-Medical Device Researches of Necmettin Erbakan University with the number 2018/1312. Participants were informed about the study's method and potential risks, and informed consent was obtained.

References

1. Bridge CA, Jones MA, Drust B. Physiological responses and perceived exertion during international taekwondo competition. *Int J Sports Physiol Perform*. 2009;4:485–493.
2. Bridge CA, Ferreira da Silva Santos J, Chaabene H, Pieter W, Franchini E. Physical and physiological profiles of taekwondo athletes. *Sports Med*. 2014;44:713–733.
3. Melhim AF. Aerobic and anaerobic power responses to the practice of taekwon-do. *Br J Sports Med*. 2001;35:231–234.
4. Mitchell JH, Haskell W, Snell P, Van Camp SP. Task Force 8: classification of sports. *J Am Coll Cardiol*. 2005;45(8):1364–1367.
5. Herrera-Valenzuela T, Zapata-Bastias J, Guajardo-Medrano M, et al. Can simulation tasks reproduce the taekwondo match physiological responses? *Arch Budo*. 2018;14:25–31.
6. Chaabene H, Negra Y, Bouguezzi R, et al. Physical and Physiological Attributes of Wrestlers: An Update. *J Strength Cond Res*. 2017;31(5):1411–1442.
7. Buchheit M, Laursen PB. High-intensity interval training, solutions to the programming puzzle: Part I: cardiopulmonary emphasis. *Sports Med*. 2013;43(5):313–338.
8. Buchheit M, Laursen PB. High-intensity interval training, solutions to the programming puzzle. Part II: anaerobic energy, neuromuscular load and practical applications. *Sports Med*. 2013;43(10):927–954.
9. Slimani M, Chaabe`ne H, Davis P, Franchini E, Cheour F, Chamari, K. Performance aspects and physiological responses in male amateur boxing competitions: A brief review. *J Strength Cond Res*. 2017; 31:1132–1141.
10. World Taekwondo. Competition rules & interpretation. http://m.worldtaekwondo.org/rules-wt/rules.html/. Published May 21, 2018. Updated May 14, 2019. Accessed May 1, 2022.
11. Davis P, Wittekind A, Beneke R. Amateur boxing: activity profile of winners and losers. *Int J Sports Physiol Perform*. 2013;8(1):84–91.
12. Özen G, Atar Ö, Yurdakul HÖ, Pehlivan B, Koç H. The effect of pre-season football training on hematological parameters of well-trained young male football players. *Pedagogy of Physical Culture and Sports*. 2020;24(6):303–309.
13. Duzova H, Karakoc Y, Gullu E, Gullu A, Koksal B, Esen B. The acute effects of single football match on whole blood viscosity and hematological variables in female soccer players. *Biomedical Research*. 2016;27(4):1423–1425.
14. Kantyka J, Herman D, Roczniok RL. Effects of aqua aerobics on body composition, body mass, lipid profile, and blood count in middle-aged sedentary women. *Human Movement*. 2015;16(1):9–14.
15. Erdoğdu M, Yüksel MF, Işık B, Boyalı E, Erdağı K, Sevindi T. The effects of acute moderate intensity training on hematological parameters in elite para-badminton athletes. *J Mens Health*. 2021;18(2):1–8.
16. Spiropoulos K, Trakada G. Hematologic and biochemical laboratory parameters before and after a marathon race. *Lung*. 2003;181(2):89–95.
17. Hammami N, Hattabi S, Salhi A, Rezgui T, Oueslati M, Bouassida A. Combat sport injuries profile: a review. *Sci. Sports*. 2018;33:73–79.
18. Chazaud B. Inflammation during skeletal muscle regeneration and tissue remodeling: application to exercise-induced muscle damage management. *Immunol Cell Biol*. 2016;94:140–145.
19. Bush JA, Kraemer WJ, Mastro AM, Triplett-McBride NT, Volek JS, Putukian M, et al. Exercise and recovery responses of adrenal medullary neurohormones to heavy resistance exercise. *Med Sci Sports Exerc*. 1999;31(4):554–559.

20. Chen CY, Liao YH, Chou CC, Sung YC, Tsai SC. Initial systemic inflammatory state perturbs exercise training adaptations in elite taekwondo athletes. *PLoS One.* 2017;12:e0176140.
21. Kazemi M, Casella C, Perri G. 2004. Olympic taekwondo athlete profile. *J Can Chiropr Assoc*. 2009; 53(2):144–152.
22. Dill DB, Costill DL. Calculation of percentage changes in vol-umes of blood, plasma, and red cells in hydration. *J Appl Physiol.* 1974;37(2):247–248.
23. Franchini E, Cormack S, Takito MY. Effects of High-Intensity Interval Training on Olympic Combat Sports Athletes' Performance and Physiological Adaptation: A Systematic Review. *J Strength Cond Res.* 2019;33(1):242–252.
24. Kim J, Lee N, Trilk J, et al. Effects of sprint interval training on elite Judoists. *Int J Sports Med*. 2011;32(12):929–934.
25. Lee N, Kim J, Hyung GA, et al. Training Effects on Immune Function in Judoists. *Asian J Sports Med.* 2015;6(3):e24050.
26. Monks L, Seo MW, Kim HB, Jung HC, Song JK. High-intensity interval training and athletic performance in Taekwondo athletes. *J Sports Med Phys Fitness.* 2017;57(10):1252–1260.
27. Boyalı E, Sevindi T, Yüksel MF, Demir H. The effects of preparation period exercises on the hematological parameters of the taekwondo athletes. *Physical Education of Students*. 2019;23:9–15.
28. Çakmakçı E. Effects of camp term on some hematological parameters in male taekwondoers. *Nigde University Journal of Physical Education and Sport Sciences*. 2009;3(1):21–29.
29. Erdağı K, Yüksel MF, Sevindi T. The effects of maximal power training on hematologic parameters of female weight-lifters. *The Journal of Turkish Sport Sciences*. 2020;24(6):41–48.
30. Wardyn GG, Rennard SI, Brusnahan SK, et al. Effects of exercise on hematological parameters, circulating side population cells, and cytokines. *Exp Hematol.* 2008;36(2):216–223.
31. Lippi G, Salvagno GL, Danese E, Tarperi C, Guidi GC, Schena F. Variation of Red Blood Cell Distribution Width and Mean Platelet Volume after Moderate Endurance Exercise. *Advances in Hematology*. 2014;2014:1–4.
32. Demiriz M, Erdemir İ, Kayhan RF. Effects of anaerobic interval training with different rest interval on aerobic capacity, anaerobic threshold and blood parameters. *Int. J of Sport Exer and Train Sci*. 2015;1(1):1–8.
33. Belviranli M, Okudan N, Kabak B. The Effects of Acute High-Intensity Interval Training on Hematological Parameters in Sedentary Subjects. *Med Sci (Basel).* 2017;19;5(3):15.
34. Chou CC, Sung YC, Davison G, Chen CY, Liao YH. Short-term high-dose vitamin C and E supplementation attenuates muscle damage and inflammatory responses to repeated taekwondo competitions: a randomized placebo-controlled trial. *Int J Med Sci.* 2018;15:1217–1226.
35. Peake JM, Neubauer O, Walsh NP, Simpson RJ. Recovery of the immune system after exercise. *J Appl Physiol* (1985). 2017;122(5):1077–1087.
36. Chuang SJ, Sung YC, Chen CY, Liao YH, Chou CC. Can Match-Mimicking Intermittent Practice Be Used as a Simulatory Training Mode of Competition Using Olympic Time Frame in Elite Taekwondo Athletes? *Front Physiol.* 2019;10:244.
37. Liao YH, Sung YC, Chou CC, Chen CY. Eight-week training cessation suppresses physiological stres but rapidly impairs health metabolic profiles and aerobic capacity in elite Taekwondo athletes. *PloS one.* 2016;11(7): e0160167.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.9

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 316–322

ORIGINAL PAPER

Correlation of the end-tidal CO_2 value with arterial blood gas parameters – evaluation of the treatment efficacy of COPD exacerbation in the emergency department

Fatma Çakmak [1], Erdal Tekin [2]

[1] Department of Emergency Medicine, Health Sciences University, Erzurum Regional Training and Research Hospital, Erzurum, Turkey
[2] Department of Emergency Medicine, Ataturk University Faculty of Medicine, Erzurum, Turkey

ABSTRACT

Introduction and aim. Painful, invasive, and expensive arterial blood gas (ABG) analysis is required in the diagnosis, follow-up, treatment, and even discharge of patients with chronic obstructive pulmonary disease (COPD). This study aimed to compare the end-tidal carbon dioxide ($ETCO_2$) value, which allows non-invasive, painless, low-cost, and continuous monitoring, with ABG parameters, in the evaluation of the treatment efficacy of COPD exacerbation.

Material and methods. The study was prospectively conducted with patients who presented to the emergency department with COPD exacerbation. ABG analysis and $ETCO_2$ measurement were simultaneously performed in patients with COPD exacerbation at the time of arrival and after treatment, and were statistically compared.

Results. The study included a total of 216 patients, of whom 57.4% were male. The mean age of the patients was 67.3±13.9 years. The $ETCO_2$ values of the patients at arrival and after COPD exacerbation treatment were 39.2±10 and 37.3±9, respectively, and a statistically significant difference was determined (p=0.001). The partial pressure of pCO_2 values measured at arrival and after treatment were 40.85±10.54 and 38.74±9.25, respectively, and it was statistically significant (p=0.001). A strong positive and statistically significant correlation was found between the $ETCO_2$ and pCO_2 values both at arrival and after COPD exacerbation treatment (r=0.840; p<0.001 and r=0.872; p<0.001, respectively). The Bland-Altman plot was constructed for the agreement between $ETCO_2$ and pCO_2 at both evaluation times.

Conclusion. $ETCO_2$ measurement could accurately predict the pCO_2 of patients with COPD at arrival and after COPD exacerbation treatment. Also, ETCO2 may be useful in cases where pCO_2 cannot be used.

Keywords. arterial blood gas, capnograph, chronic obstructive pulmonary disease, end tidal carbon dioxide, partial pressure of carbon dioxide

Introduction

Chronic obstructive pulmonary disease (COPD) is a leading public health problem worldwide. Although COPD is a slowly progressive, irreversible disease, it is important because of its preventable nature and the possibility of stopping of the progression.[1-3] However, COPD exacerbations adversely affect the patient's health status, hospitalization, and length of stay.[4] In these patients, arterial blood gas (ABG) measurement is one of the valuable tests used to determine the patient's oxygenation, ventilation, and acid-base status during and after an exacerbation. However, ABG is an invasive and

Corresponding author: Erdal Tekin, e-mail: dret25@gmail.com

Received: 23.05.2022 / Revised: 19.06.2022 / Accepted: 21.06.2022 / Published: 30.09.2022

Çakmak F, Tekin E. *Correlation of the end-tidal CO_2 value with arterial blood gas parameters – evaluation of the treatment efficacy of COPD exacerbation in the emergency department.* Eur J Clin Exp Med. 2022;20(3):316–322. doi: 10.15584/ejcem.2022.3.9.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

painful procedure and may need to be repeated in certain cases. Therefore, alternative methods have been used in the evaluation of diagnosis, and follow-up of these patients.[5,6] One of these alternative methods is the measurement of end–tidal carbon dioxide ($ETCO_2$).

$ETCO_2$ is determined by non-invasively with a capnometer by measuring the partial pressure of expiratory carbon dioxide from the air inhaled and exhaled during respiration.[7] This parameter is generally used in the emergency department (ED) to confirm the location of the endotracheal tube, monitor spontaneous return during cardiopulmonary resuscitation in patients with cardiac arrest, and monitor ventilation status in procedural sedoanalgesia.[8,9] In addition, it can provide instant information concerning the metabolic status, perfusion, and most importantly ventilation of critically ill patients in the ED.[4] Studies have emphasized that $ETCO_2$ can be used to evaluate the severity of obstructive airway disease in the ED.[7,10]

Aim

In this study, we aimed to compare the $ETCO_2$ value measured simultaneously with the ABG in patients who came to the ED with COPD exacerbation, at the time of admission and after COPD exacerbation treatment was applied.

Material and methods

Study design

This single-center, prospective clinical study was conducted with patients who presented to the ED with COPD exacerbation, between June 27th, 2019 and December 27th, 2019. The tertiary university hospital, where the study was carried out, provides health services to approximately 4.5 million people in 12 neighboring provinces. The study protocol was in accordance with the Declaration of Helsinki and Good Clinical Practices. Informed consent was obtained from all patients participating in the study. Local ethics committee approval was obtained for the study (decision number: 27.06.2019/05-28).

Patient selection

The eligible participants and sample population were the patients who were admitted to the ED with COPD exacerbations during the study period. Patients with shortness of breath due to reasons other than COPD exacerbation, those younger than 18 years, those with contraindications to ABG sampling (such as coagulopathy, local infection, and thrombus), those that did not provide consent for participation in the study, pregnant patients, intubated patients or patients that required intubation during their follow-up, patients who could not tolerate capnometry for the $ETCO_2$ measurement, those with anatomical disabilities, and non-compliant patients were excluded from the study.

ABG sampling

In the ABG sampling procedure, after the radial artery pulse of the patient was palpated, the most suitable site for the intervention was determined and cleaned with 10% povidone iodine. Then, arterial puncture was performed by the blood gas determination set containing lithium heparin. All interventions in the ED were undertaken by the same physician. The ADL 800 Flex device was used for the ABG analyses. The blood gas analyzer was calibrated according to the manufacturer's recommendations.

Study protocol

Patients presenting to the ED and included in the study were asked to breathe through a nasal cannula connected to a capnometer for at least 30 seconds, during which ABG samples were obtained. The $ETCO_2$ value was simultaneously recorded with ABG parameters. The $ETCO_2$ measurement was performed non-invasively by the same physician using a capnograph/pulse oximeter device, Capnostream®20p portable bedside monitor. To make an accurate measurement, the patient was monitored for at least 6 minutes (240 seconds) with a capnograph/pulse oximeter.[11] During the procedure, SpO_2, rhythm (rate and rhythm) and $ETCO_2$ were monitored and respiratory rate were recorded. Then, COPD exacerbation treatment was applied to the patients in line with the guidelines of the Global Initiative on Chronic Obstructive Pulmonary Disease.[12] After the initial evaluation and exacerbation treatment, the patients were asked to breathe again with a capnometer, and the $ETCO_2$ value, respiratory rate, body temperature, heart rate, arterial blood pressure, and oxygen saturation values were recorded again simultaneously with the ABG parameters.

Treatment efficacy

The efficacy of treatment was evaluated by comparing vital signs, ABG parameters, and $ETCO_2$ measured at the time of arrival at the ED and after COPD exacerbation treatment.

Outcome measures

The primary outcome of the study was the correlation between ABG parameters and $ETCO_2$, which were obtained at the time of arrival at the ED and after COPD exacerbation treatment. The secondary outcome was the treatment efficacy evaluated by comparing the pre-treatment and post-treatment vital signs, ABG parameters, and $ETCO_2$.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Science (SPSS), Version 20.0 (IBM Corp., Armonk, New York, USA). Data were

presented as mean, standard deviation, percentage and frequency. The normality of the distribution of continuous variables was analyzed by using the Shapiro-Wilk test. In the comparisons of two dependent groups, the paired-samples t-test was used for the normally distributed data, and the Wilcoxon test for data without a normal distribution. In the comparison of two continuous variables, the Pearson correlation test was conducted if the normal distribution was determined, and the Spearman correlation test otherwise. The agreement between the $ETCO_2$ value and ABG parameters was evaluated by using the Bland-Altman method. The statistical significance level was taken as $p<0.05$.

Results

Patient population and characteristics

During the study, a total of 556 patients were presented to the ED due to COPD exacerbation. After applying the inclusion and exclusion criteria, a total of 216 patients were included in the study. The flow chart of the study is given in Figure 1.

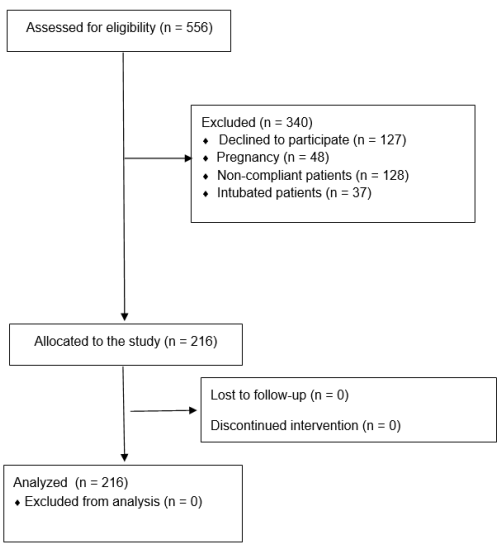

Fig. 1. Flow diagram of the study

Table 1 presents the demographic and clinical features of the patients. The mean age of the patients was 67.3±13.9 years, and 124 (57.4%) were male. All of the patients included in the study were COPD patients. The second most common disease detected in the participants was hypertension (n=107, 49.5%).

Comparison of pre-treatment and post-treatment evaluations

Figure 2 presents the comparison of the clinical features, $ETCO_2$ values and ABG parameters of the patients at the time of arrival and after COPD exacerbation treatment. There was a statistical significance between the pre- and post-treatment vital signs, $ETCO_2$ values and ABG parameters ($p<0.05$).

Table 1. Demographic and clinical characteristics of the patients (n=216) [a]

Variable	Value
Mean age (years)	67.3 ± 13.9
Gender	
Male, n (%)	124 (57.4%)
Female, n (%)	92 (42.6%)
Condenser use, n (%)	110 (50.9%)
Smokers, n (%)	191 (88.4%)
Smoking, pack-day	1.2 ± 0.1
Clinical characteristics	
Respiratory rate (breaths/min)	25.1 ± 7.2
Body temperature (°C)	36.9 ± 0.3
Heart rate (beats/min)	99.8 ± 19.9
Systolic arterial blood pressure (mmHg)	139.9 ± 19.9
Diastolic arterial blood pressure (mmHg)	84.2 ± 12.3
Oxygen saturation (%)	80.1 ± 10.6
$ETCO_2$	39.2 ± 10
Chronic diseases other than COPD	
Hypertension, n (%)	107 (49.5%)
Diabetes mellitus, n (%)	69 (31.9%)
Congestive heart failure, n (%)	56 (25.9%)
Other, n (%)	43 (19.9%)

[a] COPD – chronic obstructive pulmonary disease, $ETCO_2$ – end-tidal carbon dioxide

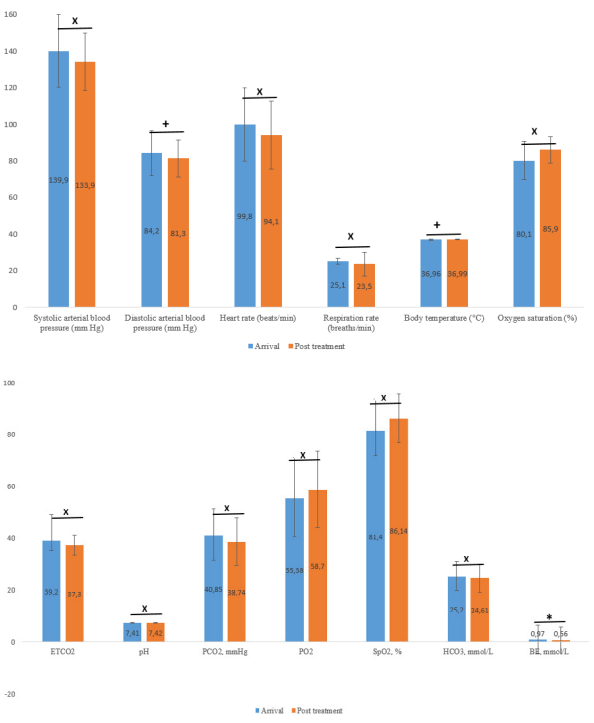

Fig. 2. Comparison of the patients' vital signs, $ETCO_2$ values and ABG parameters measured at arrival and after treatment (X: p=0.001, +: p=0.002, ++: p=0.042, *: p=0.015)

The relationship between the $ETCO_2$ value and ABG parameters measured at arrival is shown in Figure 3. There was a positive, strong and statistically significant correlation between $ETCO_2$ and pCO_2 ($r=0.840$, $p<0.001$).

Figure 4 shows the relationship between the $ETCO_2$ values and ABG parameters measured after COPD exacerbation treatment. Likewise, a positive, strong and statistically significant correlation was found between $ETCO_2$ and pCO_2 (r=0.872, p<0.001).

According to the Bland-Altman analysis, the mean ± standard deviation of the difference between the pCO_2 and $ETCO_2$ values measured at the time of arrival was 1.64±5.73 [95% confidence interval (CI): 0.87-2.41], and the upper and lower limits were determined as 12.87 and -9.59, respectively (Figure 5a). In the Bland-Altman analysis of the difference between the post-treatment pCO_2 and $ETCO_2$, the mean ± standard deviation was found to be 1.45±4.41 (95% CI: 0.86–2.04), and the upper and lower limits were 10.09 and -7.19, respectively (Figure 5b).

Discussion

In this study, the $ETCO_2$ value was compared with ABG parameters in patients applied with COPD exacerbation, at the time of arrival and after the treatment. In order to determine the efficacy of COPD exacerbation treatment, the vital signs and ABG parameters of the patients were examined before and after treatment, and the differences were found to be statistically significant. When the correlation of the $ETCO_2$ value with the ABG parameters was evaluated, $ETCO_2$ had a strong correlation with pCO_2 both at arrival and after treatment, and had a moderately positive and statistically significant correlation with HCO_3. In addition, there was a clear agreement between $ETCO_2$ and pCO_2 both before and after treatment according to the Bland-Altman graph.

ABG evaluation is of great importance in the hospitalization and discharge decisions after the treatment of patients with COPD exacerbation. The ABG analysis is an invasive and painful procedure that may be accompanied by possible complications.[13] Therefore, the analysis of ABG is difficult in some cases.[14] During the study, it was also observed that patients did not want to give an ABG sample for the second time.

Capnography, which is a non-invasive, painless and uncomplicated method, provides instant information concerning the patient's status by measuring the $ETCO_2$ value and respiratory rate in patients with acute respiratory problems, such as COPD exacerbations.[15] In the literature, comparisons have been made between $ETCO_2$ and pCO_2 in the evaluation of respiratory status of the patients who have undergone sedation and general anesthesia, except for COPD cases, and the results have been reported to be statistically significantly.[8,16-18] In the current study, it was shown that the $ETCO_2$ value could be used in the follow-up of patients with COPD and in the evaluation of the efficacy of COPD exacerbation treatment. Dogan et al., who investigated the accuracy of the $ETCO_2$ level in the prediction of the severity of COPD exacerbation in the ED, found the presence of a relationship between $ETCO_2$ and pCO_2, and pO_2, similar to the results of our study.[10]

Cinar et al. investigated whether there was a difference between the ABG pCO_2 value and the $ETCO_2$ value in patients applied to the ED due to acute dyspnea, and reported a positive, strong and statistically significant correlation between these two parameters (r=0.911; p<0.001).[19] The authors also emphasized that

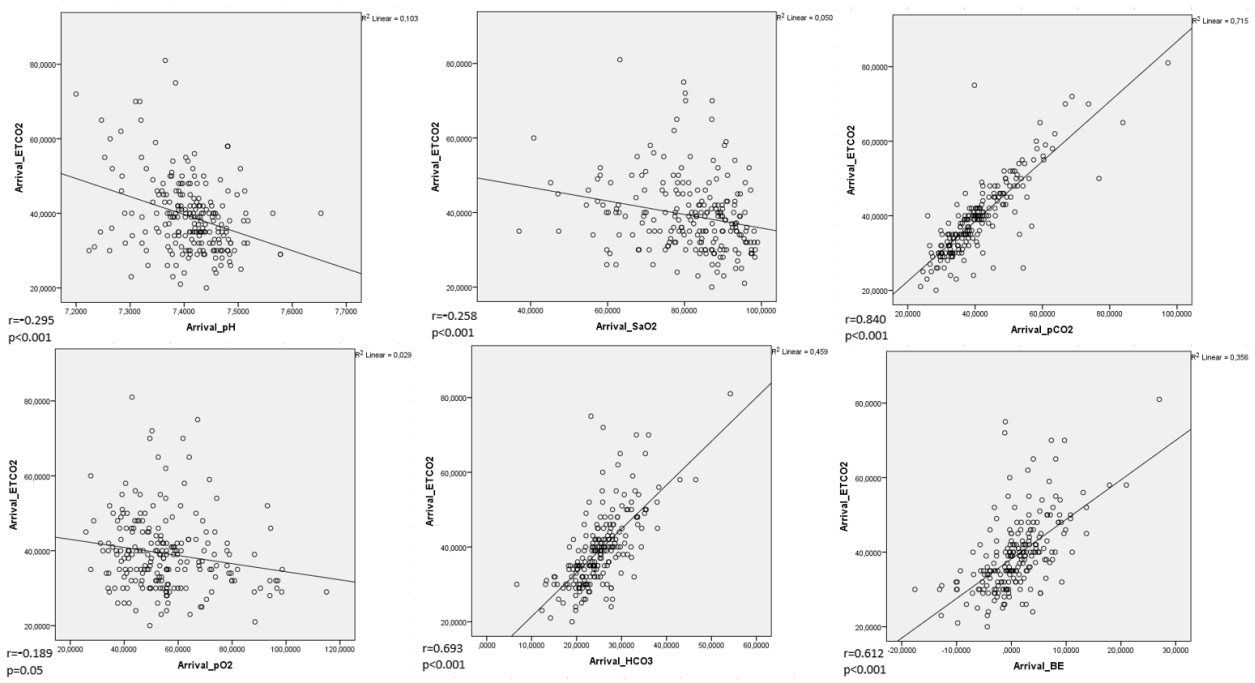

Fig. 3. Correlation of the patients' $ETCO_2$ values with arterial blood gas parameters at arrival

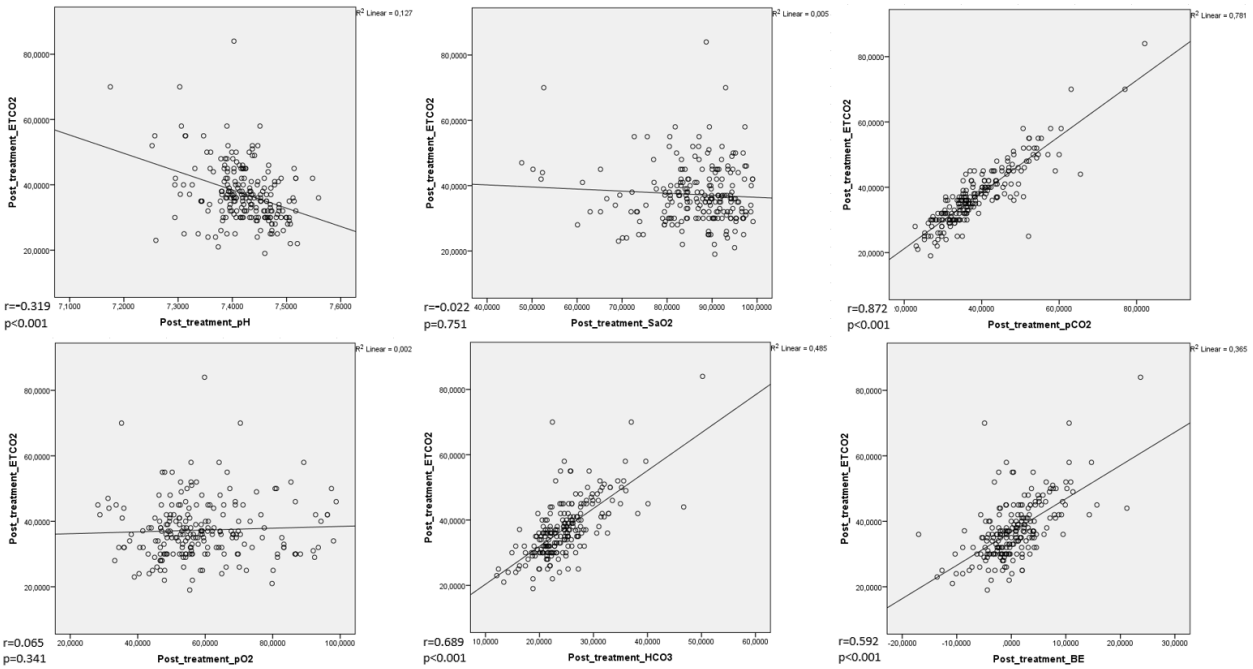

Fig. 4. Correlation of the patients' post-treatment $ETCO_2$ values with arterial blood gas parameters

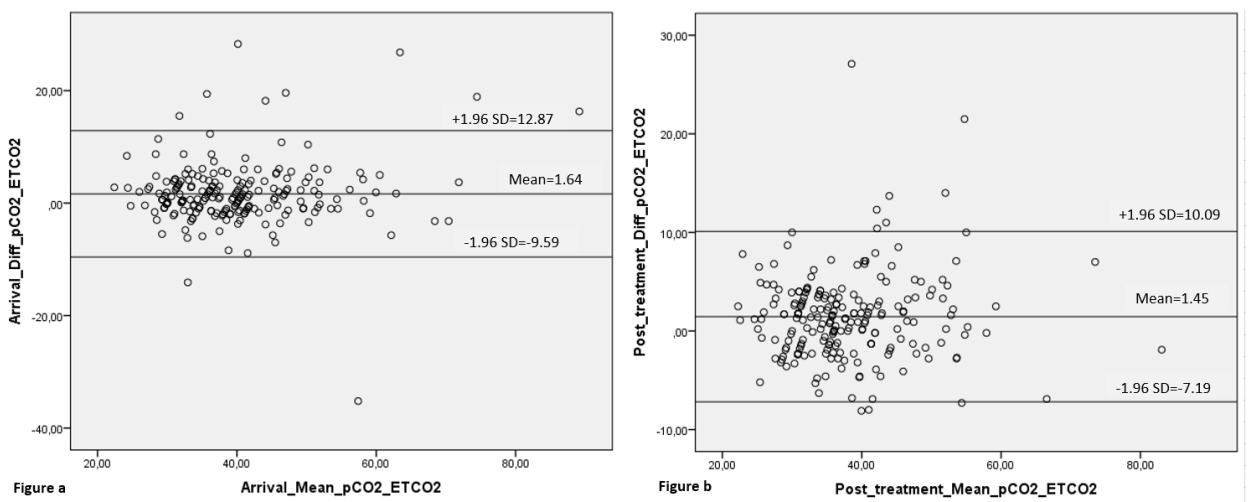

Fig. 5. Bland-Altman graph (a. agreement between $ETCO_2$ and pCO_2 at arrival, b. agreement between $ETCO_2$ and pCO_2 after COPD exacerbation treatment)

$ETCO_2$ could accurately predict the pCO_2 value of patients presenting to the ED with acute dyspnea. In another study conducted with adult asthmatic patients with acute exacerbation, Corbo et al. found a significant correlation between pCO_2 in blood gas and $ETCO_2$ measured by capnography.[20] Kartal et al. investigated the value of $ETCO_2$ measurement for patients with COPD in the ED and concluded that there was a moderate correlation between the $ETCO_2$ and pCO_2 levels, but unlike other studies, $ETCO_2$ measurement was not sufficient to predict the pCO_2 level in this patient group.[4] Similarly, Pekdemir et al. reported that the $ETCO_2$ value was significantly lower than the pCO_2 value and that there was a weak correlation between these two parameters.[21] In a review of $ETCO_2$, it was emphasized that, $ETCO_2$ was an important tool in evaluating the severity of obstructive airway disease.[7] In acute respiratory problems, ETCO2 can detect airway obstruction and hypoventilation at an early stage. Thus, they are able to provide earlier medical intervention to these patients.

Taghizadieh et al. compared $ETCO_2$ with bicarbonate (HCO_3), an ABG parameter, and emphasized that, although $ETCO_2$ could be used in the primary diagnosis of metabolic acidosis, ABG was still the gold standard for guiding diagnosis and treatment.[22] However, Kartal et al. suggested that the $ETCO_2$ level could indicate the severity of metabolic acidosis and mortality.[4] In another study comparing $ETCO_2$ with HCO_3 in pa-

tients with COPD exacerbation, Taghizadieh et al. detected no statistically significant difference between the $ETCO_2$ and HCO_3 levels (r=0.04; p=0.136).[23] In the same study, the authors found a low negative correlation between $ETCO_2$ and pH (r=-0.249; p<0.001), and a moderate positive correlation between $ETCO_2$ and pCO_2 (r=0.611; p<0.001). In the current study, the $ETCO_2$ and HCO_3 levels of the patients were positively correlated both at arrival and after treatment, and this correlation was statistically significant. In addition, we observed a low level of negative correlation between $ETCO_2$ and pH both at arrival and after treatment. According to the data obtained from our study, there was a strong positive correlation, similar distribution and agreement between the values of $ETCO_2$ and PCO_2 measured at the time of arrival. More importantly, this correlation was even stronger in the post-treatment $ETCO_2$ and PCO_2 measurements of the patients, showing less bias and lower difference, and a much better agreement.

Limitations

The first limitation of the study was that it was conducted in a single center. Secondly, patients with concomitant metabolic diseases, including diabetes mellitus and acute renal failure, which affect metabolic parameters, such as pH and HCO_3, were not excluded from the sample. Since real-world COPD patients may have some other comorbidities, and our study aimed to study all COPD patients, patients with or without certain concomitant metabolic diseases, such as diabetes or acute renal failure, were not excluded. Subsequently, factors such as cardiomegaly and elevated pulmonary artery pressure that could affect the correlation between pCO_2 and $ETCO_2$ were not evaluated. Also, body temperature, which may affect the pCO_2 level, was not taken into consideration. Lastly, the cigarette consumption of the patients was calculated as package per day. This, in turn, does not report how much COPD patients actually consume package cigarettes per year.

Conclusion

It was determined that $ETCO_2$ measurement which was non-invasive, painless, repeatable and low-cost capnography procedure may provide information about the ventilation, circulation and metabolism of patients during COPD exacerbation at the time of arrival and after treatment. Considering the similar and strongly related properties of $ETCO_2$ to PCO_2 and HCO_3, we believe that it may be used during the follow-up of patients.

Declarations

Funding

The authors declared that this study has received no financial support or any funding.

Author contributions

Conceptualization, F.Ç. and E.T.; Methodology, F.Ç. and E.T.; Software, F.Ç.; Validation, F.Ç. and E.T.; Formal Analysis, F.Ç. and E.T.; Investigation, F.Ç.; Resources, F.Ç.; Data Curation, F.Ç. and E.T.; Writing – Original Draft Preparation, F.Ç. and E.T.; Writing – Review & Editing, F.Ç. and E.T.; Visualization, F.Ç.; Supervision, E.T.

Conflicts of interest

The authors have no conflict of interest to declare.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Ethics approval

Local ethics committee approval was obtained from the Ethics Committee of Faculty of Medicine, University of Ataturk, Erzurum, Turkey, with the decision number of 27.06.2019/05-28.

References

1. Stanford RH, Korrer S, Brekke L, Reinsch T, Bengtson LG. Validation and assessment of the COPD treatment ratio as a predictor of severe Exacerbations. *Chronic Obstr Pulm Dis*. 2020;7(1):38–48.
2. Vogelmeier CF, Román-Rodríguez M, Singh D, Han MK, Rodríguez-Roisin R, Ferguson GT. Goals of COPD treatment: Focus on symptoms and exacerbations. *Respir Med*. 2020;166:105938.
3. Arslan S, Delice O, Katran ZY. The predictive value of qSOFA and CURB-65 scores in predicting the hospitalization need and mortality in patients with acute exacerbation of chronic obstructive pulmonary disease. *Ital J Emerg Med*. 2020;9(3);169–177.
4. Kartal M, Goksu E, Eray O, et al. The value of ETCO2 measurement for COPD patients in the emergency department. *Eur J Emerg Med*. 2011;18(1):9–12.
5. Mummery V, Rogers E, Padmanaban V, Matthew D, Woodcock T, Bloch S. Transcutaneous carbon dioxide measurement is not a reliable alternative to arterial blood gas sampling in the acute medical setting. *Eur Respir J*. 2019;53(4):1801726.
6. Huttmann SE, Windisch W, Storre JH. Techniques for the measurement and monitoring of carbon dioxide in the blood. *Ann Am Thorac Soc*. 2014;11(4):645–652.
7. Aminiahidashti H, Shafiee S, Zamani Kiasari A, Sazgar M. Applications of End-Tidal Carbon Dioxide (ETCO2) Monitoring in Emergency Department; a Narrative Review. *Emerg (Tehran)*. 2018;6(1):e5.
8. Long B, Koyfman A, Vivirito MA. Capnography in the Emergency Department: A Review of Uses, Waveforms, and Limitations. *J Emerg Med*. 2017;53(6):829–842.

9. Cereceda-Sánchez FJ, Molina-Mula J. Capnography as instrument for airway management during basic instrumental CPR manoeuvers. *Resuscitation.* 2019;138:132–133.
10. Dogan NO, Sener A, Gunaydın GP, et al. The accuracy of mainstream end-tidal carbon dioxide levels to predict the severity of chronic obstructive pulmonary disease exacerbations presented to the ED. *Am J Emerg Med.* 2014;32(5):408–411.
11. Galia F, Brimioulle S, Bonnier F, et al. Use of Maximum End-Tidal CO2 Values to Improve End-Tidal CO2 Monitoring Accuracy. *Respir Care.* 2011;56(3):278–283.
12. Global Strategy for Diagnosis, Management and Prevention of COPD. The Global Initiative for Chronic Obstructive Lung Diseases (GOLD). 2020 report. https://goldcopd.org/gold-reports/. Accessed September 24, 2021.
13. Tyagi D, Govindagoudar MB, Jakka S, Chandra S, Chaudhry D. Correlation of PaCO(2) and ETCO(2) in COPD Patients with Exacerbation on Mechanical Ventilation. *Indian J Crit Care Med.* 2021;25(3):305–309.
14. Ekström M, Engblom A, Ilic A, Holthius N, Nordström P, Vaara I. Calculated arterial blood gas values from a venous sample and pulse oximetry: Clinical validation. *PLoS One.* 2019;14(4):e0215413.
15. Schwarz SB, Windisch W, Magnet FS, et al. Continuous non-invasive PCO2 monitoring in weaning patients: Transcutaneous is advantageous over end-tidal PCO2. *Respirology.* 2017;22(8):1579–1584.
16. Bhavani-Shankar K, Philip JH. Defining segments and phases of a time capnogram. *Anesth Analg.* 2000;91(4):973–977.
17. Onodi C, Bühler PK, Thomas J, Schmitz A, Weiss M. Arterial to end-tidal carbon dioxide difference in children undergoing mechanical ventilation of the lungs during general anaesthesia. *Anaesthesia.* 2017;72(11):1357–1364.
18. Kummer DJ, Walden BJ. Capnography Monitoring for Patients Undergoing Moderate Sedation: An SGNA Fellowship Project. *Gastroenterology Nursing.* 2019;42(1):49–54.
19. Cinar O, Acar YA, Arziman İ, Kilic E, Eyi YE, Ocal R. Can mainstream end-tidal carbon dioxide measurement accurately predict the arterial carbon dioxide level of patients with acute dyspnea in ED. *Am J Emerg Med.* 2012;30(2):358–361.
20. Corbo J, Bijur P, Lahn M, Gallagher EJ. Concordance between capnography and arterial blood gas measurements of carbon dioxide in acute asthma. *Ann Emerg Med.* 2005;46(4):323–327.
21. Pekdemir M, Cinar O, Yilmaz S, Yaka E, Yuksel M. Disparity between mainstream and sidestream end-tidal carbon dioxide values and arterial carbon dioxide levels. *Respir Care.* 2013;58(7):1152–1156.
22. Taghizadieh A, Pouraghaei M, Moharamzadeh P, Ala A, Rahmani F, Basiri Sofiani K. Comparison of end-tidal carbon dioxide and arterial blood bicarbonate levels in patients with metabolic acidosis referred to emergency medicine. *J Cardiovasc Thorac Res.* 2016;8(3):98–101.
23. Taghizadieh A, Rahmani F, Soleimanpour H, Aminifazl H. Comparison of end tidal carbon dioxide and arterial blood bicarbonate levels in patients with exacerbation chronic obstructive pulmonary disease. *Thrita.* 2015;4(2):e26169.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.10

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 323–329

ORIGINAL PAPER

Circulatory and ventilatory power markers in patients with diabetes mellitus – influence of glycemic control

Letícia Menegalli Santos, Claudio Donisete da Silva, Laura Beatriz Lorevice, Clara Italiano Monteiro, Paula Angelica Ricci, Audrey Borghi-Silva, Renata Gonçalves Mendes

Cardiopulmonary Physiotherapy Laboratory, Department of Physical Therapy, Federal University of Sao Carlos, Sao Carlos, Sao Paulo, Brazil

ABSTRACT

Introduction and aim. Cardiorespiratory function has been shown to be impaired in individuals with type 2 diabetes mellitus (T2DM). Some deficiencies in cardiopulmonary exercise test (CPET)-derived variables are known, however, the influence of glycemic control on cardiovascular integrity indices as circulatory power (CP) and ventilatory power (VP), deserve to be instigated. The aim was to investigate the influence of glycemic control on CP and VP indices in T2DM.

Material and methods. T2DM individuals of both sexes aged between 40 and 64 years were allocated into two groups: Good glycemic control (GGC, n=11; HbA1c≤7%) and insufficient glycemic control (IGC, n=26; HbA1c>7%). All participants underwent a CPET on a treadmill using a gas analyzer and a laboratory blood test. CP values were obtained by the product of peak of oxygen uptake and systolic blood pressure (SBP) and VP by dividing SBP by the ventilatory efficiency (VE/VCO2 slope). The level of significance was set at $p<0.05$.

Results. No baseline differences were found between the groups, except for the expected fasting glucose and glycated hemoglobin. No differences were found between GGC and IGC groups for CP (4756.05±1061.67 and 4434.15±1247.83 mmHg.ml.kg^{-1}min^{-1}, p=0.460) and VP (5.85±1.08 and 5.86±1.31 mmHg, p=0.978), respectively.

Conclusion. CP and VP were similar in individuals with T2DM regardless of glycemic control. Predictive ability of these variables in health outcomes deserves to be further investigated in T2DM.

Keywords. cardiopulmonary exercise test, glycemic control, type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by persistent hyperglycemia, resulting from a deficiency in the production of insulin by the pancreas, in its action, or both mechanisms.[1] The number of people diagnosed with DM has increased in several countries. In 2019, the worldwide prevalence of DM was 463 million people, with a forecast of reaching 700 million in 2045.[1]

One aspect of health impairment in patients diagnosed with DM type 2 (T2DM), contributing to a higher risk of cardiovascular disease (CVD), is related to the inability to perform activities of daily living when compared to individuals without the disease[2] and low cardiorespiratory functional capacity, that can be accessed through oxygen uptake (VO2), as studies show a strong inverse association between cardiorespiratory function-

Corresponding author: Renata Gonçalves Mendes, e-mail: renatamendes@ufscar.br

Received: 1.06.2022 / Revised: 22.06.2022 / Accepted: 23.06.2022 / Published: 30.09.2022

Santos LM, da Silva CD, Lorevice LB et al. *Circulatory and ventilatory power markers in patients with diabetes mellitus – influence of glycemic control.* Eur J Clin Exp Med. 2022;20(3):323–329. doi: 10.15584/ejcem.2022.3.10.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

al capacity and diabetes, which is a significant risk factor for mortality in this population.[3-5]

Cardiopulmonary exercise test (CPET) is a non-invasive procedure aimed to assess patient's functional capacity being considered the gold standard for aerobic capacity or cardiorespiratory and metabolic performance assessment.[6] Peak oxygen consumption ($VO2_{PEAK}$) is the most representative parameter of cardiorespiratory physical fitness, whose strong association with aerobic physical performance certifies it as a tool for the prescription of aerobic physical training.[7] However, for some groups of patients, such as diabetic patients who have very low $VO2_{PEAK}$ ranges, this variable may lose its prognostic value a little, giving way to other important variables also obtained by CPET.

Circulatory power (CP) and ventilatory power (VP) has been demonstrated important marker of individual's cardiovascular integrity level.[8] According to the authors Forman et al, low CP values indicate a worse prognosis of the disease, while high VP values indicate a better prognosis.[9] CP assesses the central and peripheral components of cardiac work, being defined as a product of peak $VO2_{PEAK}$ and peak systolic blood pressure (SBP_{PEAK}) while the VP index combines the assessment of the hemodynamic system with ventilatory efficiency during exercise, being defined by the division of PAS_{PEAK} by VE/VCO2 slope representing the ventilatory efficiency for the production of carbon dioxide.[10,11] For some variables, there are already known and tabulated values, among the most used is the American Heart Association functional classification table published in 1972, which is based on the $VO2_{PEAK}$ obtained in a CPET. Regarding the variables that will be addressed in this study, Mezzani presents a table with CP normality values for healthy people, however, there are still no comparative values with the diabetic population.[12] Regarding VP, we did not find tables with normal values in the literature.

We can also consider glycemic control as an important factor for DM control. Adequate glycemic control delays the onset and progression of microvascular complications, in addition to reducing the risk of cardiovascular events by 42% and by 57% of non-fatal myocardial infarction, stroke, and death.[13] When intensified, glycemic control can prevent and/or delay the onset of chronic DM complications, and the glycemic index and glycemic load are useful factors to predict the glycemic response to foods.[14] Also, it is already know the impact of poor glycemic control in the aerobic capacity demonstrated by low VO2PEAK and workload achieved in CPET.[15]

In this way, glycated hemoglobin (HbA1c) reflects the average concentration of glucose in the blood in recent weeks, instead of the concentration of glucose in the blood at that moment representing the percentage of hemoglobin that is bound to glucose.[16] HbA1c levels above 7% indicate poor glycemic control and are associated with a progressively higher risk of chronic complications, hence the current concept of diabetes mellitus treatment defined by the Brazilian Diabetes Society (SBD) stipulated the value of 6.5% as the upper limit of the acceptable value for a patient with well-controlled DM.[17,18]

Aim

This study aimed to explore the influence of glycemic control on CP and VP indices in T2DM patients. We hypothesize that the worst glycemic profile would translate into a worse PC and VP.

Material and methods

This was a cross-sectional, observational study, followed by STROBE statement and was developed at the Laboratory of Cardiopulmonary Physiotherapy (LACAP) at the Federal University of São Carlos (UFSCar).

Selection of participants and ethical aspects

The study included individuals of both sexes with a previous diagnosis of T2DM[19] and aged between 40 and 64 years, residents in the city of São Carlos - São Paulo, Brazil, who were allocated into two groups: good glycemic control group (GGC) and insufficient glycemic control (IGC) according to value for dividing the groups at 7% HbA1c.[17] The study did not include smokers, alcoholics, or illicit drug users, participants who presented: changes in the electrocardiogram [ischemia, overloads, severe arrhythmias (such as ventricular tachycardia) and conduction disorders], both at rest and during the clinical physical exercise test, participants with neurological and orthopedic disorders, participants who did not have sufficient level of understanding to understand the routine of the protocols.

The recruitment of participants was carried out through dissemination in electronic and printed media, and patients registered in the Basic Health Units. After identifying the eligible participants, they were invited to participate in the study and after their acceptance, they performed all the assessments described below.

Participants were also informed and oriented about the procedures they would be submitted to, and the methods used in this study and the non-invasive nature of the experiments. Information was also provided to participants about the confidentiality of data collected during the study and about the preservation of their identities. After clarifying all the doubts raised by the participants and freely accepting to participate in the research, all signed an informed consent form, following the norms of the National Health Council (resolution 466/2012).

Sample characterization

All participants underwent an anamnesis to obtain personal data such as full name, address, age, body mass, height, daily life, and eating habits. Participants were also asked about medications in use, family history, and history.

A laboratory blood tests were performed at Medical Laboratory Dr. Maricondi Sao Carlos – Brazil, Biochemical analyses, including fasting serum lipids (total cholesterol and fractions and triglyceride), glucose profile (glycemia and fasting insulin Homeostasis Model Assessment index – HOMA and HbA1c and complete blood count. Samples were drawn between 7:00 a.m. and 10:00 a.m. and participants were instructed to fast for 8 to 12 hours.

Cardiopulmonary exercise test (CPET)

CPET was performed in the presence of a cardiologist, in order to assess the aerobic capacity of the participants as well as to obtain the variables of interest for this study ($VO2_{PEAK}$ and VE/VCO2 slope) in addition, systolic blood pressure was monitored by the auscultatory method using a sphygmomanometer (Tycos/Bic, Brazil) and a stethoscope (Littmann® Classic III, Brazil). CPET, considered the gold standard for aerobic capacity assessment, was performed on a treadmill (Super ATL, Porto Alegre, Rio Grande do Sul, Brazil) applying the Bruce steps incrementally. There was an increase in speed and inclination every 3 minutes. To analyze the ergospirometric variables, an Oxycon Mobile® gas analyzer (Mijnhardt/Jäger, Würzburg, Germany) was used. Volunteers were encouraged to perform the test until exhaustion and the criteria for test interruption were those described by Balady.[20]

Operationalization of variables

During CPET, the following variables were collected for analysis: $VO2_{PEAK}$, peak carbon dioxide production ($VCO2_{PEAK}$), respiratory exchange rate (RER) were defined as the mean of the last 30 seconds of exercise.[21] Blood pressure was measured at the end of each stage of the test and at the time of peak exercise. The VE/VCO2 slope was calculated from the beginning to the peak of the exercise and the value considered to be VE/VCO2 slope was obtained through linear regression between these variables. CP values were obtained by the product of $VO2_{PEAK}$ by SBP_{PEAK}[22] and VP by dividing the SBP_{PEAK} values by the VE/VCO2 slope[10] (Fig. 1).

Statistical analysis

For statistical analysis, the Sigma Plot 11.0 software will be used (Systat, USA, 2011). The normality of data distribution will be verified by the Shapiro-Wilk test. For comparison between groups according to population characteristics, laboratory tests, and CP and VP indices obtained in CPET, t-test will be used for data with normal distribution and Mann-Whitney test for non-normal distribution. Data are presented as mean ± standard deviation for data with normal distribution and median and interquartile range for data with non-normal distribution.

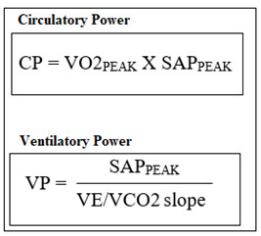

Fig. 1. Circulatory and ventilatory power equation[a]

[a] CP – circulatory power, SAP_{PEAK} – peak of systolic arterial pressure, VE/VCO2 slope – relationship between minute ventilation and carbon dioxide production, $VO2_{PEAK}$ – oxygen uptake, VP – ventilatory power

To assess the relationship between glycemic control and, VP and CP Pearson correlation test was used. Classification to correlation coefficient was the proposed by MUNRO, 2001 with a small correlation being considered: values from 0 to 0.25; low from 0.26 to 0.49; moderate from 0.50 to 0.69; high from 0.70 to 0.89; and very high from 0.90 to 1.00.[23] The level of significance was set at $p<0.05$.

Results

Thirty-seven patients diagnosed with T2DM participated in this study and were divided into two groups (GGC, n=11 and IGC, n=26). Baseline characteristics of the groups are described in table 1. No baseline differences were found between the two groups, except for the expected fasting glucose and glycated hemoglobin.

Regarding the cardiovascular parameters during cardiopulmonary the results are described in table 2.

In the interest variables (CP and VP), no differences were found between the studied groups and the results are described in table 2 and figure 2A and 2B. Furthermore, there was no relationship between the main variables and glycemic control, either within each group or total number of participants (Table 3).

Discussion

The present study aimed to investigate the influence of glycemic control on aerobic functional capacity through circulatory power and ventilatory power in patients diagnosed with T2DM. After the patients were submitted to the cardiorespiratory exercise test, we can consider that, for the studied sample, it was not possible to identify differences between the variables. The initial hypothesis of this study was based on the premise that patients

Table 1. Sample characterization

	GGC (n=11)	IGC (n=26)	p	TOTAL (n=37)
Age (years)	53 ± 8	54 ± 8	0.69	54 ± 8.26
Gender (M/F)	7M/4F	19M/7F		26M/11F
Body mass (kg)	84.06 ± 14.95	87.10±15.46	0.58	86.20 ± 5.17
Height (m)	1.7 ± 0.13	1.72 ± 0.1	0.68	1.72 ± 0.11
BMI (kg/m^2)	28.47 (25.4–30.89)	29.61 (25.74–30.96)	0.79	29.1 ± 4.7
Fasting glucose (mg/dl)	125 (115.25–129.75)	147.5 (127–170)	**0.01**	148 ± 50.32
Glycated hemoglobin (%)	5.87 ± 1.16	8.98 ± 1.53	**<0.001**	8.06 ± 2.02
Total cholesterol (mg/dl)	172.18 ± 44.47	184.59±45.56	0.45	180.9 ± 44.99
HDL (mg/dl)	44 (38–55)	42.5 (32–50.6)	0.22	44.93 ± 14.69
LDL (mg/dl)	94.72 ± 44.73	112.46 ± 39.93	0.24	105.5 ±4 2.08
VLDL (mg/dl)	27.09 ± 12.35	33.92 ± 14.58	0.19	31.77 ±14.11
Triglycerides (mg/dl)	114 (82–184.25)	193 (116–232)	0.13	181.98 ± 122
Diabetes time (years)	2 (1–5.5)	7 (4–10)	**0.03**	6.65 ± 5.6
Hypertension, n (%)	4 (36)	6 (23)	0.44	10 (27)
Obesity, n (%)	4 (36)	11 (38)	0.78	15 (41)

[a] Data presented as mean ± SD and median (quartile 1 – quartile 3), BMI – body mass index, GGC – good glycemic control group, HDL – high-density lipoprotein, IGC – insufficient glycemic control group, LDL – low-density lipoprotein, VLDL – very-low-density lipoprotein

Table 2. Cardiovascular parameters during cardiopulmonary testing[a]

	GGC (n=11)	IGC (n=26)	p	TOTAL (n=37)
Rest				
HR_{REST} (bpm)	76 ± 11	82 ± 11	0.13	80.84 ± 11.05
SBP_{REST} (mmHg)	130 (111.5–130)	130 (120–140)	0.21	131.68 ± 17.16
DBP_{REST} (mmHg)	80 (72.5–90)	80 (80–90)	0.66	81.78 ± 8.42
Peak exercise				
VO_{2Peak} (ml/kg/min)	23.4 (19.57–27.02)	21.4 (19.3–25)	0.33	22.51 ± 4.46
VE/VCO2 slope	35.52 (32.82–38.88)	34.3 1(31.8–37.11)	0.58	35.27 ± 6.32
OUES	1.94 ± 0.51	1.84 ± 0.42	0.54	1.87 ± 0.45
HR_{PEAK} (bpm)	163 ± 18	155 ± 20	0.28	158.13 ± 19.25
% HR_{MAX}	97.77 ± 8.92	95.35 ± 10.44	0.47	96.13 ± 9.96
SBP_{PEAK} (mmHg)	201 ± 19	201 ± 30	0.98	201.08 ± 26.93
DBP_{PEAK} (mmHg)	90 (90–107.5)	100 (90–110)	0.59	98.35 ± 12.36
RER	1.13 (1.08–1.23)	1.12 (1.07–1.20)	0.48	1.14 ± 0.1
VP (mmHg)	5.85 ± 1.08	5.86 ± 1.31	0.98	5.86 ± 1.24
CP (mmHg.ml.kg^{-1}min^{-1})	4756.05 ± 1061.67	4434.15 ± 1247.83	0.46	4529.85 ± 1190.23

[a] CP – circulatory power, DBP_{PEAK} – peak diastolic blood pressure, DBP_{REST} – resting diastolic blood pressure, GGC – good glycemic control group, HR_{PEAK} – peak heart rate, HR_{REST} – resting heart rate, IGC – insufficient glycemic control group, RER – respiratory exchange ratio, SBP_{PEAK} – peak systolic blood pressure, SBP_{REST} – resting systolic blood pressure, VE/VCO2 slope – ventilatory efficiency index slope of the ventilatory equivalent of carbon dioxide, VO_{2PEAK} – peak oxygen uptake, VP – ventilatory power

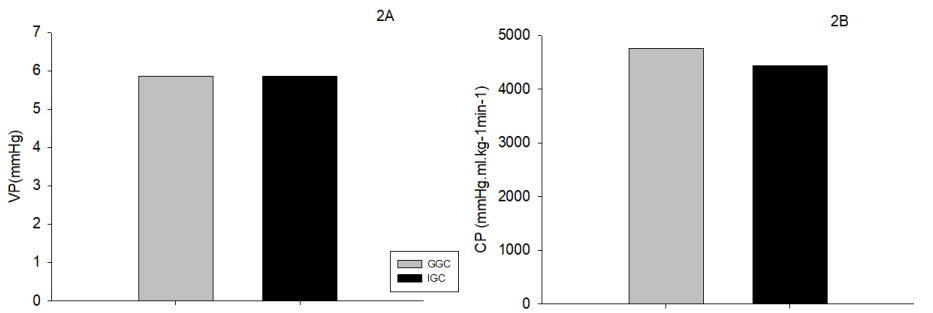

Fig. 2. Circulatory and ventilatory powers comparation [a]

[a] CP – circulatory power, GGC – good glycemic control, IGC – insufficient glycemic control, VP – ventilatory power

with insufficient glycemic control would have lower CP and VP values when compared to patients with good glycemic control, however, when analyzing the results of both groups, the variables presented values without significant differences, which was not expected.

Table 3. Correlation coefficient analysis between glycemic control (HbA1c) and ventilatory power and circulatory power[a]

	r	p
IGC		
VP	-0.205	0.314
CP	0.045	0.825
GGC		
VP	-0.398	0.225
CP	-0.443	0.172
TOTAL		
VP	-0.167	0.322
CP	-0.128	0.451

[a] CP – circulatory power, GGC – good glycemic control group, IGC – insufficient glycemic control group, VP – ventilatory power

The literature suggests that the increase in HbA1c has been shown to be an independent predictor of cardiovascular disease in adult patients with T2DM.[24] Complications, both macro and microvascular, including diabetic neuropathy, are strongly related to the increase in HbA1c[17] and among the symptoms related to this type of neuropathy is exercise intolerance[25] which leads to low adherence to physical exercise programs resulting in a reduction in cardiorespiratory capacity assessed by peak oxygen consumption when compared to a control group.[26] In this context, our findings corroborate those found in the literature, since the patients included in this study also had reduced aerobic functional capacity according to the American Heart Association.[27]

In this way, insufficient glycemic control can compromise physical fitness accessed by maximal oxygen uptake as confirmed by Niranjen et al. when comparing healthy patients with controlled and uncontrolled diabetic patients.[28] We expected to find the same differences comparing CP and VP since mechanisms such as autonomic control and compromised pulmonary responses and arterial stiffness may affect these variables as well as physical fitness.[15,29]

Regarding CP and VP, we have not yet found reference values for the T2DM population that can provide us with comparative information to our findings, however, one study compared patients with coronary artery disease with healthy individuals finding, in this population, higher values for the two variables.[9] Comparing the values found in our study with those described by Castello-Simões, we can say that the T2DM population has CP and VP values lower than the healthy population.[9] Likewise, Mezzani describes that CP is an interesting parameter provided by the cardiopulmonary exercise test capable of non-invasively evaluating the systolic function of the left ventricle during incremental exercise, in this study the author brings values of reference for the healthy population ranging from 5680 to 7050 $mmHg.ml.kg^{-1}min^{-1}$.[12] Thus, the patients involved in this study had lower CP values compared to the reference values mentioned.

We had the hypothesis of this study that T2DM patients who were part of the group with worse glycemic control would have worse values of CP and VP, due to all the adverse effects caused by this lack of control, which has already been described above, however, we did not find differences between the groups studied. We believe that regardless the glycemic control, low aerobic functional capacity of T2DM patients in both groups may have reflected in the values of CP and VP since VO-2PEAK presents a significant correlation with the studied variables.

Our study also had some limitations. First, the sample size was relatively small, especially for the group with good glycemic control. Second, the lack of a control group with apparently healthy subjects to compare CP and VP, since there are no reference values for these variables for the T2DM population.

As clinical importance, we can highlight that given the already known importance of glycemic control in several complications in T2DM, this study intended raise awareness regarding the disease control, however, although we did not observe a direct influence on the PC and PV indices, sample's particularities and size must be considered, so that further studies will be able to consolidate these findings.

Also, the study brings to light the CP and VP, important markers of cardiovascular integrity level to be addressed in rehabilitation programs.[9] In addition, we also reinforce the need for studies to determine indicative cutoff values for patients diagnosed with T2DM.

Conclusion

In conclusion, CP and VP were similar in individuals with T2DM regardless of glycemic control. The predictive ability of these variables in health outcomes deserves to be further investigated in T2DM.

Acknowledgements

The authors gratefully acknowledge all participants of this study.

Declarations

Funding

This study was supported by the National Council for Scientific and Technological Development (CNPq)

and São Paulo Research Foundation (FAPESP; Grant: 2017/19853-4).

Author contributions

Conceptualization, R.G.M., C.D.S. and L.M.S.; Methodology, R.G.M., C.D.S., L.M.S., L.B.L., C.I.M. and P.A.R.; Formal Analysis, L.M.S. and C.D.S.; Investigation, L.M.S., C.D.S., L.B.L., C.I.M. and P.A.R.; Resources, R.G.M. and A.B.S.; Data Curation, L.M.S. and C.D.S.; Writing – Original Draft Preparation, L.M.S.; Writing – Review & Editing, R.G.M., C.D.S. and L.B.L.; Supervision, R.G.M.; Project Administration, R.G.M.; Funding Acquisition, R.G.M. and A.B.S.

Conflicts of interest

The authors declare no conflicts of interest.

Data availability

The data that support the findings of this study are available on request from the corresponding author, RGM. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

Ethics approval

The study was approved by the Human Research Ethics Committee of University (process number 2.814.754) and all individuals read and signed the free and informed consent form.

References:

1. Saeedi P, Petersohn I, Salpea P, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract*. 2019;157:107843.
2. Anjos DM da C dos, Araújo IL, Barros VM, Pereira DAG, Pereira DS. Avaliação da capacidade funcional em idosos diabéticos. *Fisioterapia e Pesquisa*. 2012;19(1):73–78.
3. Gregg EW, Beckles GL, Williamson DF, et al. Diabetes and physical disability among older U.S. adults. *Diabetes Care*. 2000;23(9):1272–1277.
4. Wei M, Gibbons LW, Mitchell TL, Kampert JB, Lee CD, Blair SN. The association between cardiorespiratory fitness and impaired fasting glucose and type 2 diabetes mellitus in men. *Ann Intern Med*. 1999;130(2):89–96.
5. Silva DAS, Naghavi M, Duncan BB, Schmidt MI, de Souza M de FM, Malta DC. Physical inactivity as risk factor for mortality by diabetes mellitus in Brazil in 1990, 2006, and 2016. *Diabetol Metab Syndr*. 2019;11:23.
6. Pereira DAG, Rodrigues RS, Samora GAR, et al. Capacidade funcional de indivíduos com insuficiência cardíaca avaliada pelo teste de esforço cardiopulmonar e classificação da New York Heart Association. *Fisioterapia e Pesquisa*. 2012;19(1):52–56.
7. Miranda HS. O Imaginário nas escolas de Reggio Emilia. In: *SEMINÁRIO EDUCAÇÃO, IMAGINAÇÃO E AS LINGUAGENS ARTÍSTICOCULTURAIS DA UNESC*. Vol 1. UNESC; 2005.
8. Wang N, Liu Y, Ma Y, Wen D. High-intensity interval versus moderate-intensity continuous training: Superior metabolic benefits in diet-induced obesity mice. *Life Sci*. 2017;191:122–131.
9. Castello-Simões V, Minatel V, Karsten M, et al. Circulatory and Ventilatory Power: Characterization in Patients with Coronary Artery Disease. *Arquivos Brasileiros de Cardiologia*. 2015;104(6):476–485.
10. Forman DE, Guazzi M, Myers J, et al. Ventilatory power: a novel index that enhances prognostic assessment of patients with heart failure. *Circ Heart Fail*. 2012;5(5):621–626.
11. Hirashiki A, Adachi S, Nakano Y, et al. Circulatory power and ventilatory power over time under goal-oriented sequential combination therapy for pulmonary arterial hypertension. *Pulm Circ*. 2017;7(2):448–454.
12. Mezzani A. Cardiopulmonary Exercise Testing: Basics of Methodology and Measurements. *Ann Am Thorac Soc*. 2017;14(1):3–11.
13. Queiroz KC, Silva IN, Alfenas R de CG. Associação entre fatores nutricionais e o controle glicêmico de crianças e adolescentes com diabetes melito tipo 1. *Arquivos Brasileiros de Endocrinologia & Metabologia*. 2010;54(3):319–325.
14. Silva FM, Steemburgo T, Azevedo MJ de, Mello VD de. Papel do índice glicêmico e da carga glicêmica na prevenção e no controle metabólico de pacientes com diabetes melito tipo 2. *Arquivos Brasileiros de Endocrinologia & Metabologia*. 2009;53(5):560–571.
15. El-Habashy MM, Agha MA, El-Basuni HA. Impact of diabetes mellitus and its control on pulmonary functions and cardiopulmonary exercise tests. *Egyptian Journal of Chest Diseases and Tuberculosis*. 2014;63(2):471–476.
16. Rossaneis MA, Andrade SM de, Gvozd R, et al. Fatores associados ao controle glicêmico de pessoas com diabetes mellitus. *Ciência & Saúde Coletiva*. 2019;24(3):997–1005.
17. Stolar M. Glycemic Control and Complications in Type 2 Diabetes Mellitus. *The American Journal of Medicine*. 2010;123(3):3–11.
18. Netto AP, Andriolo A, Fraige Filho F, et al. Atualização sobre hemoglobina glicada (HbA1C) para avaliação do controle glicêmico e para o diagnóstico do diabetes: aspectos clínicos e laboratoriais. *Jornal Brasileiro de Patologia e Medicina Laboratorial*. 2009;45(1):31–48.
19. Matheus. Diretrizes e Posicionamentos. *Sociedade Brasileira de Diabetes*. https://www.diabetes.org.br/profissionais/publicacoes/diretrizes-e-posicionamentos-1. Accessed September 13, 2018.
20. Balady GJ, Arena R, Sietsema K, et al. Clinician's Guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association. *Circulation*. 2010;122(2):191–225.

21. Wasserman K. *Principles of Exercise Testing and Interpretation*. 3rd ed. Lippincott, Williams E Comercial Wilkins; 1999.
22. Cohen-Solal A, Tabet JY, Logeart D, Bourgoin P, Tokmakova M, Dahan M. A non-invasively determined surrogate of cardiac power ('circulatory power') at peak exercise is a powerful prognostic factor in chronic heart failure. *Eur Heart J*. 2002;23(10):806–814.
23. Munro, B. H. *Correlation. Statistical Methods for Health Care Research*. 4th ed. Lippincott; 2001.
24. Morgan CL, Currie CJ, Peters JR. Relationship between diabetes and mortality: a population study using record linkage. *Diabetes Care*. 2000;23(8):1103–1107.
25. LA, Russell JW. Physical activity and dietary interventions in diabetic neuropathy: a systematic review. *Clin Auton Res*. 2019;29(4):443–455.
26. Poitras VJ, Hudson RW, Tschakovsky ME. Exercise intolerance in Type 2 diabetes: is there a cardiovascular contribution? *Journal of Applied Physiology*. 2018;124(5):1117–1139.
27. American Heart Association. *Committee on Exercise: Exercise Testing and Training of Apparently Healthy Individuals: A Handbook for Physicians*. 1972.
28. Niranjan V, McBrayer DG, Ramirez LC, Raskin P, Hsia CCW. Glycemic Control and Cardiopulmonary Function in Patients With Insulin-Dependent Diabetes Mellitusfn1fn1This work was supported by the American Lung Association of Texas. C.C.W. Hsia is a recipient of the Established Investigator Award from the American Heart Association. *The American Journal of Medicine*. 1997;103(6):504–513.
29. Italiano Monteiro C, Polaquini Simões R, Domingues Heubel A, et al. Arterial stiffness can predict cardiorespiratory fitness in type 2 diabetic patients? *European Journal of Clinical and Experimental Medicine*. 2022;(1):28–35.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.11

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 330–335

ORIGINAL PAPER

The role of the hematological inflammatory index and systemic immuno-inflammation index in acute cholecystitis

Serdar Özdemir, İbrahim Altunok, Abuzer Özkan, Mehmet Muzaffer İslam, Abdullah Algın, Serkan Emre Eroğlu, Gökhan Aksel

Department of Emergency Medicine, University of Health Sciences Umraniye Training and Research Hospital, Istanbul, Turkey

ABSTRACT

Introduction and aim. Acute cholecystitis is one of the most common hepatobiliary emergencies. We aimed to investigate the role of the initial hematological inflammatory index and systemic immuno-inflammation index in predicting short-term mortality in patients with acute cholecystitis.

Material and methods. This study with a retrospective observational design was conducted at the emergency department of a tertiary teaching hospital. Patients admitted to our clinic between June 15, 2021, and March 15, 2022, according to the Tokyo criteria were included in the sample. The hematological inflammatory index and systemic immuno-inflammation index were calculated using the hematological test results of the patients evaluated at the emergency department. Survivor and non-survivor groups were formed according to all-cause 30-day mortality. The differences between survivor and non-survivor groups were investigated.

Results. A total of 194 patients were included in the final analysis. The median age of the study population was 59 (25^{th}–75^{th} percentiles: 46.75–72) years. The rate of all cause-short-term mortality was 7.7. There were significant differences between the survivor and non-survivor groups in terms of the neutrophil count and the systemic immuno-inflammation index (p=0.007, 0.034, respectively; Mann-Whitney U test). No significant difference was found in the remaining laboratory parameters (lymphocyte count, platelet count, and hematological inflammatory index) (p=0.220, 0.489, 0.367 respectively; Mann-Whitney U test).

Conclusion. The systemic immuno-inflammation index was determined to be significantly higher in the non-survivor group than in the survivor group among the patients with acute cholecystitis. However, there was no significant difference between these two groups in relation to the hematological inflammatory index.

Keywords. acute cholecystitis, neutrophil, lymphocyte, platelet, mortality

Introduction

Acute cholecystitis is a disease caused by the acute inflammation of the gallbladder. Inflammatory changes that occur in this disease can range from a mild symptomatic to a severe clinical picture, including acute pancreatitis, acute cholangitis, and even empyema or gangrene.[1] Acute cholecystitis constitutes approximately 1–3% of patient presentations with abdominal pain. The cause is gallstones in 90–95% of cases.[1,2] To diagnose acute cholecystitis, it is necessary to evaluate the medical history, physical examination findings, laboratory results, and radiological imaging findings together. There is not a single clinical or laboratory parameter that will diagnose or exclude the diagno-

Corresponding author: Serdar Özdemir, e-mail: dr.serdar55@hotmail.com

Received: 23.06.2022 / Accepted: 19.07.2022 / Published: 30.09.2022

Özdemir S, Altunok İ, Özkan A et al. *The role of the hematological inflammatory index and systemic immuno-inflammation index in acute cholecystitis*. Eur J Clin Exp Med. 2022;20(3):330–335. doi: 10.15584/ejcem.2022.3.11.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

sis. The Tokyo criteria (TG18 Diagnostic Criteria and Severity Grading of Acute Cholecystitis) published in 2007 and updated in 2013 and 2018 are used as diagnostic criteria.[3]

In infections, trauma, inflammatory diseases, and similar conditions, a series of changes occur as a response at or away from the inflammation area. This response is called the acute phase response, including neuroendocrine, hematopoietic, and metabolic changes.[4] Proteins with increasing or decreasing serum concentrations (acute phase reactants) and some hematological parameters are used in the clinical evaluation of the inflammatory acute phase response and response to therapy. The most studied hematological parameters are the neutrophil count, white blood cell count, and lymphocyte count. To determine the ideal marker, researchers have worked on a combination of these parameters.[5] The hematological inflammatory index (HII) and systemic immuno-inflammation (SII) are newly developed inflammatory indexes.[6,7] To the best of our knowledge, there is no study in the literature evaluating the role of HII and SII in acute cholecystitis.

Aim

The aim of our study was to investigate the role of the initial SII and HII in predicting short-term mortality in patients presenting to the emergency department with acute cholecystitis.

Material and methods

Design of study

The current study with a retrospective observational design was conducted at the adult emergency department of a tertiary teaching hospital, serving a population of approximately one million and having an average of 1,000 admissions per day.

Patient sampling

The data of patients with the acute cholecystitis ICD code, who presented to the Emergency Department of University of Health Sciences Umraniye Training and Research Hospital between June 15, 2021, and March 15, 2022, were obtained from the hospital computer-based patient information system. Patients who did not meet the Tokyo 2018 criteria and those with incomplete data were excluded from the study.

Data collection

Demographic data, comorbidities, and laboratory parameters were gathered from the patient information system of the hospital. Comorbidities were noted as congestive heart failure, diabetes mellitus, asthma, hypertension, chronic obstructive pulmonary disease, and chronic renal failure. The white blood cell count, hemoglobin, red cell distribution width, neutrophil count, lymphocyte count, platelet count, mean platelet volume, blood urea nitrogen, creatinine, and albumin values were documented. The neutrophil lymphocyte ratio, SII, and HII were calculated. HII was calculated by multiplying the platelet count by 100 and dividing the result by the product of the neutrophil count and lymphocyte count. SII was calculated by multiplying the neutrophil-lymphocyte ratio by the platelet count.

Statistical analysis

Statistical analysis was performed using Jamovi (The Jamovi Project, Version 1.6.21.0; 2020). The fit of the parameters to the normal distribution was determined with the Shapiro-Wilk test. Categorical data were presented as numbers and percentages, and continuous data as median and 25th and 75th percentile values. The correlation between categorical data and mortality was investigated using the chi-square test, and the relationship between continuous data and mortality was determined with the Mann-Whitney U test. The ability of the variables to predict mortality was examined using the receiver operating characteristic (ROC) analysis. The results of this analysis were presented with positive and negative predictive values, and the cut-off point specified as the area under the curve (AUC). Values above 0.7 were considered significant as promising AUC values.[8,9] Values above 0.05 were accepted for the significant p value.

Ethics

Ethical approval for the study was obtained from the University of Health Sciences Ümraniye Training and Research Hospital ethics committee with 03.31.2022 date and 114 number. Informed consent was waived within the knowledge of the local ethics committee, as the study did not include any personal information of the patients and had a retrospective design.

Results

A total of 194 patients were included in the final analysis. The median age of the study population was 59 (25th–75th percentiles: 46.75–72) years, and 93 (47.9%) patients were female. The rate of all cause-short-term mortality was 7.7. The descriptive data of the study population and comparison of these characteristics between the survivor and non-survivor groups are presented in Table 1.

There were significant differences between the survivor and non-survivor groups in terms of the neutrophil count [10.23 (7.48-13.97) versus 14.78 (9.46-23.2) $10^3/\mu L$, p = 0.007] and SII [1726.37 (974.21-2746.58) versus 2381.1 (1509.74-6835.21), p = 0.034] (Mann-Whitney U test). No significant difference was observed between the two groups in relation to the remaining laboratory parameters: lymphocyte count

Table 1. Baseline characteristics and laboratory parameters of the enrolled patients and their comparison between the survivor and non-survivor groups

Variables	Total n = 194	Survivor n = 179 (92.3%)	Non-survivor n = 15 (7.7%)	p
	n (%)/Median (25^{th}-75th percentiles)	n (%)/Median (25^{th}-75^{th} percentiles)	n (%)/Median (25^{th}-75^{th} percentiles)	
Age	59 (46.75 – 72)	58 (45 – 69)	79 (72 – 86)	<0.001
<65 years	122 (62.9%)	120 (98.4%)	2 (1.6%)	<0.001
≥65 years	72 (37.1%)	59 (81.9%)	13 (18.1%)	
Gender				
Female	93 (47.9%)	85 (91.4%)	8 (8.6%)	0.663
Male	101 (52.1%)	94 (91.3%)	7 (6.9%)	
Comorbidities				
Chronic obstructive pulmonary disease	10 (5.2%)	8 (80%)	2 (20%)	0.175
Hypertension	83 (42.8%)	74 (89.2%)	9 (10.8%)	0.161
Diabetes mellitus	47 (24.2%)	43 (91.5%)	4 (8.5%)	0.818
Coronary artery disease	39 (20.1%)	33 (84.6%)	6 (15.4%)	0.085
Congestive heart failure	14 (7.2%)	11 (78.6%)	3 (21.4%)	0.081
Asthma	12 (6.2%)	11 (91.7%)	1 (8.3%)	0.936
History of malignancy	8 (4.1%)	8 (100%)	0	0.403
Hyperlipidemia	41 (21.1%)	38 (92.7%)	3 (7.3%)	0.911
Laboratory parameters				
White blood cell count (10^3/μL)	13.1 (10.07 – 17.06)	12.97 (9.88 – 16.8)	16.8 (12.3 – 25.6)	0.012
Neutrophil count (10^3/μL)	10.38 (7.53 – 14.24)	10.23 (7.48 – 13.97)	14.78 (9.46 – 23.2)	0.007
Lymphocyte count (10^3/μL)	1.59 (0.49 – 0.71)	1.61 (1.17 – 2.18)	1.24 (0.98 – 2.12)	0.22
Hemoglobin (g/dL)	10.5 (11.8 – 14.9)	13.6 (12 – 14.9)	11.2 (9.3 – 12.8)	<0.001
Hematocrit (%)	40.5 (36.3 – 44.5)	40.9 (37.1 – 44.7)	33 (28.1 – 37)	<0.001
Red blood cell distribution width (%)	13.6 (13.1 – 14.5)	13.5 (13 – 14.2)	17.2 (16.9 – 19.3)	<0.001
Platelet count (10^3/μL)	270 (225 – 321)	271 (229 – 321)	244 (197 – 370)	0.489
Mean platelet volume (fL)	9.7 (8.97 – 10.5)	9.8 (9.1 – 10.6)	8.12 (7.26 – 9.53)	<0.001
Plateletcrit (%)	0.26 (0.22 – 0.32)	0.26 (0.22 – 0.32)	0.21 (0.15 – 0.27)	0.03
Blood urea nitrogen (mg/dL)	30.6 (22.2 – 40.1)	29.6 (21.5 – 37.3)	55.6 (36.3 – 70.6)	<0.001
C-reactive protein, (mg/dL)	51.64 (8.5 – 154.88)	45.8 (8.1 – 145.9)	135 (112 – 188)	0.006
Albumin (g/dL)	42.9 (38.7 – 45)	43 (39.4 – 45)	30 (23 – 33)	<0.001
Total bilirubin (mg/dL)	0.84 (0.53 – 1.43)	0.81 (0.51 – 1.39)	1.2 (0.77 – 3.56)	0.018
Direct bilirubin (mg/dL)	0.27 (0.15 – 0.55)	0.26 (0.14 – 0.51)	0.55 (0.27 – 2.61)	0.006
Indirect bilirubin (mg/dL)	0.48 (0.31 – 0.79)	0.48 (0.3 – 0.78)	0.86 (0.43 – 1.24)	0.025
Neutrophil-lymphocyte ratio	6.51 (3.95 – 10.05)	6.37 (3.89 – 9.53)	12.31 (9.27 – 16.58)	0.002
Platelet-lymphocyte ratio	162.62 (119.65 – 240.16)	162.29 (12.83 – 233.98)	201.02 (100.67 – 365)	0.559
C-reactive protein/albumin ratio	1.22 (0.2 – 4.03)	1.08 (0.17 – 3.52)	4.3 (3.95 – 7.45)	<0.001
Blood urea nitrogen/albumin ratio	0.71 (0.51 – 0.99)	0.69 (0.48 – 0.94)	2.06 (1.4 – 3.03)	<0.001
Systemic immune-inflammation index	1764.96 (1001.77 – 2778.77)	1726.37 (974.21 – 2746.58)	2381.1 (1509.74 – 6835.21)	0.034
Hematologic inflammatory index	1.68 (1.08 – 2.5)	1.7 (1.13 – 2.54)	1.5 (0.92 – 2.46)	0.367

[1.61 (1.17-2.18) versus 1.24 (0.98-2.12) 10^3/μL, p = 0.220], platelet count [3 (1-22) versus 9 (2-21) 10^3/μL, p= 0.489], and HII [1.7 (1.13-2.54) versus 1.5 (0.92-2.46), p = 0.367] (Mann-Whitney U test). The initial laboratory parameters of the enrolled patients and their comparison between the survivor and non-survivor groups are shown in Table 1.

The ROC curve analysis was performed to determine the predictive ability of the neutrophil count, lymphocyte count, platelet count, SII, and HII for short-term mortality. The cut-off values of these parameters according to the best Youden's index, as well as their sensitivity, specificity, AUC, and 95% confidence interval values are presented in Table 2 and Figure 1.

Table 2. Accuracy of the investigated laboratory parameters in predicting short-term mortality in patients with acute cholecystitis [a]

Variables	AUC	Accuracy	95% CI	Cut-off value	Sensitivity	Specificity	PPV	NPV	PLR	NLR	p value
Neutrophil count	0.708	0.933	0.639–0.771	>16.82	46.67	91.62	31.8	95.3	5.57	0.58	0.014
Lymphocyte count	0.596	0.923	0.523–0.665	≤1.39	60.00	64.80	12.5	95.1	1.70	0.62	0.246
Platelet count	0.554	0.923	0.481–0.625	≤230	46.67	73.74	13.0	94.3	1.78	0.72	0.574
Systemic immuno--inflammation	0.666	0.918	0.593–0.884	>4049.3	40.00	91.62	28.6	94.8	4.77	0.65	0.041
Hematological inflammatory index	0.570	0.923	0.498–0.641	≤1.53	66.67	56.42	11.4	95.3	1.53	0.59	0.428

[a] AUC – area under the curve, CI – confidence interval, PPV – positive predictive value, NPV – negative predictive value, PLR – positive likelihood ratio, NLR – negative likelihood ratio

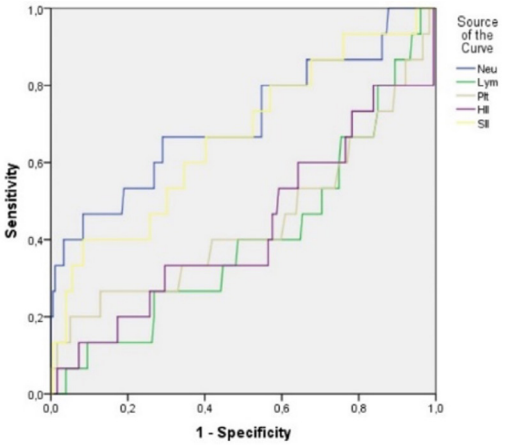

Fig. 1. Receiver operating characteristic curves of the hematological inflammatory index (HII), systemic immuno-inflammation index (SII), neutrophil count (Neu), lymphocyte count (Lym), and platelet count (Plt) for the prediction of short-term mortality in patients with acute cholecystitis

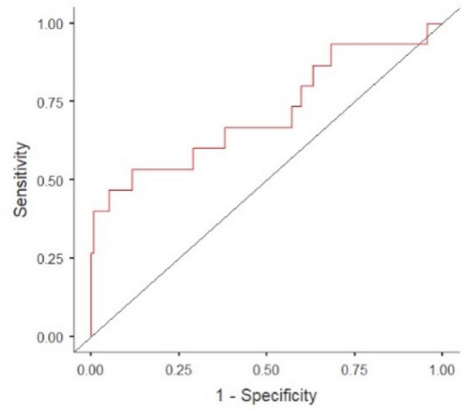

Fig. 2. Receiver operating characteristic curve of the multivariate logistic regression model for the prediction of short-term mortality in patients with acute cholecystitis

With the multivariate regression model created for the prediction of short-term mortality, the AUC value was calculated as 0.714 (accuracy: 0.943, sensitivity: 0.999, and specificity 0.267, p<0.001) (Figure 2).

Discussion

In the current study, we investigated the role of the initial SII and HII values in predicting short-term mortality in patients with acute cholecystitis. According to the results of the univariant analysis, there was no significant difference in the HII value between the survivor and non-survivor groups. The SII value was found to be significantly higher in the mortality group, and it was able to predict short-term mortality in acute cholecystitis with high specificity (91.62%). Another valuable finding for the literature was that the created multivariate regression model detected the risk of short-term mortality in acute cholecystitis with high accuracy (0.943).

Neutrophils, one of the primary response agents to acute inflammation, constitute 50–70% of circulating leukocytes, and an increase in neutrophils is usually expected in acute cholecystitis.[10] In a study by Naidu et al., it was shown that the neutrophil count was higher in the patient group whose histology was compatible with acute cholecystitis among patients who had undergone cholecystectomy for acute cholecystitis compared to those without this disease (10.1 K/uL versus 6.0 K/uL).[11] In another study, Sato et al. reported that the neutrophil values of patients with acute cholecystitis significantly differed between the three groups formed according to disease severity determined by the TG18 criteria.[12] In the current study, the neutrophil count was found to be significantly higher in the mortality group. These results show that the neutrophil value can be used as a guide for both the diagnosis of acute cholecystitis and the determination of severity and prediction of mortality.

Lymphocytes are among markers representing immunity.[13] In the current literature, there are conflicting results in studies evaluating the role of the lymphocyte count in acute cholecystitis. In a study by Sato et al., evaluating the effectiveness of the lymphocyte count in predicting the severity of acute cholecystitis, it was suggested that this parameter did not have a place in this prediction.[12] These results were later validated by Mahmood et al.[14] On the other hand, Ertok et al. found that

the lymphocyte count was significantly lower in patients with acute cholecystitis compared to the control group.[15] In our study, the lymphocyte counts, and mortality were unrelated. The results of the mentioned studies reveal that there is no relationship between acute cholecystitis and lymphocyte count.

Platelet plays a role in inflammation, in addition to being an important element of the coagulation cascade. The platelet count is a well-known predictor of many infectious diseases, especially sepsis.[16] On the other hand, there are controversial publications concerning the effects of platelets on acute cholecystitis. Contrary to expectations, Sayit et al. reported increased platelet values in patients with acute cholecystitis compared to the control group.[17] Sato et al. found a low platelet count in severe acute cholecystitis cases.[14] In contrast, Woo et al. revealed that the platelet count was not affected in patients with acute cholecystitis compared to severe cases.[18] In the current study, the platelet count was unaffected. The results of the mentioned studies indicate that there is no relationship between acute cholecystitis and platelet count.

SII and HII are new and inexpensive biomarkers that can be easily calculated using the neutrophil, platelet, and lymphocyte counts.[19-22] These two indexes are parameters that show the balance between inflammatory and immune responses. The role of SII has been investigated in many malignant diseases, asthma, coronary disease, ischemic stroke, and as a systemic inflammation and prognostic marker. High SII values have been associated with poor outcome in coronary diseases, stroke, and malignant diseases.[19-21] A logical explanation for this has been suggested in the literature as SII being a marker of a strong inflammatory response and a weak immune response. On the other hand, HII is a newly developed and less studied indicator compared to SII. Şahinli and Türker proposed HII as a new prognostic marker in patients that underwent resection for gastric cancer.[22] According to the best of our knowledge, our study is the first in the literature to evaluate the role of SII and HII in predicting short-term mortality in patients with acute cholecystitis.

There are several important limitations to our study. The retrospective design is the most important limitation. In addition, acute cholecystitis represents a heterogeneous group, including patients with or without stones, gangrenous cholecystitis, and gallbladder empyema. However, in the current study, we were not able to perform subgroup analyses. The limited sample and single-center design can be considered as other limitations that could limit the generalizability of our findings.

Conclusion

In conclusion, SII was determined to be significantly higher in the non-survivor group than in the survivor group among the patients with acute cholecystitis. In terms of HII, there was no significant difference between the survivor and non-survivor groups with acute cholecystitis. SII is an easily accessible, inexpensive parameter that assists clinicians in the clinical follow-up of patients with acute cholecystitis. However, we consider that our results should be validated through large multicenter studies to increase their generalizability.

Declarations

Funding

The authors declared that this study has received no financial support or any funding.

Author contributions

Conceptualization, S.Ö. and İ.A.; Methodology, S.Ö., İ.A., A.A., M.M.İ. and A.Ö.; Software, S.Ö.; Validation, S.Ö., İ.A., A.A. and G.A.; Formal Analysis, S.Ö., M.M.İ., G.A. and S.E.E.; Investigation, S.Ö.; Resources, S.Ö., A.Ö., M.M.İ., A.A. and S.E.E.; Data Curation, S.Ö., İ.A., M.M.İ. and G.A.; Writing – Original Draft Preparation, S.Ö., İ.A., A,Ö., M.M.İ., A.A., S.E.E. and G.A.; Writing – Review & Editing, S.Ö., G.A. and S.E.E.; Visualization, S.Ö., İ.A., A,Ö., M.M.İ., A.A., S.E.E. and G.A.; Supervision, S.Ö., İ.A., A,Ö., M.M.İ., A.A., S.E.E. and G.A.; Project Administration, S.Ö.; Funding Acquisition, S.Ö., İ.A., A,Ö., M.M.İ., A.A., S.E.E. and G.A.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

Study was approved by the institutional review board, and a waiver of authorization was given (Ethics Committee decision no. 114, date: 03.31.2022).

References

1. Gallaher JR, Charles A. Acute Cholecystitis: A Review. *JAMA*. 2022;327(10):965–975.
2. Lam R, Zakko A, Petrov JC, Kumar P, Duffy AJ, Muniraj T. Gallbladder Disorders: A Comprehensive Review. *Dis Mon.* 2021;67(7):101130.
3. Yokoe M, Hata J, Takada T, et al. Tokyo Guidelines 2018: diagnostic criteria and severity grading of acute cholecystitis (with videos). *J Hepatobiliary Pancreat Sci.* 2018;25(1):41–54.
4. Ozdemir S, Ozkan A. Acute Phase Response. *Eurasian Journal of Critical Care.* 2021;3(3):81.
5. Ozkan A. Ideal predictor studies. *J Exp Clin Med.* 2022;39(2):595–596.
6. Walzik D, Joisten N, Zacher J, Zimmer P. Transferring

clinically established immune inflammation markers into exercise physiology: focus on neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and systemic immune-inflammation index. *Eur J Appl Physiol.* 2021;121(7):1803–1814.
7. Sahinli H, Türker S. The hematologic inflammatory index is a new prognostic marker in patients resected for gastric cancer. *J Cancer Res Ther.* 2020;16:S144–S149.
8. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics.* 1988;44(3):837–845.
9. Ozdemir S, Algın A. Interpretation of the Area Under the Receiver Operating Characteristic Curve. *Experimental and Applied Medical Science.* 2022;3(1);310–311.
10. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. *J Immunol.* 2004;172(5):2731–2738.
11. Naidu K, Beenen E, Gananadha S, Mosse C. The Yield of Fever, Inflammatory Markers and Ultrasound in the Diagnosis of Acute Cholecystitis: A Validation of the 2013 Tokyo Guidelines. *World J Surg.* 2016;40(12):2892–2897.
12. Sato N, Kinoshita A, Imai N, et al. Inflammation-based prognostic scores predict disease severity in patients with acute cholecystitis. *Eur J Gastroenterol Hepatol.* 2018;30(4):484–489.
13. Chandrashekara S, Mukhtar Ahmad M, Renuka P, Anupama KR, Renuka K. Characterization of neutrophil-to-lymphocyte ratio as a measure of inflammation in rheumatoid arthritis. *Int J Rheum Dis.* 2017;20(10):1457–1467.
14. Mahmood F, Akingboye A, Malam Y, Thakkar M, Jambulingam P. Complicated Acute Cholecystitis: The Role of C-Reactive Protein and Neutrophil-Lymphocyte Ratio as Predictive Markers of Severity. *Cureus.* 2021;13(2):e13592.
15. Ertok İ, Karakayalı O, Kocasaban DU. Akut Kolesistit--Kolelitiazis Ayırıcı Tanısında Nötrofil/Lenfosit Oranının Klinik Önemi. *Kocaeli Medical J.* 2016;5(3):6–11.
16. Ozdemir S, Algın A. The Role of Platelet Indices in Predicting Short-Term Mortality in Elderly Patients with Pulmonary Embolism. *J Contemp Med.* 2021;11(6): 833–837.
17. Sayit AT, Gunbey PH, Terzi Y. Is the Mean Platelet Volume in Patients with Acute Cholecystitis an Inflammatory Marker? *J Clin Diagn Res.* 2015;9(6):TC05–TC07.
18. Woo SH, Lee WJ, Seol SH, Kim DH, Choi SP. The accuracies of abdominal computed tomography and the neutrophil-to-lymphocyte ratio used to predict the development of clinically severe acute cholecystitis in elderly patients visiting an emergency department. *Niger J Clin Pract.* 2018;21(5):645–652.
19. Kubota K, Ito R, Narita N, et al. Utility of prognostic nutritional index and systemic immune-inflammation index in oral cancer treatment. *BMC Cancer.* 2022;22(1):368.
20. Öcal L, Keskin M, Cerşit S, et al. Systemic immune-inflammation index predicts in-hospital and long-term outcomes in patients with ST-segment elevation myocardial infarction. Coron Artery Dis. 2022;33(4):251–260.
21. Zhang F, Niu M, Wang L, et al. Systemic-Immune-Inflammation Index as a Promising Biomarker for Predicting Perioperative Ischemic Stroke in Older Patients Who Underwent Non-cardiac Surgery. *Front. Aging Neurosci.* 2022;14:865244.
22. Sahinli H, Türker S. The hematologic inflammatory index is a new prognostic marker in patients resected for gastric cancer. *J Cancer Res Ther.* 2020;16:S144–S149.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.12

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 336–341

ORIGINAL PAPER

The incidence of obesity among children in Turkey – obesity awareness, physical activity and other associated factors

Aysel Topan [1], Ayse Ferda Ocakcı [2], Tulay Kuzlu Ayyıldız [1], Aylin Kurt [3], Sümeyye Ozdemir [1]

[1] Nursing Department, Faculty of Health Science, Zonguldak Bülent Ecevit University, Zonguldak, Turkey
[2] Nursing Department, Faculty of Nursing, Koç University, Istanbul, Turkey
[3] Nursing Department, Faculty of Health Science, Bartın University, Bartın, Turkey

ABSTRACT

Introduction and aim. Obesity is the leading one among the most common nutritional disorders seen during childhood period. We aimed to determine the incidence of obesity, obesity awareness, physical activity and associated factors in school-age children.

Material and methods. The sampling was composed of 725 students who were studying in secondary schools during the 2016–2017 academic year. Data were collected with "Personel Information Form", "Obesity Awareness Scale" and "Physical Activity Questionnaire for Older Children". The mean age was 12.30±1.32 (10–15), 53% of the students were females and 60.1% of students have a person with obesity in the family.

Results. We determined that 18.3% of the students were with obesity. The body mass index of students who have a balanced diet compared to those who have not is statistically significantly lower ($p<0.05$). There was a significant relationship between students' body mass index and obesity awareness ($r=-0.084$, $p=0.024$).

Conclusion. Screening for obesity and related factors, such as obesity awareness, having a balanced diet should begin in school-aged children for childhood obesity prevention.

Keywords. awareness, children, obesity, pediatric obesity, physical exercise

Introduction

Obesity is the leading one among the most common nutritional disorders seen during childhood period. Obesity is one of the serious public health problems since impaired glucose tolerance and type 2 diabetes are frequently seen among children with obesity as in adults and many associated diseases that seriously threaten life such as hypertension, cardiovascular diseases, degenerative arthritis and thrombophlebitis occur commonly during childhood as well as adulthood period.[1]

Various obesity prevalences have been reported from distinct regions of the world today; and according to the statement of World Health Organization (WHO), 42 millions of overweight children below 5 years old have been living on earth since 2010, and almost 35 million of these children live in developing countries.[2] Obesity was most common in America, Caribbeans, Middle and North Africa by a rate of 20% and higher.[3]

Improper dietary habits, sedentary lifestyle, genetic and hormonal factors and lack of physical activity had roles in the emergence of childhood obesity. Prevention from obesity is based on changing unhealthy dietary and exercise habits into healthy behaviors on behalf of

Corresponding author: Aylin Kurt, e-mail: aylinkurt67@gmail.com

Received: 26.05.2022 / Revised: 1.07.2022 / Accepted: 19.07.2022 / Published: 30.09.2022

Topan A, Ocakcı AF, Ayyıldız TK, Kurt A, Ozdemir S. *The incidence of obesity among children in Turkey – obesity awareness, physical activity and other associated factors.* Eur J Clin Exp Med. 2022;20(3):336–341. doi: 10.15584/ejcem.2022.3.12.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

childhood obesity.[5] These principles are generally evaluted as part of obesity awareness. In recent years, studies examining the effect of obesity awareness on obesity have attracted attention.[4,5]

Awareness about obesity has been reported to be an indicator for future increase in an individual's body weight management and their quality of life.[6] Obesity awareness contains physical activity and nutrition beside obesity awareness. At this point, it is required to determine and improve obesity awareness and physical activity levels among the children and adolescents. Although there are studies in the literature that examine the obesity levels of students, studies examining the relationship between body mass index (BMI) and obesity awareness are quite limited.[6–8]

Aim

This study was carried out to determine relationship between obesity, obesity awareness, physical activity and other associated factors in school age children. The questions of this study: (1) What was the BMI levels of the students? (2) Is there a difference BMI levels of students according to personal characteristics (gender, having person with obesity in the family, having breakfast everyday, having dinner everyday and having balanced diet)? (3) Are there relationships between BMI levels, obesity awareness and physical activity of students?

Material and methods

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by Human Research the Ethics Committee of the Zonguldak Bülent Ecevit University (Date: 26.01.2017, No: 192). Data collection was performed based on the voluntary participation of the individuals and their parents enrolled in the study. We informed the students and their parents about the aim of the study and the confidentiality of all data, and they gave their written consents.

Setting

The study used a cross-sectional study design in which the data were collected via questionnaires during 2016–2017 academic year with 725 school-aged children and analyzed using descriptive statistics in Zonguldak, Turkey. This study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies.[9]

Participants

The total number of students in five secondary schools is 972. The sampling method was not used in this study and all students volunteering to particiapte in this study were included. A total of 725 (participation rate: 74.6%) students participated in the study. Criteria for inclusion in the study; being a student at 5, 6, 7 and 8th grades and volunteering to participate in the study. Exclusion Criteria: not volunteering to participate in the study.

Data collection

Data were collected face to face from students. Data were collected with "Personel Information Form", "Obesity Awareness Scale" and "Physical Activity Questionnare for Older Children".

Personel information form

This form prepared by the reseachers by scanning the literature.[10,11] This form consists of questions related to the sociodemografic variables of students age, gender, height, weight and diet. The children's height and weight were measured by the researchers during 2016–2017 academic year.

Obesity awareness scale (OAS)

In order to determine of obesity awareness of the children "Obesity Awareness (OAS) Scale" were used. This scale was developed and validated for Turkey by Kafkas and Özen.[12] The validity and reliability value of the scale is 0.87. It consists of twenty items and questions assessing obesity awareness, nutrition and physical activity knowledge of the children. Each statement was scored from 0 to 4. Original form of the scale consisted of 23 items and three domains including obesity awareness (8 items) nutrition (7 items) and physical activity (8 items). The scores were added to give a final score (0–80). Higher scores indicate an increase in the awareness of obesity.[13] Cronbach Alpha value was found to be 0.85 in this study.

Physical activity questionnare for older children (PAQ-C 4-8)

This questionnaire was developed by Trocker, Bailey, Faulkner, Kowalski and McGrath; and its validity and reliability studies were performed by Tanır and Yoncalık.[14] It gives an idea about the general physical activity habits of the participants (8–14 year old children who were studying at 4–8 grades.). The scale consists of 9 questions (Cronbach's alpha = 0.76) graded in 1-5 Likert type. In calculating the physical activity scores of the participants in the study, the average of all questions is taken. Five points for each question indicate the highest physical activity level and one point indicates the lowest physical activity level.[14] Cronbach's Alpha value was found to be 0.89 in this study.

Table 1. Disturibution of students' personal characteristics (n=725)

Personal characteristics	$\bar{X}$ ± SD (Min – **Max)**	
Age	12.30 ± 1.32 (10 – 15)	
	n	**%**
Gender		
Male	384	53
Female	341	47
BMI		
Thin (<18.9 kg/m^2)	195	26.9
Normal (19.0-24.9 kg/m^2)	305	42.1
Overweight (25.0-29.9 kg/m^2)	92	12.7
With obesity (>30 kg/m^2)	133	18.3
Person with obesity in the family		
Yes	283	39.9
No	436	60.1
Having breakfast everday		
Yes	481	66.3
No	55	7.6
Sometimes	189	26.1
Having lunch everday		
Yes	447	65.8
No	41	5.7
Sometimes	207	28.6
Having dinner everday		
Yes	23	88.3
No	640	3.2
Sometimes	62	8.6
Having balanced diet		
Yes	401	55.3
No	74	10.2
Sometimes	250	34.5

Data analysis

Statistical Package for the Social Sciences (SPSS) 22.0 package program (IBM, Armonk, NY, USA) was used to analyze the data. Descriptive data are indicaed by number, percentage, mean and standard deviation. Whether the data was normally distributed was checked with skewness and kurtosis, and parametric test were used. In comparison of quantitative data, one-way analysis of variance was used for more than two groups with the difference of student t test between two groups. Bonferroni post hoc test was used to determine where the significant difference originated. The extent of the relationship between BMI and obesity awareness was examined by simple linear regression analysis. Analysis results were analyzed as $p<0.05$ significant levels.

Results

The mean age was 12.30 ± 1.32 (10–15), 53% of the students were females and 60.1% of students have a person with obesity in the family. Additionally, 66.3% of students have breakfast everyday, 65.8% of students have lunch everyday, 88.3% of students have dinner everyday and 55.3% have a balanced diet. Altogether, 12.7% were overweight and 18.3% were with obesity (Table 1).

A comprison of students according to personel characteristics in terms of BMI is given in Table 2.

Table 2. Comparisons of students' BMI based on their personal characteristics and (n=725)

Personal characteristics	BMI $\bar{X}$ ± SD	Statistical analysis	Significant difference (post hoc)*
Gender			
Male	19.54 ± 4.25	t = -1.195	
Female	19.93 ± 4.5	p = 0.232	
Person with obesity in the family			
Yes	19.20 ± 3.89	t = -4.012	
No	20.52 ± 4.91	p < 0.001	
Having breakfast everday			
Yes	19.28 ± 4.23		1-2
No	21.23 ± 4.1	F = 8.429	(p = 0.005)
Sometimes	20.43 ± 4.62	p < 0.001	1-3 (p = 0.006)
Having lunch everday			
Yes	19.74 ± 4.38		
No	19.64 ± 3.86	F = 0.016	
Sometimes	19.70 ± 4.47	p = 0.984	
Having dinner everday			
Yes	19.51 ± 4.21	F = 7.981	1-2
No	20.10 ± 4.1	p < 0.001	(p < 0.001)
Sometimes	21.80 ± 5.45		
Having balanced diet			1-2 (p < 0.001)
Yes	19.16 ± 4.16	F = 13.529	
No	21.91 ± 5.5	p < 0.001	1-3 (p = 0.002)
Sometimes	19.99 ± 4.1		

There was a statistically significant difference in terms of BMI according to having person with obesity in the family, having breakfast everyday, having dinner everyday and having balanced diet ($p<0.05$). BMI of students who have a person with obesity in the family compared to who have not is statistically significantly higher ($p<0.001$). BMI of students who have berakfast everyday compared to who have not ($p=0.005$) and have sometimes ($p=0.006$) are statistically significantly lower. BMI of students who have balanced diet compared to who have not ($p<0.001$) and have sometimes ($p=0.002$) is statistically significantly lower.

The total score average of the students' OAS was 57.70±8.7 (20–80) and the highest score among the subdimensions was obesity awareness 22.34±3.91 (8–42). The total score average of the students' PAQ-C was 22.34 ± 3.91 (8–42) (Table 3).

Table 3. Students' obesity awareness scale and physical activity questionnare for older children total scores (n=725)

Scales	$\bar{X}$ ± SD	Min-Max
Obesity awareness scale		
Awareness	22.34 ± 3. 34	8 – 42
Nutrition	20.57 ± 3.48	7 – 28
Physical activity	14.78 ± 2.68	5 – 20
Total score	57.70 ± 8.7	20 – 80
Physical activity questionnare for older children		
Total score	22.34 ± 3.91	8 – 42

There was a significant releationship between students' BMI and obesity awareness (r=-0.084, p=0.024). There was no significant releationship between students' BMI and physical activity (p<0.05) (Table 4).

Table 4. Correlations between students' BMI, obesity awareness scale and physical activity questionnare for older children total scores (n=725) [a]

	Obesity awareness scale total score	Physical activity questionnare for older children total score
BMI		
r*	-0.084	-0.034
p	0.024**	0.365

[a] *Pearson correlation test, **p<0.05

According to the results of the regression analysis, when the significance level corresponding to the F value is examined, the model established is statistically significant (F=5.124; p<0.05). Looking at the beta coefficient value, t value and significance level of the independent variable; OAS has a statistically significant effect on BMI (t=2.264, p<0.05). In this study, 0.7% of the change on the BMI is explained (Regulated R^2=0.007). One unit increase in the OAS variable causes a decrease of 0.084 on the BMI (β=0.084, p<0.05) (Table 5).

Discussion

This study was carried out to determine relationship between obesity, obesity awareness, physical activity and and other associated factors in school age children. Although there are studies in the literature that examine the obesity levels of students, studies examining the relationship between BMI and obesity awareness are quite limited.[6–8] In this study, 12.7 % students were overweight and 18.3% were with obesity. There was a statistically significant difference in terms of BMI according to having person with obesity in the family, having breakfast everyday, having dinner everyday and having balanced diet (p<0.05). There was a significant releationship between students' BMI and obesity awareness (r=0.084, p=0.024). One unit increase in the OAS variable causes a decrease of 0.084 on the BMI (β=-0.084, p<0.05). There was no significant releationship between students' BMI and physical activity (p<0.05).

In this study, 12.7 % students in this study were overweight and 18.3% were with obesity. In a meta analysis which was carried out among the children in Turkey between 1990–2015, prevalence of excess weight has increased from 0.6% to 7.3% by a 11.6-fold increase during 1990–1995 and 2011–2015.[14] This result appears to be an estimated outcome according to the previous literature. Obesity is increasing in Turkey as well as in whole world.

Obesity awareness of the students was found to be at a moderate level [57.70±8.7 (20–80)] in this study. In the study by examining knowledge levels and awareness about obesity, it was reported that only 25.4% of the children had awareness among 528 school-aged children.[4]

There was a relationship between BMI and obesity awareness. A negative correlation was determined between students' BMI and obesity awareness (r=-0.084, p=0.024). A negative correlation was reported o between BMI and obesity awareness in an study conducted with overweight children aged 10–14 years (r=-0.180, p=0.001).[6] As awareness increases, BMI decreases in this study. Children pay attention to their nutrition or physical activity level.

As a result of this study, there was no significant releationship between students' BMI and physical activity. As awareness increases, children pay attention to their nutrition in this study. However, Wang et al. have reported a negative correlation between physical activity level and BMI among 742 children between 8-13 years old.[15] One of the issues addressed in the scope of obesity awareness is physical activity in the literature.[16,17] One of the factors that can be evaluated in the scope of obesity awareness is physical activity.[18]

Table 5. Associations between students' BMI and obesity awareness scale total scores of the students* (n=725) [a]

Dependent variables	Independent variables	β	SD	Beta	t	p	F	Model (p)	R^2	Durbin Watson
BMI	**Constant**	17.298	1.087	–	15.915	0.000	5.124	0.024**	0.007	1.762
	Obesity Awareness	0.042	0.019	-0.084	2.264	0.024				

[a] * Simple linear regression analysis, ** p<0.05

As a result of this study, BMI of students who have a person with obesity in the family compared to who have not is statistically significantly higher ($p<0.001$). Families and consumption are at the nexus of the problem, as childhood weight issues depend significantly on family-related influences (genetic predispositions, physical activities, and household food consumption practices.[19,20] Some children with obesity can show higher number of nuclear abnormalities compared with children with normal weight.[21]

In this study, BMI of students who have berakfast everyday compared to who have not are lower. Also, BMI of students who have balanced diet compared to who have not are lower. One of the significant factors increasing the risk of obesity is dietary habits. Most children tend to skip meals.[22] Especially balanced diet should be frequently evaluated in order to identify and improve dietary habits.[23]

Conclusion

In conclusion, 12.7% were overweight and 18.3% were with obesity. There was a statistically significant difference in terms of body mass index according to having person with obesity in the family, having breakfast everyday, having dinner everyday and having balanced diet. There was a significant releationship between students' BMI and obesity awareness.

Obesity awareness and having a balanced diet are crucial in childhood obesity prevention. Screening for obesity and related factors, such as obesity awareness, having a balanced diet should begin in school-aged children for childhood obesity prevention and health promotion and disease prevention. Also, trainings which were given by health care providers and school health nurses for the prevention of childhood obesity would be effective in increasing obesity awareness. To prevent the obesity and improve obesity awareness in children, it is important to direct and infom the children about nutrition and having a balanced diet. Efforts are needed to develop and implement interventions and policies that may promote school nurse active engagement in related factors (such as balanced diet and obesity awareness) in childhood obesity prevention practices. In addition, studies examining the interventions and programs to prevent the childhood obesity should be conducted.

Declarations

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Author contributions

Conceptualization, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Methodology, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Software, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Validation, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Formal Analysis, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Investigation, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Resources, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Data Curation, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Writing – Original Draft Preparation, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Writing – Review & Editing, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Visualization, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Supervision, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Project Administration, A.T., A.F.O., T.K.A., A.K. and S.Ö.; Funding Acquisition, A.T., A.F.O., T.K.A., A.K. and S.Ö.

Conflicts of interest

The authors declare that there are no conflict of interests.

Data availability

Data available on request from the authors.

Ethics approval

The study was approved by ethics committee of Zonguldak Bülent Ecevit University Human (Date: 26.01.2017, No: 192).

References

1. Gülmez R, Demirel F, Emir S. Obez çocuk ve ergenlerde obeziteye eşlik eden endokrin ve metabolik bozukluklar ve ilişkili faktörler. *Turkish J Pediatr Dis.* 2015;2:104–112.
2. World Health Organization. Childhood overweight and obesity. 2016. https://www.who.int/. Accessed July 21, 2022.
3. Ezzati M. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: A pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet.* 2017;390:2627–2642.
4. Alasmari HD, Al-Shehri AD, Aljuaid TA, Alzaidi BA, Alswat KA. Relationship Between Body Mass Index and Obesity Awareness in School Students. *J Clin Med Res.* 2017;9(6):520–524.
5. Bhattacharya P, Gogoi N, Roy A. Prevalence and awareness of obesity and its risk factors among adolescents in two schools in a Northeast Indian city. *Int J Med Sci Public Heal.* 2016;5(6):1111.
6. Bozbulut R, Ertaş-Öztürk Y, Döğer E, Bideci A, Köksal E. Increased obesity awareness and adherence to healthy lifestyle-diet reduce metabolic syndrome risk in overweight children. *J Am Coll Nutr.* 2020;39(5):432–437.
7. Sheinbein DH, Stein RI, Hayes JF, et al. Factors associated with depression and anxiety symptoms among children seeking treatment for obesity: A social-ecological approach. *Pediatr Obes.* 2019;14(8):1–9.
8. Park S, Choo J, Chang SO. Healthy eating for obese children from socioeconomically disadvantaged families: Its ecological factors and strategies. *J Transcult Nurs.* 2019;30(3):268–279.

9. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *J Clin Epidemiol.* 2008;61(4):344–349.
10. Mölbert SC, Sauer H, Dammann D, et al. Multimodal body representation of obese children and adolescents before and after weight-Loss treatment in comparison to normal-Weight children. *PLoS One.* 2016;11(11):1–14.
11. AlBlooshi A, Shaban S, AlTunaiji M, et al. Increasing obesity rates in school children in United Arab Emirates. *Obes Sci Pract.* 2016;2(2):196–202.
12. Kafkas ME, Özen G. The Turkish Adaptation of the Obesity awareness Scale: A validity and reliability study. *İnönü Univ J Phys Educ Sport Sci.* 2014;1(2):1–15.
13. Tanır H, Yoncalık O. The relationship between elementary school eight grade students' physical activity level and various anthropometric characteristics with their academic achievement. *Nigde Univ J Phys Educ Sport Sci.* 2014;8(1):1–10.
14. Alper Z, Ercan İ, Uncu Y. A meta-analysis and an evaluation of trends in obesity prevalence among children and adolescents in Turkey: 1990 through 2015. *J Clin Res Pediatr Endocrinol.* 2018;10(1):59–67.
15. Wang JJ, Baranowski T, Lau WP, Chen TA, Pitkethly AJ. Validation of the physical activity questionnaire for older children (PAQ-C) among Chinese children. *Biomed Env Sci.* 2016;29(3):177–186.
16. Herbenick SK, James K, Milton J, Cannon D. Effects of family nutrition and physical activity screening for obesity risk in school-age children. *J Spec Pediatr Nurs.* 2018;23(4):1–5.
17. Wilk P, Clark AF, Maltby A, Smith C, Tucker P, Gilliland JA. Examining individual, interpersonal, and environmental influences on children's physical activity levels. *SSM - Popul Heal.* 2018;4(2017):76–85.
18. Friedenreich CM, Ryder-Burbidge C, McNeil J. Physical activity, obesity and sedentary behavior in cancer etiology: epidemiologic evidence and biologic mechanisms. *Mol Oncol.* 2021;15(3):790–800.
19. Moore ES, Wilkie WL, Desrochers DM. All in the family? Parental roles in the epidemic of childhood obesity. *J Consum Res.* 2017;43(5):824–859.
20. Bahreynian M, Qorbani M, Khaniabadi BM, Motlagh ME, Safari O. Association between obesity and parental weight status in children and adolescents. *J Clin Res Pediatr Endocrinol.* 2017;9(2):111–117.
21. Arreola E, Martínez O, Armendáriz P, Maldonado M, Lara B. Evaluation of genetic damage and eating habits in children with normal weight and obesity in school age. *Nutr Hosp.* 2019;36(2):309–314.
22. Demir G, Süer O, Kaya S. Investigation of milk and dairy products consumption of adolescents. *J Curr Res Heal Sect.* 2018;8(2):303–314.
23. Larson N, Miller JM, Eisenberg ME, Watts AW, Story M, Neumark-Sztainer D. Multicontextual correlates of energy-dense, nutrient-poor snack food consumption by adolescents. *Appetite.* 2017;112:23–34.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.13

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 342–347

ORIGINAL PAPER

Predictive value of blood urea nitrogen to serum albumin ratio in estimating in-hospital mortality in patients with upper gastrointestinal bleeding

Erdem Kurt [1], Sebnem Zeynep Eke Kurt [2]

[1] Department of Emergency Medicine, Istanbul Training and Research Hospital, Istanbul, Turkey
[2] Department of Emergency Medicine, Taksim Training and Research Hospital, Istanbul, Turkey

ABSTRACT

Introduction and aim. The aim of this study was to examine the usability of blood urea nitrogen to serum albumin ratio (BAR) as a prognostic predictor of in-hospital mortality in patients with gastrointestinal (GI) bleeding.

Material and methods. The electronic medical records of the patients who applied to the emergency department due to upper GI bleeding during the study period were reviewed. The receiver operating characteristic (ROC) curve and the area under the curve (AUC) were used to evaluate each discriminant cut-off value to estimate mortality.

Results. The study included 225 patients. The median (IQR) age of the patients was 75.0 (68.0–84.0) and 94 (41.8%) were female. AUC was determined as 0.784±0.055 (95% CI, 0.677–0.892) for BAR ($p<0.001$) in terms of in-hospital mortality. The cut-off value of BAR for this outcome was calculated as 16.26. In this cut-off value, sensitivity was 71.43%, specificity 82.84%, positive predictive value (PPV) 30.00% and negative predictive value (NPV) 96.57%.

Conclusion. BAR is a useful tool that can be used to predict the in-hospital mortality of patients with GI bleeding. Patients with GI bleeding with a BAR above 16.26 will require more aggressive and timely intervention.

Keywords. albumin, blood urea nitrogen, mortality, upper gastrointestinal bleeding

Introduction

Upper gastrointestinal (GI) bleeding is one of the most common causes of emergency department (ED) visits worldwide.[1–3] There are more than 800,000 ED visits in the United States each year due to the disease, and half of these are hospitalized.[4] Endoscopic and pharmacological advances have resulted in reductions in mortality from GI bleeding.[5,6] However, despite diagnostic and therapeutic advances, the mortality rate due to GI bleeding still varies between 5-10%.[7] Therefore, risk identification strategies are important in EDs.

It is aimed to predict the prognosis of the disease and to recognize critically ill patients early by using scoring systems and laboratory values in GI diseases.[8,9] There are scoring systems used to predict mortality, length of hospital stay and endoscopy requirement in GI bleeding.[10,11] However, their use in ED is difficult due to the lack of endoscopy units in every hospital and the complex structures of the scores.

Urea is formed by the liver metabolization of its nitrogen-containing products and is excreted by the kidneys. Clinicians often use blood urea nitrogen (BUN) to measure the amount of nitrogen from urea in the blood as an index of kidney function. BUN is a biomarker that provides valuable information about the clinical status of patients such as renal hypoperfusion, low cardiac out-

Corresponding author: Erdem Kurt, e-mail: erdemkurt@live.com

Received: 29.06.2022 / Revised: 20.07.2022 / Accepted: 22.07.2022 / Published: 30.09.2022

Kurt E, Kurt SZE. *Predictive value of blood urea nitrogen to serum albumin ratio in estimating in-hospital mortality in patients with upper gastrointestinal bleeding.* Eur J Clin Exp Med. 2022;20(3):342–347. doi: 10.15584/ejcem.2022.3.13.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

put, dehydration and neurohumoral activity.[12] Albumin plays an important role in many physiological mechanisms, including the regulation of osmotic pressure. It takes part in the transport of molecules such as hormones, cholesterol, calcium, iron, bilirubin, free fatty acids and drugs.[13] It has been shown by various studies that the BUN albumin ratio (BAR) increases in many diseases.[14,15]

Aim

The aim of this study was to examine predictive value of blood urea nitrogen to serum albumin ratio in estimating in-hospital mortality in patients with upper gastrointestinal bleeding who visited the ED.

Material and methods

Between June 1, 2021 and June 1, 2022, this retrospective cohort research was done in the ED of a tertiary care hospital. The institutional review board authorized the analysis and waived permission (Ethics Committee Ruling number: 2011/KAEK/50/211).

All patients over the age of 18 who visited to ED within the period determined for the study and were diagnosed with upper GI bleeding were included in the study. By scanning the hospital electronic medical records; vital parameters, comorbid diseases, medications, length of hospital stay and in-hospital mortality were recorded in a pre-created dataset. After all the data were processed by the first researcher, the second researcher controlled them. The BAR was defined as the BUN value divided by the albumin value, and this value was calculated. The definition of upper GI bleeding was based on the presence of at least one of the following three features: hematemesis, melena, or solid clinical evidence and laboratory support for acute blood loss from the upper gastrointestinal (UGI) tract. Patients with a diagnosis other than upper GI bleeding, patients with deficient BUN and/or albumin values, patients transferred from another hospital, patients who died or were discharged in the ED were excluded from the study. Death within the hospital during index admission defined as in-hospital mortality and interval between hospital admission (admissions from ED) and discharge defined as the length of hospital stay.[16] The primary study outcome was all-cause in-hospital mortality. The secondary study outcome was the relationship between BAR and length of the hospital stay.

Statistical analysis

The descriptive statistics were presented in median values and interquartile ranges (IQR; 25% to 75%) for the quantitative variables; and frequencies and percentages for the categorical variables. Normality tests were carried out by using one-sample Kolmogorov–Smirnov and Shapiro-Wilk tests and through histogram graphs. Patients were divided into two groups as survivors and non-survivors and all variables were compared according to groups. The frequencies of categorical variables were compared using Pearson's chi-square and Fisher's exact test as appropriate. The median values of the quantitative variables were compared using the Mann-Whitney U test. Receiver operating characteristic (ROC) analysis was performed to evaluate the predictive power of the BAR in terms of in-hospital mortality. In the light of the ROC analysis, the optimum cut-off points were calculated for BAR according to Youden's index. The correlations of BAR, in-hospital mortality and length of hospital stay variables were evaluated using point-biserial correlation and Spearman's rho correlation. A 2-sided P-value of 0.05 was regarded as statistically significant (except correlation analyses – correlation is significant at the 0.01 level). The area under the curve (AUC) was used to evaluate each discriminant cut-off value to predict in-hospital mortality. AUC of the non-diagnostic test is 0.50. If it is a perfect test, with zero false positives and zero false negatives, the value of the field would be 1.00. If the value under the curve is 0.90–1.00, it is excellent, 0.80–0.90 is good, 0.70–0.80 is medium, 0.60–0.70 is weak, 0.50–0.60 is unsuccessful. All data analyses were performed using SPSS statistical software (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.).

Results

A total of 291 patients were included in the study. Because 34 patients were diagnosed other than upper GI bleeding, 8 patients had deficient BUN and/or albumin values, 17 patients were transferred from another hospital, 2 patients died in ED and 5 patients were discharged from ED; were not included in the study. The study was completed with 225 patients. The median (IQR) age of the patients was 75 (68–84) years and 94 (41.8%) were female. In the first evaluation; the Glasgow coma scale/score (GCS) of 217 patients (96.4%) was 15, six (2.7%) of them had a GCS of 14, and two (0.9%) had a GCS of 13. The median (IQR) systolic blood pressure of the patients was 122 (115–131) mmHg and the pulse rate was 97 (87–105) bpm. The comorbidities of the patients enrolled in the study were examined, the first three were acute coronary syndrome (38.7%), diabetes mellitus (26.2%), and congestive heart failure (20.4%). Fourty-seven (20.9%) of the patients had no comorbidities, 71 (31.6%) had one, 61 (27.1%) had two, and 46 (20.4%) had three or more comorbidities. 5.8% of the patients were using steroids, 24.4% were using anticoagulants, 32.9% were using antiplatelet agents and 13.8% were using nonsteroidal anti-inflammatory drugs. While 74 (32.9%) of the patients did not use any medication, 130 (57.8%) were using one drug, 20 (8.9%) were using two drugs, and 1 (0.4%) was using three drugs. In terms of gastrointestinal (GI) bleeding symptoms melena

Table 1. Demographic, laboratory and clinical characteristics of the patients, n=225 [A]

Variables	Total (%)
Gender[a]	
female	94 (41.8)
male	131 (58.2)
Age[b], years	75.0 (68.0–84.0)
GCS[a]	
13	2 (0.9)
14	6 (2.7)
15	217 (96.4)
SBP[b], mmHg	122 (115–131)
Pulse rate[b], bpm	97 (87–105)
Comorbidities[a]	
Acute coronary syndrome	87 (38.7)
Congestive heart failure	46 (20.4)
Peripheral vascular disease	11 (4.9)
Cerebrovascular disease	24 (10.7)
Dementia	18 (8)
Hemiplegia or paraplegia	1 (0.4)
Chronic pulmonary disease	27 (12)
Rheumatologic disease	0 (0)
Peptic ulcer	23 (10.2)
Diabetes mellitus	59 (26.2)
Chronic renal failure	21 (9.3)
Liver disease	10 (4.4)
Malignancy	27 (12)
Leukemia or Lymphoma	3 (1.3)
Number of comorbidities[a]	
0	47 (20.9)
1	71 (31.6)
2	61 (27.1)
2<	46 (20.4)
Drugs[a]	
Steroids	13 (5.8)
Anticoagulants	55 (24.4)
Antiplatelet agents	74 (32.9)
Nonsteroidal anti-inflammatory	31 (13.8)
Number of drugs[a]	
0	74 (32.9)
1	130 (57.8)
2	20 (8.9)
3	1 (0.4)
GI bleeding symptoms[a]	
hematemesis	77 (34.2)
melena	184 (81.8)
hematochezia	6 (2.7)
Number of GI bleeding symptoms[a]	
0	6 (2.7)
1	172 (76.4)
2	46 (20.4)
3	1 (0.4)
Laboratory[b]	
Hemoglobin, g/dL	8.7 (6.6-10.2)
BUN, mg/dL	30.5 (18.7-50.5)
Creatinine, mg/dL	0.99 (0.79-1.37)
Albumin, g/dL	3.4 (2.96-3.79)
BUN/Albumin, mg/g	8.92 (5.61-15.5)
Length of hospital stay[b], days	5 (5-7)
In-hospital mortality[a]	21 (9.3)

[A] GCS – Glasgow coma scale, SBP – systolic blood pressure, GI – gastro-intestinal, BUN – blood urea nitrogen, [a] n (%), [b] median (IQR)

was the leading one with 81.8%. Six (2.7%) of the patients had no GI bleeding symptom (These patients were included in the study because they had decreased hemoglobin values or symptoms of GI bleeding during their observation in the ED) 172 (76.4%) had one, 46 (20.4%) had two, and 1 (0.4%) had all three (hematemesis, melena and hematochezia) symptoms. The median length of hospital stay was 5 (5–7) days and 21 patients (9.3%) died. The demographic, laboratory and clinical characteristics of the patients were shown in Table 1.

The patients were divided into two groups as survivors and non-survivors, and all variables were compared over these two groups. There was no significant difference between the groups in gender, age, GCS, SBP, pulse rate, number of comorbidities, drugs used, GI bleeding symptoms, number of symptoms and length of hospital stay variables (p>0.05 for all). Chronic renal failure was found in 23.8% of the non-survivor group and 76.2% of the survivor group (p=0.033). No significant difference was found between the groups in other comorbidities (p>0.05 for all). Laboratory tests were compared between groups; BUN, creatinine and BUN/albumin levels were significantly higher in the non-survivor group (p<0.001, p=0.008 and p<0.001; respectively); and albumin was significantly lower in non-survivors (p<0.001). There was no difference between the groups in terms of hemoglobin levels and also the length of hospital stay days (p=0.356 and 0.172, respectively). The comparisons of all these parameters were shown in Table 2.

ROC analyses were performed to evaluate the power of the BAR to predict in-hospital mortality (Figure 1).

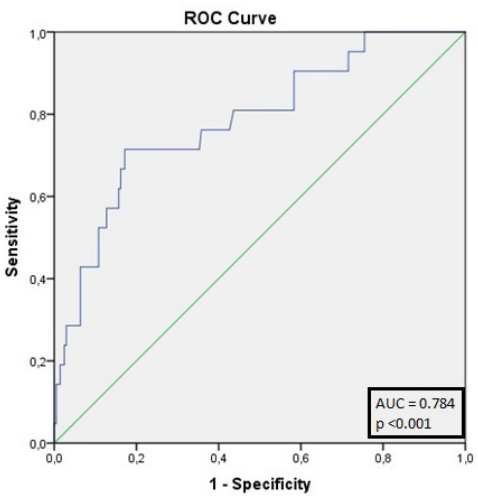

Fig. 1. ROC analysis of BUN/Albumin ratio in terms of in-hospital mortality (AUC of BUN/albumin=0.784±0.055 (95% CI=0.677–0.892), (p<0.001))

The area under the curves (AUCs) was determined as 0.784±0.055 (95% CI, 0.677–0.892) for BAR (p<0.001). According to the result of the ROC analyses, the optimum cut-off points of the BAR were determined

using Youden's index. The cut-off value of BAR for this outcome was calculated as 16.26. In this cut-off value, sensitivity was 71.43%, specificity 82.84%, PPV 30.00% and NPV 96.57% (Table 3).

Table 3. Optimum cut-off points* of BUN/albumin ratio in terms of in-hospital mortality [A]

Cut-off point	Sens (%)	Spec (%)	PPV (%)	NPV (%)	AUC	Youden's Index
16.15	71.43	81.86	28.85	96.53	0.784	0.533
16.18	71.43	82.35	29.41	96.55	0.784	0.538
16.26	71.43	82.84	30.00	96.57	0.784	0.543

[A] BUN – blood urea nitrogen, Sens – sensitivity, Spec – specificity, PPV – positive predictive value, NPV – negative predictive value AUC – area under the curve, *Cut-off points with the three highest Youden's index value were shown

Correlation analysis was performed to evaluate the relationships between BAR, in-hospital mortality and length of hospital stay parameters (Table 4). There was a fair positive correlation between BAR and in-hospital mortality (R=0.366, p<0.001), and there was also a poor positive correlation between BAR and lenght of the hospital stay (R=0.244, p<0.001).

Table 4. Correlation matrix of BUN/albumin, in-hospital mortality and length of hospital stay [A]

Correlations	BUN/albümin	In-hospital mortality	Length of hospital stay
BUN/albumin	-		
In-hospital mortality	R=0.366 p<0.001*	-	
Length of hospital stay	R=0.244 p<0.001**	R=0.103 p=0.125*	-

[A] BUN – blood urea nitrogen, *Point-biserial correlation, correlation is significant at the 0.01 level (2-tailed), **Spearman's rho correlation, correlation is significant at the 0.01 level (2-tailed)

Discussion

This study examined the relationship between BAR and in-hospital mortality and length of the hospital stay in patients with GI bleeding who visited the ER. It was concluded that BAR can be used as a good predictor in patients with GI bleeding.

GI bleeding is a disease condition that is frequently seen in EDs and can be seen in clinical presentations with high mortality. Although there are some clinical scoring systems used to determine the severity of GI bleeding; these scoring systems are not always useful in emergency practice. For this reason, laboratory parameters that can be viewed quickly in the ED can guide physicians in patient management.

There are studies in the literature in which albumin and BUN values are used as prognostic tools in patients

Table 2. The comparison of parameters between survivors and non-survivors [A]

Variables	Survivors, n=204 (%)	Non-survivors, n=21 (%)	p value
Gender[a]			0.719*
female	86 (91.5)	8 (8.5)	
male	118 (90.1)	13 (9.9)	
Age[b], years	75.0 (68.0-83.0)	81.0 (75.0-85.0)	0.061**
GCS[a]			0.165***
<15	6 (66.7)	2 (33.3)	
15	198 (91.2)	19 (8.8)	
SBP[b], mmHg	122 (115-132)	121 (102-124)	0.103**
Pulse rate[b], bpm	97 (87-105)	104 (93-109)	0.058**
Comorbidities[a]			
Acute coronary syndrome	77 (37.7)	10 (47.6)	0.376*
Congestive heart failure	40 (19.6)	6 (28.6)	0.392***
Peripheral vascular disease	9 (4.4)	2 (9.5)	0.274***
Cerebrovascular disease	23 (11.3)	1 (4.8)	0.708***
Dementia	16 (7.8)	2 (9.5)	0.678***
Hemiplegia or paraplegia	1 (0.5)	0 (0.0)	1.000***
Chronic pulmonary disease	25 (12.3)	2 (9.5)	1.000***
Rheumatologic disease	0 (0.0)	0 (0.0)	-
Peptic ulcer	20 (9.8)	3 (14.3)	0.458***
Diabetes mellitus	52 (25.5)	7 (33.3)	0.437*
Chronic renal failure	16 (7.8)	5 (23.8)	0.033***
Liver disease	9 (4.4)	1 (4.8)	1.000***
Malignancy	25 (12.3)	2 (9.5)	1.000***
Leukemia or Lymphoma	3 (1.5)	0 (0.0)	1.000***
Number of comorbidities[a]			0.199***
0	45 (22.1)	2 (9.5)	
1	65 (31.9)	6 (28.6)	
2	56 (27.5)	5 (23.8)	
2<	38 (18.6)	8 (38.1)	
Drugs[a]			
Steroids	11 (5.4)	2 (9.5)	0.347***
Anticoagulants	47 (23.0)	8 (38.1)	0.126*
Antiplatelet agents	66 (32.4)	8 (38.1)	0.594*
Nonsteroidal anti-inflammatory	26 (12.7)	5 (23.8)	0.181***
Number of drugs[a]			0.036***
0	71 (34.8)	3 (14.3)	
1	117 (57.4)	13 (61.9)	
2	15 (7.4)	5 (23.8)	
3	1 (0.5)	0 (0.0)	
GI bleeding symptoms[a]			
hematemesis	70 (34.3)	7 (33.3)	0.928*
melena	166 (81.4)	18 (85.7)	0.773***
hematochezia	5 (2.5)	1 (4.8)	0.448***
Number of GI bleeding symptoms[a]			0.890***
0	6 (2.9)	0 (0.0)	
1	156 (76.5)	16 (76.2)	
2	41 (20.1)	5 (23.8)	
3	1 (0.5)	0 (0.0)	
Laboratory[b]			
Hemoglobin, g/dL	8.8 (6.7-10.3)	8.4 (5.7-10.0)	0.356**
BUN, mg/dL	29.2 (18.2-46.3)	57.9 (37.9-73.8)	<0.001**
Creatinine, mg/dL	0.96 (0.78-1.28)	1.21 (0.96-2.91)	0.008**
Albumin, g/dL	3.42 (3.01-3.84)	2.96 (2.42-3.36)	0.001**
BUN/Albumin, mg/g	8.5 (5.3-15.1)	20.6 (11.5-31.6)	<0.001**
Length of hospital stay[b], days	5 (5-7)	6 (5-10)	0.172**

[A] GCS – Glasgow coma scale, SBP – systolic blood pressure, GI – gGastro-intestinal, BUN – blood urea nitrogen, [a] n (%), [b] median (IQR), *Pearson Chi-Square test, ** Mann-Whitney U test, ***Fischer's Exact test

with GI bleeding. Albumin level decreases in chronic diseases. It also gives information about the nutritional and dehydration status of patients.[17] The serum BUN level increases in cases of severe hemorrhage and dehydration. For these reasons, these two laboratory parameters are used as variables of GI bleeding risk scores. BUN is a variant of the Glasgow Blatchford score, while albumin is a variant of the AIMS65 score.[11]

BAR increases in various critical diseases. In a retrospective study conducted by Huang et al. in 1370 patients diagnosed with COVID-19 in 2021, it was concluded that BAR is an independent predictor for the risk of critical illness in COVID-19 patients, with superior performance than CURB-65.[15] In the retrospective study of Zhao et al. in 1827 patients diagnosed with acute myocardial infarction in 2022; they concluded that BAR was calculated as 11.06 (7–18.59) ($p<0.001$) in the mortality group and 10.42 (7–16.71) in the four-year mortality group, and that a higher BAR value could be used as an independent predictor for four-year mortality.[18] In a study by Lee et al. in patients with lower GI bleeding in 2021, BUN ≥ 30 mg/dL and albumin ≤ 3.0 g/dL were associated with all-cause mortality.[19] In the study of Bae et al., the data of 596 geriatric patients with GI bleeding were analyzed and BAR and AIMS65 scores were compared. The study concluded that BAR was as successful as AIMS65 in estimating in-hospital mortality with an AUC of 0.770.[20] In our study, BAR was found to be successful in estimating in-hospital mortality with an AUC of 0.784. At a cut-off value of 16.26, its sensitivity was calculated as 71.43%, specificity 82.84%, PPV 30.00% and NPV 96.57%. In the light of this information, our study was found to be compatible with the studies in the literature.

The main limitations of our study are that it is single-center and retrospective. The lack of data in the medical records, the fact that the tests we used in our study were not requested, and the prognosis information could not be obtained by being transferred to another hospital caused many patients to be excluded from the study.

Conclusion

Simple, inexpensive, rapid and noninvasive tests should be used to diagnose, treat, and predict prognosis in patients with GI bleeding in EDs. BAR is a useful tool that can be used to predict the outcome of patients with GI bleeding. Patients with GI bleeding with a BAR above 16.26 will require more aggressive and timely intervention.

Declarations

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Author contributions

Conceptualization, E.K. and S.Z.E.K.; Methodology, E.K.; Software, E.K.; Validation, E.K. and S.Z.E.K.; Formal Analysis, S.Z.E.K.; Investigation, E.K.; Resources, S.Z.E.K.; Data Curation, E.K.; Writing – Original Draft Preparation, E.K..; Writing – Review & Editing, E.K. and S.Z.E.K.; Visualization, S.Z.E.K.; Supervision, E.K.; Project Administration, E.K.; Funding Acquisition, S.Z.E.K.

Conflicts of interest

Authors declare that they have no conflicts of interest.

Data availability

The authors agree to the conditions of publication including the availability of data and materials in our manuscript.

Ethics approval

This study was approved by the local ethics committee (2011/KAEK/50/211).

References

1. Lau JYW, Barkun A, Fan D ming, Kuipers EJ, Yang Y sheng, Chan FKL. Challenges in the management of acute peptic ulcer bleeding. *Lancet*. 2013;381(9882):2033–2043.
2. Rajan SS, Sawe HR, Iyullu AJ, et al. Profile and outcome of patients with upper gastrointestinal bleeding presenting to urban emergency departments of tertiary hospitals in Tanzania. *BMC Gastroenterol*. 2019;19(1):212.
3. Banister T, Spiking J, Ayaru L. Discharge of patients with an acute upper gastrointestinal bleed from the emergency department using an extended Glasgow-Blatchford Score. *BMJ Open Gastroenterol*. 2018;5(1):e000225.
4. Peery AF, Crockett SD, Murphy CC, et al. Burden and cost of gastrointestinal, liver, and pancreatic diseases in the United States: Update 2018. *Gastroenterology*. 2019;156(1):254–272.e11.
5. Leontiadis GI, Sharma VK, Howden CW. Proton pump inhibitor therapy for peptic ulcer bleeding: Cochrane collaboration meta-analysis of randomized controlled trials. *Mayo Clin Proc*. 2007;82(3):286–296.
6. Baradarian R, Ramdhaney S, Chapalamadugu R, et al. Early intensive resuscitation of patients with upper gastrointestinal bleeding decreases mortality. *Am J Gastroenterol*. 2004;99(4):619–622.
7. Laine L, Yang H, Chang SC, Datto C. Trends for incidence of hospitalization and death due to GI complications in the United States from 2001 to 2009. *Am J Gastroenterol*. 2012;107(8):1190–1195.
8. Ak R, Doğanay F, Unal Akoğlu E, et al. Predictive value of scoring systems for the diagnosis of acute appendicitis in emergency department patients: Is there an accurate one? *Hong Kong J Emerg Med*. 2020;27(5):262–269.

9. Tekyol D, Kılıç M. Usage of the Ranson criteria and neutrophil-lymphocyte ratio measured on presentation to the emergency department in the prediction of mortality in acute pancreatitis. *Signa Vitae*. 2022:1–5.
10. Gu L, Xu F, Yuan J. Comparison of AIMS65, Glasgow–Blatchford and Rockall scoring approaches in predicting the risk of in-hospital death among emergency hospitalized patients with upper gastrointestinal bleeding: a retrospective observational study in Nanjing, China. *BMC Gastroenterol*. 2018;18(1):98.
11. Ak R, Hökenek NM. Comparison of AIMS65 and Glasgow Blatchford scores in predicting mortality in patients with upper gastrointestinal bleeding. *Rev Assoc Med Bras (1992)*. 2021;67(5):766–770.
12. Arihan O, Wernly B, Lichtenauer M, et al. Blood Urea Nitrogen (BUN) is independently associated with mortality in critically ill patients admitted to ICU. *PLoS One*. 2018;13(1):e0191697.
13. Aziz M, Fatima R, Lee-Smith W, Assaly R. The association of low serum albumin level with severe COVID-19: a systematic review and meta-analysis. *Crit Care*. 2020;24:255.
14. Ryu S, Oh SK, Cho SU, et al. Utility of the blood urea nitrogen to serum albumin ratio as a prognostic factor of mortality in aspiration pneumonia patients. *Am J Emerg Med*. 2021;43:175–179.
15. Huang D, Yang H, Yu H, et al. Blood urea nitrogen to serum albumin ratio (BAR) predicts critical illness in patients with coronavirus disease 2019 (Covid-19). *Int J Gen Med*. 2021;14:4711–4721.
16. Lingsma HF, Bottle A, Middleton S, Kievit J, Steyerberg EW, Marang-van de Mheen PJ. Evaluation of hospital outcomes: the relation between length-of-stay, readmission, and mortality in a large international administrative database. *BMC Health Serv Res*. 2018;18(1):116.
17. Nicholson JP, Wolmarans MR, Park GR. The role of albumin in critical illness. *Br J Anaesth*. 2000;85(4):599–610.
18. Zhao D, Liu Y, Chen S, et al. Predictive Value of Blood Urea Nitrogen to Albumin Ratio in Long-Term Mortality in Intensive Care Unit Patients with Acute Myocardial Infarction: A Propensity Score Matching Analysis. *Int J Gen Med*. 2022;15:2247–2259.
19. HS, Moon HS, Kwon IS, et al. Validation and modification of existing mortality prediction models for lower gastrointestinal bleeding: a retrospective study. *BMC Gastroenterol*. 2021;21(1):450.
20. Bae SJ, Kim K, Yun SJ, Lee SH. Predictive performance of blood urea nitrogen to serum albumin ratio in elderly patients with gastrointestinal bleeding. *Am J Emerg Med*. 2021;41:152–157.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.14

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 348–354

ORIGINAL PAPER

Nonthyroidal illness syndrome as independent predictor of hospital mortality in the elderly hospitalized patients with COVID-19 pneumonia – single-center observation

Aleksandra Młodożeniec [1], Renata Orłowska-Florek [1,2], Adrianna Czarnożycka-Wróbel [1], Krzysztof Gargasz [2], Agnieszka Gala-Błądzińska [1,2]

[1] Department of Internal Medicine, Nephrology and Endocrinology, St. Queen Jadwiga Clinical District Hospital No. 2, Rzeszów, Poland
[2] Medical College of Rzeszow University, Institute of Medical Sciences, Rzeszów, Poland

ABSTRACT

Introduction and aim. Elderly patients with COVID-19 are at increased risk for adverse outcomes. This study aims to evaluate the prevalence of nonthyroidal illness syndrome (NTIS) in hospitalized patients with COVID-19 pneumonia, its independent impact on patients' survival. Furthermore, to investigate selected inflammatory biomarkers in those patients and to determine whether they predict mortality associated with the disease.

Material and methods. In this single-centered, retrospective study, the medical records of 53 patients with confirmed SARS-CoV-2 infection who attended the provincial hospital between October 2020 and January 2021 were reviewed. Demographic data, laboratory values, comorbidities, treatments, and clinical outcomes were collected. We compared the data in survivor and non-survivor groups.

Results. Of 393 adult patients with SARS-CoV-2 pneumonia, 53 (13,49%) met the inclusion criteria and were included. The median age was 72±12.2 years, 26 patients (49%) were men. The NTIS prevalence was 62.3% and showed a strong independent correlation with disease severity and mortality in COVID-19 patients (p=0.01). The interleukin-6, white blood cells, ferritin and neutrophil ratios also differed significantly statistically between survivors and non-survivors.

Conclusion. NTIS and the lowering level of FT3 pose an independent prognostic marker of clinical deterioration and higher mortality in elderly patients with COVID-19.

Keywords. COVID-19, elderly, mortality, NTIS, pneumonia

Introduction

COVID-19 is an acute infectious disease of the respiratory system caused by the coronavirus of the severe acute respiratory syndrome (SARS-CoV-2). Most patients have mild symptoms and recover without special treatment. However, some of them develop serious complications and need hospital care. Most of the patients requiring hospitalization due to COVID-19 pneumonia constitute the geriatric population. Infection-hospitalization ratio estimates ranged from 0.4% for those younger than 40 years to 9.2% for those older than 60 years.[1] The mortality rate for patients with COVID-19 admitted to hospitals is high.[2] SARS-CoV-2 is known to have direct effects on endocrine glands, including the pituitary and thyroid gland. The virus has been detected in the pituitary gland after mortem. The hypothalamic-pi-

Corresponding author: Agnieszka Gala-Błądzińska, e-mail: aggala@ur.edu.pl

Received: 21.06.2022 / Revised: 29.07.2022 / Accepted: 29.07.2022 / Published: 30.09.2022

Młodożeniec A, Orłowska-Florek R, Czarnożycka-Wróbel A, Gargasz K, Gala-Błądzińska A. *Nonthyroidal illness syndrome as independent predictor of hospital mortality in the elderly hospitalized patients with COVID-19 pneumonia – single-center observation.* Eur J Clin Exp Med. 2022;20(3):348–354. doi: 10.15584/ejcem.2022.3.14.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

tuitary-thyroid axis may be one of the most susceptible to disturbance in patients with COVID-19.[3] The mRNA of the SARS-CoV-2 receptor, ACE-2 is expressed in thyroid cells.[4] Three main mechanisms for thyroid function disorders in patients with COVID-19 are: a direct viral effect on thyroid cells; an indirect effect of the systemic inflammatory immune response; and the most common thyroid dysfunction in the shape of Non-thyroidal illness syndrome (NTIS).[4] NTIS also known as "sick euthyroid syndrome or low T3 syndrome" is usually described as a transient biochemical deviation of thyroid hormone levels and is common in the hospitalized older population with acute or chronic critical illness. About 93% of total triiodothyronine (T3) is produced by the peripheral conversion from total thyroxine (T4) so this syndrome is considered to be a condition with impaired peripheral conversion of T4 to T3.[5,6]

One of the theories of the NTIS pathogenesis in COVID-19 includes suppression of hypothalamic thyrotropin-releasing hormone, and as a result reduced secretion of TSH. Sometimes distinguishing between NTIS and central hypothyroidism can be difficult.[7] We still do not know whether hormone responses represent an adaptive, physiological response or they are a maladaptive response that contributes to the worsening of the disease.[8]

The most common hormone pattern in NTIS is low T3 and free triiodothyronine (FT3) with low or normal free thyroxine (FT4) and normal or decreased levels of thyrotropin (TSH). The elevated plasma reverse (rT3) may occur.[9]

The mechanism of this syndrome is not yet well defined, so there is controversy about the indication of the treatment of this condition with hormone replacement therapy.[10] Sciacchitano et al. indicated the method which could represent the system in recognizing the efficacy of T3 treatment in NTIS.[11] NTIS occurs more frequently among patients with more severe COVID-19. It is clinically relevant whether the occurrence of NTIS on admission predicts adverse clinical outcomes in COVID-19 patients and whether this syndrome can be useful for clinicians in comprehensive geriatric assessment for management decisions.[12] Despite many studies, the prognostic role of thyroid hormone abnormalities in older patients remains uncertain.

Aim

The aim of this study was to investigate the frequency of NTIS in hospitalized elderly patients with COVID-19 pneumonia and to evaluate if it is an independent risk factor of the deterioration and mortality of those patients. Furthermore, we were additionally looking for the role of selected inflammatory biomarkers and their independent impact on patient's survival.

Material and methods

Ethics approval

This retrospective study was approved by the Bioethics Committee of the University of Rzeszów (Reference No. 12/05/2020). The procedures used in this study adhere to the tenets of the Declaration of Helsinki. We collated data mainly from electronic patient histories. Although personal identification numbers were used to match the datasets, these were subsequently anonymized.

Study desing

We retrospectively analyzed laboratory data from patients hospitalized in St. Queen Jadwiga Clinical District Hospital No. 2 Rzeszów, in the Department of Internal Medicine, Nephrology, and Endocrinology from October 2020 to January 2021 with confirmed SARS-CoV-2 infection (n=393). For this analysis, we selected the data of 53 patients (13.49%) with COVID-19 pneumonia. Hospitalization due to COVID-19 pneumonia was the criterion for inclusion in the study. All patients were tested positive for SARS-CoV-2 using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) with samples from the respiratory tract, throat, and nose swab. All of them were symptomatic and showed common respiratory tract symptoms such as dyspnea, cough, or gastric symptoms of COVID-19.

Table 1. Biochemical blood parameters tested at the hospitalization, the methodology of determinations and the range of reference values*

Findings	Reference range	Determination method
FT3, pg/mL	2.3–4.2	chemiluminescent immunoassay CLIA
FT4, ng/dL	0.89–1.76	
TSH, uIU/mL	0.55–4.78	
WBC, 10^9/L	4–11	fluorescence flow cytometry using a laser
Neutrophils, 10^9/L	1.9–7.5	
Lymphocytes, 10^9/L	0.9–4.5	
CRP, mg/L	< 10	immunoturbidimetry
Ferritin, ng/mL	10–291	chemiluminescent immunoassay CLIA
Interleukin-6, pg/mL	< 4.4	
PCT, ng/mL	< 0.03	

* Abbreviations: FT3 – free triiodothyronine; FT4 – free thyroxine; TSH – thyrotropin; CRP – C-reactive protein; PCT – procalcitonin; WBC – white blood cell count, CLIA – clinical laboratory improvement amendments

All patients with COVID-19 infection were initially in stage II of the course of the disease.[13] Patients with a history of thyroid disease, patients treated with thyroid drugs, and those who recently received iodinated contrast were excluded. Most of the patients eligible for the study had comorbidities, the most common being

hypertension (n=44, 83%), heart disease (n=32, 60.4%) (including coronary artery disease, condition after myocardial infarction or coronary angiography with coronary artery bypass surgery, heart failure or arrhythmias) and diabetes (n=22, 41.5%). Each patient had blood tests at the beginning of hospitalization, before initiation of COVID-19 treatments. Laboratory tests were performed in the hospital laboratory. All patients were treated for COVID-19 in accordance with the recommendations of the Polish Association of Epidemiologists and Infectiologists in the period from October 2020 to January 2021.[14,15]

Table 1 presents the biochemical parameters tested in the blood serum at the beginning of hospitalization, the methodology of determinations, and the range of reference values.

Statistical analysis

Statistical analysis was performed with the STATISTICA 13.1 statistical program (StatSoft Inc. 2016, Tulsa, OK, USA). Differences between categorical variables were evaluated using Pearson's Chi-square test. Yates' correction was used at frequencies lower than five. The Shapiro-Wilk test was used for the assessment of the distribution of continuous variables. Due to non-normal distribution, continuous variables were compared using Mann Whitney's U test for two groups, or Kruskal-Wallis one-way analysis of variance with additional post hoc comparisons for three or more groups. A correction for multiple tests was applied. Correlations between variables were measured for normal distribution with Pearson's correlation, otherwise with Spearman's correlation, p values < 0.05 were considered statistically significant.

Results

The final study population included 53 patients with a laboratory-confirmed presence of SARS-CoV-2 RNA and CT confirmed COVID-19 related pneumonia. The median age was 72±12.2 years, 26 patients (49%) were men. The 45 (84.9%) of included patients were elderly persons according to classification.[16] All deaths due to COVID-19 pneumonia were over the age of sixty. The 51 (96%) patients had chronic diseases. The demographic and clinical characteristics of all included patients divided into non-survivors and survivors are presented in Table 2.

Table 3 presents the relationship between thyroid hormones (TH) levels, selected inflammatory markers and in-hospital mortality of patients with COVID-19 pneumonia.

Our analysis showed the appearance of NTIS in 33 (62.3%) of all patients, 11 (33.3%) of whom died. Based on statistical analysis, serum levels of FT3 were observed to decrease and showed a strong independent correlation with disease severity and mortality prognosis in COVID-19 patients. The observed relationship is presented in Figure 1.

Table 2. Selected demographic and clinical data results in survivors and non-survivors of COVID-19 patients*

Parameter	Non-survivors (n=14)	Survivors (n=39)	p
Demographics, n (%)			
Male sex	7 (50%)	19 (48.72%)	0.93
Female sex	7 (50%)	20 (51.28%)	
Age, median years (min-max)	79.5 (60–91)	72 (37–90)	0.06
< 60	0	8 (20.51%)	0.16
60 +	14 (100%)	31 (79.49%)	
Comorbidities, n (%)			
Hypertension	11 (78.57%)	33 (84.62%)	0.92
Heart disease	13 (92.86%)	19 (48.72%)	0.01
COPD	2 (14.29%)	5 (12.82%)	0.75
T2DM	4 (28.57%)	18 (46.15%)	0.41
CKD	8 (57.14%)	8 (20.51%)	0.01
Drugs used, n (%)			
Metformin	2 (14.29%)	5 (12.82%)	0.75
Insulin	6 (42.86%)	12 (30.77%)	0.41
ACE-I	6 (42.86%)	20 (51.28%)	0.59
ARB	1 (7.14%)	1 (2.56%)	0.96

*Abbreviations: COPD – chronic obstructive pulmonary disease; CKD – chronic kidney disease; T2DM – diabetes mellitus type 2; ACE-I – angiotensin converting enzyme inhibitors; ARB – angiotensin receptor blockers; p < 0.05 was considered statistically significant

Table 3. Relationship between TH levels, selected inflammatory markers and in-hospital mortality of patients with COVID-19 pneumonia*

Predictive parameter	Non-survivors (n=14)			Survivors (n=39)			p
	median	min.	max.	median	min.	max.	
FT3, pg/mL	1.8	1.2	2.5	2.2	1.3	3.4	0.01
FT4, ng/mL	1.1	0.6	1.5	1.3	0.6	1.8	0.05
TSH, uIU/mL	0.8	0.2	3.2	0.7	0.2	2.8	0.37
Interleukin 6, pg/mL	41.9	4.8	620	15	2.7	559	0.05
CRP, µmol/L	76	4	159	49	4	366.2	0.39
PCT, ng/mL	0.165	0.05	2	0.11	0.003	17	0.16
Ferritin, ng/mL	221.25	22	994	570	12	16000	0.01
WBC, 10^9/L	10.9	7.78	195	6.75	1.01	25.97	0.002
Neutrophils, cells/µL	7860	752	15050	5120	897	23770	0.04
Lymphocyte, cells/µL	1020	210	2150	950	10	11210	0.99

*Abbreviations: FT3 – free triiodothyronine; FT4 – free thyroxine; TSH – thyrotropin; CRP – C-reactive protein; PCT – procalcitonin; WBC – white blood cell count; min. – minimum; max. – maximum

The lowered FT3 level was statistically significant (p=0.01). The FT4 levels were marginally lower in the non-survivors compared to survivor patients (p=0.05). Spearman's correlations between FT3 and the age of pa-

tients was statistically significant (R = -0.31). The observed relationship is presented in Figure 2.

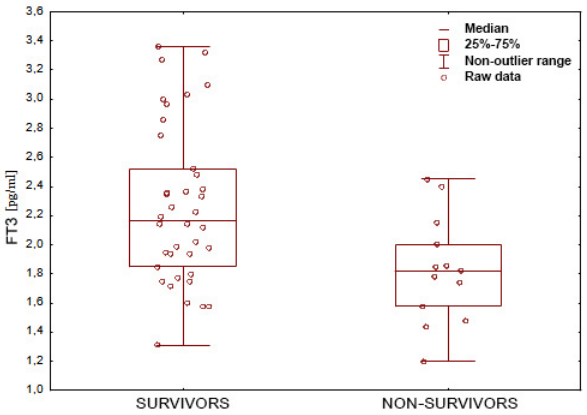

Fig. 1. Relationship between the level of FT3 and mortality in patients with COVID-19 pneumonia

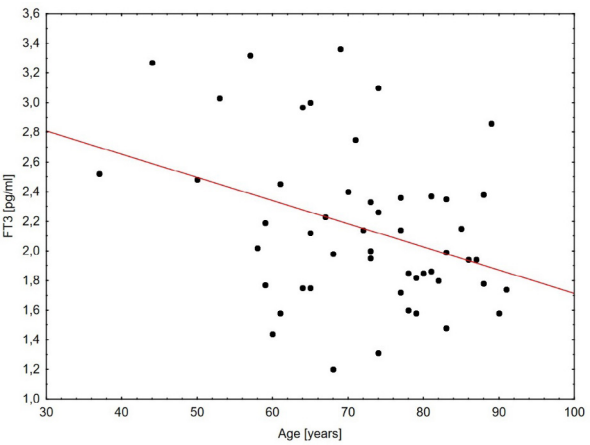

Figure 2. Relationship between the level of FT3 and age in patients with COVID-19 pneumonia

The risk of in-hospital death in elderly COVID-19 patients was also higher for the increasing values of selected inflammatory markers (Table 3). It was observed that WBC (p=0.002), neutrophils (p=0.04) and IL-6 (p=0.05) could be predictors of deterioration patients with COVID-19. The median Il-6 for deceased patients was statistically significantly higher than for those who survived (41.9 pg/mL and 15 pg/mL, respectively). These markers can also be useful in the assessment of the risk of death.

The relationship between Il-6 and mortality in patients with COVID-19 pneumonia is shown in Figure 3.

Unexpectedly a decrease in ferritin was observed in non-survivor elderly patients with COVID-19 pneumonia compared to survivors (p=0.01). There were no statistically significant differences in the level of CRP and PCT between survivors and non-survivors in our study.

In the tested sample, the relationship between lymphocytes and FT3 was also not statistically significant (Spearman's rank order correlation R = 0.13 p> 0.05).

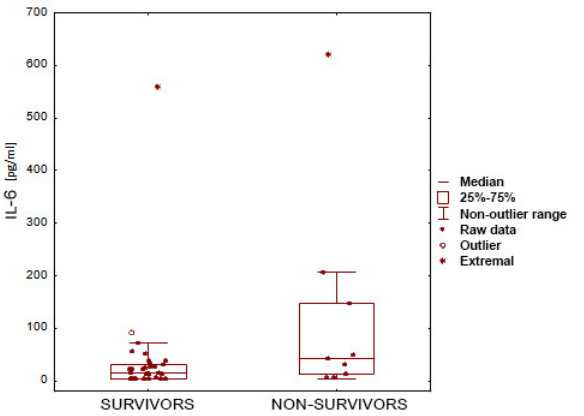

Figure 3. Relationship between Il-6 and mortality in patients with COVID-19 pneumonia

Discussion

Most of the patients requiring hospitalization due to COVID-19 pneumonia constitute the geriatric population. Some of the risk factors for mortality reported in patients with COVID-19, except age, are male gender, duration from onset to admission, admission SARS-CoV-2 viral load, comorbidities and higher levels of inflammatory markers.[17,19-21] The study by Alizadehsani et al. indicate that age, blood group, heart disease, anosmia and dry cough are the most crucial factors in the mortality of patients with COVID-19.[18] The coexistence of chronic diseases, poor physical condition, lymphopenia, bacterial co-infection and smoking history also increase the ratio of death.[21,22] Many factors influence the thyroid function during COVID-19 infection. Hypercortisolemia, increased cytokines, oxidative stress can impact on thyroid axis.[23,24] Several of the commonly used medications, such as glucocorticoids, dopamine or heparin, may affect or interfere with thyroid function tests.[25,26] Patients with COVID-19 often require such treatment. Therefore, thyroid function was measured, before treatment in the enrolled patients.

It is still not clear whether the finding of low FT3 levels in patients with COVID-19 describes NTIS or if the thyroid could be a direct target of SARS-CoV-2. Wang et al. found that the TSH level of COVID-19 patients was significantly lower than that in non-COVID-19 pneumonia patients and this suggests that thyroid function abnormalities in COVID-19 patients cannot be fully explained by NTIS.[27] The mRNA encoding for the ACE-2 receptor is expressed in follicular thyroid cells, making them a potential target for SARS-COV-2 entry.[28] The potential effect of systemic inflammation on thyroid is also accent.[27,29] The inflammatory response leads among others to a reduction in deiodinase activity and a decrease in the conversion of T4 to T3. In a study by Ilera et al. all TH (T3, T4, FT3, FT4) correlated, in an opposite way, with in-

flammation parameters and worse clinical outcome.[29] The suppression of the hypothalamic-pituitary-thyroid axis is also observed.[7] Some scientists reported that the elevated C-reactive protein was independently associated with the appearance of low TSH and low FT3.[30,31] Wang et al. identified increased levels of leukocytes, neutrophils, CRP and procalcitonin, and decreased levels of lymphocytes in the thyroid dysfunction group.[27]

We did not observe a statistically significant correlation between inflammatory markers (IL-6, CRP, lymphocyte levels) with NTIS in our study. These discrepancies may have been due to the small size of our group or the age-related decreasing of immune response (84.9% of patients were over 60 years of age), but other factors should be taken into account. NTIS does not always correlate with inflammatory markers. Lui et al. reported that NTIS on admission could predict clinical deterioration in COVID19 patients, irrespective of SARS-CoV-2 viral load, age and markers of inflammation and tissue injury.[12,30]

The interesting studies analyzed thyroid function between COVID-19 patients and healthy control, and reported the potential prognostic role of low FT3, mainly in severe COVID19 pneumonia. The serum TSH and T3 levels in COVID-19 patients were significantly lower than those of the healthy group. The degree of the decrease in TSH and T3 correlated positively with the severity of COVID-19 disease.[32-35] A study by Khoo et al. detected that patients with COVID-19 had lower admission levels of TSH and FT4.[36] We noticed that low serum FT3 levels showed a strong independent correlation with disease severity and mortality in COVID-19 patients (p=0.01). Many patients with the lower FT3 levels at hospital admission had deteriorated and died, which is in line with the data from the literature.

Serum FT3 concentration is lower in patients with severe COVID-19 and appears to be associated with an increased risk of death in COVID-19 patients, so it could be a potential independent prognostic marker at hospital admission.[37-39]

NTIS is common and significantly related to mortality in acutely ill, hospitalized old patients. Thyroid hormones, especially serum FT3 determination may predict clinical outcomes in old, frail patients and perhaps they should be included in the assessment of short-term prognosis.[40,41] However, the ascertainment of a definite prognostic role of NTIS in older patients with COVID-19 is difficult because this syndrome may be due to comorbidities, not only by COVID-19 pneumonia.[42]

The inflammatory response plays an important role in the progression of COVID-19. Several inflammatory markers have been reported to be associated with the severity of COVID-19.[31] The analysis of inflammatory markers (regardless of NTIS) in our group of patients showed that IL-6, WBC and neutrophil levels are significantly higher in severe COVID-19 patients, which is comparable with the available literature.[43-45] In our study, lower ferritin is associated with increased mortality of patients. This finding is in contrast with the findings of a systematic review by Cheng et al.[46]

This discrepancy could be explained by high frequency of age-related iron deficiency in our patients or population differences.[47]

There are several limitations to our study. First, it is a retrospective and unplanned work, and most of the data was obtained from electronic patient histories. Second, applying the exclusion criteria, the study sample was not large. Third, reverse triiodothyronine was not measured, therefore partial central hypothyroidism and NTIS were difficult to distinguish.

Finally, elderly patients, especially those with comorbidities, have a high incidence of NTIS and it is difficult to determine to what extent this syndrome is due to COVID-19 pneumonia alone.

Conclusion

Our study provides data that validate the prognostic role of low levels of FT3 in COVID-19 patients. We have shown that low serum FT3 is an important independent mortality risk factor in elderly patients with COVID-19 pneumonia. Serum FT3 determination could be included in the assessment prognosis of elderly patients with COVID-19 pneumonia.

Our findings confirms that the levels of Il-6, WBC, ferritin and neutrophils are also the valuable bioindicators of in-hospital mortality in patients with COVID-19. This study may be helpful in early prediction and risk reduction of mortality in elderly patients infected with COVID-19.

Acknowledgements

We are grateful to all the medical staff at the Rzeszow Clinical District Hospital No. 2, Poland, for their dedication to care for the COVID-19 patients.

Declarations

Funding

The authors declared that this study has received no financial support or any funding.

Author contributions

Conceptualization, A.M. and R.O.F.; Methodology, R.O.F., A.M., A.C.W., A.G.B.; Software, K.G.; Formal Analysis, A.M., R.O.F., A.C.W.,A.G.B.,K.G.; Investigation, A.M, R.O.F., A.C.W.; Resources, A.M., A.C.W., R.O.F.; Data Curation, A.M., A.C.W., R.O.F., Writing – Original Draft Preparation, A.M., R.O.F., A.C.W., A.G.B.; Writing – Review & Editing, A.M.,R.O.F.,A.G.B.; Visualization, K.G.; Supervision, R.O.F.,A.G.B.; Project Administration, R.O.F.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

This retrospective study was approved by the Bioethics Committee of the University of Rzeszów (Reference No. 12/05/2020). The procedures used in this study adhere to the tenets of the Declaration of Helsinki. We collated data mainly from electronic patient histories. Although personal identification numbers were used to match the datasets, these were subsequently anonymized.

References

1. Menachemi N, Dixon BE, Wools-Kaloustian KK, et al. How Many SARS-CoV-2-Infected People Require Hospitalization? Using Random Sample Testing to Better Inform Preparedness Efforts. *J Public Health Manag Pract*. 2021;27(3):246–250.
2. Macedo A, Gonçalves N, Febra C. COVID-19 fatality rates in hospitalized patients: systematic review and meta-analysis. *Ann Epidemiol.* 2021;57:14–21.
3. Ding Y, He L, Zhang Q, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. *J Pathol.* 2004;203(2):622–630.
4. Giovanella L, Ruggeri RM, Petranović Ovčariček P et al. SARS-CoV-2-related thyroid disorders: a synopsis for nuclear medicine thyroidologists. *Eur J Nucl Med Mol Imaging.* 2021;48(6):1719–1723.
5. Gree M. The thyroid gland. 4th ed. *New York. Raven press.* 1990;26:79.
6. Kelly GS. Peripheral metabolism of thyroid hormones: a review. *Altern Med Rev.* 2000;5:306333.
7. Zheng J, Cui Z, Shi N, et al. Suppression of the hypothalamic-pituitary-thyroid axis is associated with the severity of prognosis in hospitalized patients with COVID-19. *BMC Endocr Disord*. 2021;21(1):228.
8. Luca F, Goichot B, Brue T. Les dyshormonémies des affections non thyroïdiennes [Non thyroidal illnesses (NTIS)]. *Ann Endocrinol*. 2010;71(Suppl 1):S13–S24.
9. Pappa TA, Vagenakis AG, Alevizaki M. The nonthyroidal illness syndrome in the non-critically ill patient. *Eur J Clin Invest*. 2011;41(2):212–220.
10. Peeters P. Non thyroidal illness: to treat or not to treat? *Ann Endocrinol.* 2007;68(4):224–228.
11. Sciacchitano S, Capalbo C, Napoli C, et al. Nonthyroidal illness syndrome (NTIS) in severe COVID-19 patients: role of T3 on the Na/K pump gene expression and on hydroelectrolytic equilibrium. *J Transl Med.* 2021;19(1):491.
12. Lui DTW, Lee CH, Chow WS, et al. Role of non-thyroidal illness syndrome in predicting adverse outcomes in COVID-19 patients predominantly of mild-to-moderate severity. *Clin Endocrinol*. 2021;95(3):469–477.
13. Siddiqi HK, Mehra MR. COVID-19 illness in native and immunosuppressed states: A clinical-therapeutic staging proposal. *J Heart Lung Transplant*. 2020;39(5):405–407.
14. Flisiak R, Horban A, Jaroszewicz J, et al. Management of SARS- CoV-2 infection: recommendations of the Polish Association of Epidemiologists and Infectiologists as of March 31, 2020. *Pol Arch Intern Med.* 2020;130:352–357.
15. Flisiak R, Horban A, Jaroszewicz J, et al. Management of SARS-CoV-2 infection: recommendations of the Polish Association of Epidemiologists and Infectiologists. Annex no. 1 as of June 8, 2020. *Pol Arch Intern Med*. 2020;130:557–558.
16. United Nations, Department of Economic and Social Affairs, Population Division (2015). World Population Ageing 2015 (ST/ESA/SER.A/390)
17. Wang Z, Wang Z. Identification of risk factors for in-hospital death of COVID - 19 pneumonia - lessions from the early outbreak. *BMC Infect Dis*. 2021;21(1):113.
18. Alizadehsani R, Alizadeh Sani Z, Behjati M et al. Risk factors prediction, clinical outcomes, and mortality in COVID-19 patients. *J Med Virol*. 2021;93(4):2307–2320.
19. Smilowitz NR, Kunichoff D, Garshick M, et al. C-reactive protein and clinical outcomes in patients with COVID-19. *Eur Heart J*. 2021;42(23):2270–2279.
20. Westblade LF, Brar G, Pinheiro LC, et al. SARS-CoV-2 viral load predicts mortality in patients with and without cancer who are hospitalized with COVID-19. *Cancer Cell*. 2020;38(5):661–671.e2.
21. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020;8(5):475–481.
22. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* 2020;395(10223):507–513.
23. Abo-Zenah HA, Shoeb SA, Sabry AA. Relating circulating thyroid hormone concentrations to serum interleukins-6 and -10 in association with non-thyroidal illnesses including chronic renal insufficiency. *BMC Endocr Disord*. 2008;8:1.
24. Qin SL, He Q, Hu L, et al. The relationship between inflammatory factors, oxidative stress, and DIO-1 concentration in patients with chronic renal failure accompanied with or without euthyroid sick syndrome. *J Int Med Res*. 2018;46(10):4061–4070.
25. Burch HB. Drug effects on the thyroid. *N Engl J Med*. 2019;381(8):749–761.
26. Alkemade A, Unmehopa UA, Wiersinga WM, et al. Glucocorticoids decrease thyrotropin-releasing hormone messenger ribonucleic acid expression in the paraventric-

ular nucleus of the human hypothalamus. *J Clin Endocrinol Metab*. 2005;90(1):323–327.
27. Wang W, Su X, Ding Y, et al. Thyroid function abnormalities in COVID-19 patients. *Front Endocrinol*. 2021;11:623792.
28. Rotondi M, Coperchini F, Ricci G, et al. Detection of SARS-COV-2 receptor ACE-2 mRNA in thyroid cells: a clue for COVID-19-related subacute thyroiditis. *J Endocrinol Invest*. 2021;44(5):1085–1090.
29. Ilera V, Delfino LC, Zunino A, et al. Correlation between inflammatory parameters and pituitary-thyroid axis in patients with COVID-19. *Endocrine*. 2021;74(3):455–460.
30. Lui DTW, Lee CH, Chow WS, et al. Thyroid dysfunction in relation to immune profile, disease status and outcome in 191 patients with COVID-19. *J Clin Endocrinol Metab*. 2021;106(2):e926–e935.
31. Zeng F, Huang Y, Guo Y, et al. Association of inflammatory markers with the severity of COVID-19: a meta-analysis. Int J. Infect. Dis. 2020;96:467–474.
32. Chen W, Tian Y, Li Z, et al. Potential Interaction Between SARS-CoV-2 and Thyroid: A Review. *Endocrinology*. 2021;162(3):bqab004.
33. Chen M, Zhou WB, Xu WW. Thyroid function analysis in 50 patients with COVID-19: a retrospective study. *Thyroid*. 2021;31(1):8–11.
34. Muller I, Cannavaro D, Dazzi D, et al. SARS-CoV-2-related atypical thyroiditis. *Lancet Diabetes Endocrinol*. 2020;8(9):739–741.
35. Li T, Wang L, Wang H, et al. Characteristics of laboratory indexes in COVID-19 patients with non-severe symptoms in Hefei City, China: diagnostic value in organ injuries. *Eur J Clin Microbiol Infect Dis*. 2020;39(12):2447–2455.
36. Khoo B, Tan T, Clarke SA, et al. Thyroid Function Before, During, and After COVID-19. *J Clin Endocrinol Metab*. 2021;106(2):e803–e811.
37. Llamas M, Garo ML, Giovanella L. Low free-T3 serum levels and prognosis of COVID-19: systematic review and meta-analysis. *Clin Chem Lab Med*. 2021;59(12):1906–1913.
38. Gao W, Guo W, Guo Y et al. Thyroid hormone concentrations in severely or critically ill patients with COVID-19. *J Endocrinol Invest*. 2020;44(5):1031–1040.
39. Güven M, Gültekin H. The prognostic impact of thyroid disorders on the clinical severity of COVID-19: Results of single-centre pandemic hospital. *Int J Clin Pract*. 2021;75(6):e14129.
40. Iglesias P, Ridruejo E, Muñoz A et al. Thyroid function tests and mortality in aged hospitalized patients: a 7-year prospective observational study. *J Clin Endocrinol Metab*. 2013;98(12):4683–4690.
41. De Alfieri W, Nisticò F, Borgogni T et al. Thyroid hormones as predictors of short- and long-term mortality in very old hospitalized patients. *J Gerontol A Biol Sci Med Sci*. 2013;68(9):1122–1128.
42. Bossoni S, Cossi S, Marengoni A et al. Low T3 syndrome and outcome in elderly hospitalized geriatric patients. *J Endocrinol Invest*. 2002;25:73–74.
43. Speer G, Somogyi P. Thyroid complications of SARS and coronavirus disease 2019 (COVID-19). *Endocr J*. 2021;68(2):129–136.
44. Mancini A, Di Segni C, Raimondo S, et al. Thyroid hormones, oxidative stress, and inflammation. *Mediators Inflamm*. 2016;2016:6757154.
45. Izcovich A, Ragusa MA, Tortosa F, et al. Prognostic factors for severity and mortality in patients infected with COVID-19: A systematic review. *PLoS One*. 2020;15(11):e0241955.
46. Cheng L, Li H, Li L, et al. Ferritin in the coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. *J Clin Lab Anal*. 2020;34(10):e23618.
47. Short MW, Domagalski JE. Iron deficiency anemia: evaluation and management. *Am Fam Physician*. 2013;87(2):98–104.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.15

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 355–363

ORIGINAL PAPER

Comparative analysis of patients' satisfaction level, hospitalized before and during the COVID-19 pandemic

Rena Wójcik , Anna Adam , Ewa Golonka

Department of Nursing, Faculty of Medical Sciences and Health Sciences,
University of Technology and Humanities in Radom, Poland

ABSTRACT

Introduction and aim. Measurement of the satisfaction level with health services is the most frequently used indicator, mainly because of its importance for determining the quality of the care offered. It is the key to succeed in achieving high-quality healthcare. The purpose of this study was to create a retrospective comparative analysis of the satisfaction level amongst patients hospitalized before and during the COVID-19 pandemic.

Material and methods. The study covered a total of 966 patients in 19 hospital wards, in the fourth quarter of 2019 (before the pandemic) and in the second quarter of 2021 (during the pandemic) at the Masovian Specialist Hospital in Radom. The level of patient satisfaction was assessed based on the questionnaire prepared and approved by the Quality Assurance Team in the Masovian Specialist Hospital. The statistical analysis was carried out on the basis of the STATISTICA 10.1 program, using the Pearson's chi square test, for the significance level at $p<0.05$.

Results. The high level of satisfaction of patients staying in the hospital during the pandemic applied to the widely understood medical and nursing care as well as sanitary conditions in wards, especially the cleanness of rooms, bed linens and sanitary facilities.

Conclusion. The biggest dissatisfaction of hospitalized patients during the COVID-19 pandemic involved certain restrictions of visitations and using pastoral services.

Keywords. COVID-19 pandemic, satisfaction, quality of care

Introduction

One of the fundamental aspects of proper functioning nowadays health care is the high quality of provided services including professionalism and competence of personnel staff, availability to the medical services, continuity of care, following developed procedures, adjusting healthcare to the patient's needs and patient's satisfaction.[1,2] The term „satisfaction" comes from the Latin language (which means: enough, sufficiently), it determines subjective feeling of being satisfied, which is connected to personal experiences, expectations and values.[3] It is patient's emotional reaction and answer to the experiences resulting from the care provided, especially to the constantly changing situation in the market of providing healthcare, an external system of accreditation, growing competition and care about the patient force us to start even more effective methods to manage facilities. The indicator, which is essential for determining the quality of care offered, is the measurement of patient's satisfaction level with health services**.** The analysis of obtained results from those studies enables the introduction of beneficial changes and suggests the direction of further quality-aiming activities in the region of medical services.[4-6] The current epidemiological situation in the country, connected to the appearance of the SARS-CoV-2 virus, requires from every member of the

Corresponding author: Rena Wójcik, e-mail: r.wojcik@uthrad.pl

Received: 14.04.2022 / Accepted: 3.06.2022 / Published: 30.09.2022

Wójcik R, Adam A, Golonka E. *Comparative analysis of patients' satisfaction level, hospitalized before and during the COVID-19 pandemic.* Eur J Clin Exp Med. 2022;20(3):355–363. doi: 10.15584/ejcem.2022.3.15.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

health system, adaptation to the new situation, implementation of specific measures and engaging intensified effort in order to fight the coronavirus.[7]

Aim

The purpose of this study was a retrospective comparative analysis of the satisfaction level amongst patients hospitalized before and during the COVID-19 pandemic.

Material and methods

The study covered a total of 966 patients in 19 hospital wards, in the fourth quarter of 2019 (before the pandemic) and in the second quarter of 2021 (during the pandemic) at the Masovian Specialist Hospital in Radom. Consent to review of medical records (that are survey results) was gained from the Management of the Masovian Specialist Hospital sp. z o. o. in Radom, where a periodic assessment system outlining patients' satisfaction with medical services, within the Quality Management System, is being implemented since 2018 (hospital approval: 338, date: 10.01.2022). The statistical analysis was carried out based on the STATISTICA 10.1 program (Statsoft Statistica 10.1, Lublin, Poland) using Pearson's chi-square test, for the significance level at $p<0.05$.

Results

The study took place in 19 hospital wards at the Masovian Specialist Hospital in Radom, in the fourth quarter of 2019 and in the second quarter of 2021 amongst insofar hospitalized patients.

In this patient's satisfaction survey in 2019, 574 out of 949 surveys were fully completed with the return factor reaching 60.48%. Meanwhile in 2021, in the research participated 392 patients (at 615 surveys distributed, the return indicator was 63.73%) Less quantity of respondents was probably due to the former epidemic situation in our country. The most numerous group in 2019 as well as in 2021 were people aged 40 to 65 years, (2019 – 224, 2021 – 153), with a dominance of women. Most surveys were fulfilled by patients and time spent at the hospital was from 3 to 7 days.

Conducted surveys made it possible to analysis the most significant aspects of hospital healthcare, which may influence the level of patients' satisfaction:

- actions linked to admission to the hospital and the ward,
- health-care field concerning stay on the ward: an issue of patient's rights, the topography of the ward, the daily schedule, providing meals, maintaining cleanness, pain relief effectiveness during hospitalization, possibility to use pastoral services, and opportunity of visits,
- assessment of the medical care, nurse/maternity care and rehabilitation care,
- actions associated with executing diagnostic tests,
- overall, subjective evaluation of the level of services provided at Masovian Specialist Hospital (Tab.1).

The vast majority of respondents expressed their contentment in hospital care, both before and during the COVID-19 pandemic at the Masovian Specialist Hospital in Radom. The aspect of cleanliness in the wards deserves special emphasis, which is connected to the raise of the sanitary regime during the COVID-19 pandemic. Significant increase of extremely good opinions linked to the cleanness of bed linen (up to approximately 8%) as well as neatness of sanitary facilities (up to approximately 10%) compared to the times before pandemic. Variety and adequate temperature of served meals were also appreciated.

Due to the epidemic threat, to ensure patient safety, in the second quarter of 2021 possibility of visits has been restricted, which was met with the displeasure of around 31% of hospitalized patients. Accessibility and ease of contact with doctors and nurses were slightly worse assessed (decrease from very good ratings to good ratings approximately by 10%) Increase in dissatisfaction among hospitalized patients during the COVID-19 pandemic involved certain restrictions on using pastoral services and rehabilitation care.

It is worth mentioning that despite restrictions and changes in organizations of facility's work, the level of patients' satisfaction with medical and nurse healthcare is at the same level as in the analyzed quarters. Nursing interventions were also highly rated, especially in pain relief effectiveness, reacting to worse patient's well-being and assistance in self-reliant activities. Whilst no statistically significant dependencies were concluded.

In spite of the pandemic situation, in terms of organization, the standard of care during admission to the hospital has not changed significantly compared to the period before the pandemic. Short waiting time for admission to the ward in the SOR (up to 1 hour) was confirmed by roughly 40% of respondents, yet 20% of patients had waited above 2 hours. During preliminary diagnosis before admission to the ward, almost 90% of the surveyed were treated with kindness and solicitude from the therapeutic team. Growth of good opinions in terms of providing information about the current health condition and planned treatment was observed in the second quarter of 2021. The results of patient's satisfaction with medical services in 2019 are similar to those obtained in 2021, which were dominated by the COVID-19 pandemic, which means that it was possible to manage a high level of medical services despite the tough epidemic situation.

Table 1. Satisfaction of patients hospitalized in the IV quarter of 2019 and the II quarter of 2021

					Q4 2019	**Q2 2021**
Name of the hospital: Masovian Specialist Hospital					19 wards	
Amount of distributed questionnaires:					949	615
Amount of returned questionnaires:					574	392
A.	**ADMISSION TO THE HOSPITAL – PLEASE EVALUATE**					
1.	Waiting time for admission to the ward in the Emergency Room	**up to 1 hour**	**up to 2 hours**	**above 2 hours**	**no opinion**	**no answer**
	Q4 2019	42.86% n=246	30.31% n=174	20.56% n=118	2.79% n=16	3.48% n=20
	Q2 2021	43.37% n=170	29.08% n=114	19.9% n=78	5.36% n=21	2.3% n=9
	Statistics	Chi^2=5.29, Df=4, **p=0.258**				
2.	Ensuring the care and kindness of medical staff in the Emergency Room	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	3.48% n=20	51.57% n=20	37.46 n=215	4.01% n=23	3.48% n=20
	Q2 2021	2.55% n=13	58.16% n=228	33.16% n=130	4.08% n=16	2.04% n=8
	Statistics	Chi^2=5.40, Df=4, **p=0.248**				
3.	Enough data about admission to hospital (information about the patient's condition and planned course of treatment)	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	6.62% n=38	49.13% n=282	29.97% n=172	5.05% n=29	9.23% n=53
	Q2 2021	3.32% n=13	57.91% n=227	26.79% n=105	7.14% n=28	4.85% n=19
	Statistics	Chi^2=16.78, Df=4, **p=0.002**				
B.	**ADMISSION TO THE HOSPITAL WARD – PLEASE EVALUATE**					
1.	Where you accompanied by the hospital staff on your way from the Emergency Room to the ward?	**yes**		**no**	**no option**	**no answer**
	Q4 2019	89.55% n=514		6.10% n=35	3.31% n=19	1.05% n=6
	Q2 2021	94.90% n=372		1.53% n=6	2.55% n=10	1.02% n=4
	Statistics	Chi^2=12.62, Df=3, **p=0.006**				
2.	During your administration to the ward. were you acquainted with your Patient Rights and indicated where they are available?	**yes**		**no**	**no option**	**no answer**
	Q4 2019	79.97% n=459		13.07% n=75	4.53% n=26	2.44 n=14
	Q2 2021	81.89% n=321		12.24% n=48	3.83% n=15	2.04% n=8
	Statistics	Chi^2=0.66, Df=3, **p=0.882**				
3.	Where you familiarized with the topography of the ward (location of bathrooms. doctor's office. nursing station. etc.)?	**yes**		**no**	**no option**	**no answer**
	Q4 2019	86.59% n=497		7.49% n=43	2.96% n=17	2.96% n=17
	Q2 2021	89.54 n=351		7.14% n=28	2.04 n=8	1.28% n=5
	Statistics	Chi^2=3.94, Df=3, **p=0.268**				
4.	During admission to the ward. were you informed about the daily sch	**yes**		**no**	**no option**	**no answer**
	Q4 2019	77.87% n=447		13.94% n=80	4.01% n=23	4.18% n=24
	Q2 2021	77.55% n=304		13.78% n=54	6.38% n=25	2.30% n=9
	Statistics	Chi^2=5.06, Df=3, **p=0.167**				

C.	**STAY IN THE WARD – PLEASE EVALUATE**					
1.	Cleanliness in sickrooms	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0.70%	46.34%	48.43%	2.96%	1.57%
		n=4	n=266	n=278	n=17	n=9
	Q2 2021	0.26%	48.72%	49.74%	1.02%	0.26%
		n=1	n=191	n=195	n=4	n=1
	Statistics	Chi^2=9.15, Df=4, **p=0.057**				
2.	Cleanliness of the bed linen	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0.70%	43.21%	50.52%	1.92%	3.66%
		n=4	n=248	n=290	n=11	n=21
	Q2 2021	0%	39.03%	58.42%	1.53%	1.02%
		n=0	n=153	n=229	n=6	n=4
	Statistics	Chi^2=12.87, Df=4, **p=0.012**				
3.	Cleanliness of bathrooms and toilets	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	6.1%	56.97%	24.09%	2.96%	4.88%
		n=35	n=327	n=167	n=17	n=28
	Q2 2021	2.81%	50.26%	40.56%	3.34%	2.04%
		n=11	n=197	n=159	n=17	n=8
	Statistics	Chi^2=22.57, Df=4, **p=0.0002**				
4.	Providing information on the diet used	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	2.09%	47.39%	34.32%	11.5%	4.7%
		n=12	n=272	n=197	n=66	n=27
	Q2 2021	3.06%	47.96%	35.46%	11.73%	1.79%
		n=12	n=188	n=139	n=46	n=7
	Statistics	Chi^2=6.63, Df=4, **p=0.157**				
5.	Temperature of meals	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	14.63%	55.05%	19.34%	6.62%	4.36%
		n=83	n=316	n=111	n=38	n=25
	Q2 2021	5.61%	57.4%	30.36%	5.87%	0.77%
		n=22	n=225	n=119	n=23	n=3
	Statistics	Chi^2=39.9, Df=4, **p<0.00001**				
6.	Variety of meals	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	9.76%	52.44%	23%	10.98%	3.83%
		n=56	n=301	n=132	n=63	n=22
	Q2 2021	5.36%	54.34%	30.61%	8.42%	1.28%
		n=21	n=213	n=120	n=33	n=5
	Statistics	Chi^2=17.97, Df=4, **p=0.001**				
7.	Pain relief effectiveness	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0.87%	42.16%	41.64%	9.76%	5.57%
		n=5	n=242	n=239	n=56	n=32
	Q2 2021	2.55%	39.8%	48.47%	7.4%	1.79%
		n=10	n=156	n=190	n=29	n=7
	Statistics	Chi^2=16.75, Df=4, **p=0.002**				
8.	Possibility of visits	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0.35%	37.98%	50%	3.83%	7.84%
		n=2	n=218	n=287	n=22	n=45
	Q2 2021	31.38%	19.13%	9.95%	37.04%	12.5%
		n=123	n=75	n=39	n=106	n=49
	Statistics	Chi^2=411.18, Df=4, **p<0.00001**				
9.	Possibility to use pastoral services	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0.35%	33.45%	50.17%	11.67%	4.36%
		n=2	n=192	n=288	n=67	n=25
	Q2 2021	3.06%	43.62%	36.22%	15.05%	2.04%
		n=12	n=171	n=142	n=59	n=8
	Statistics	Chi^2=34, Df=4, **p<0.00001**				
D.	**MEDICAL CARE – PLEASE EVALUATE**					
1.	Availability and ease of contact with a doctor if needed	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	2.61%	44.43%	47.04%	3.83%	2.09%
		n=15	n=255	n=270	n=22	n=12
	Q2 2021	4.34%	55.36%	33.93%	5.36%	1.02%
		n=17	n=217	n=133	n=21	n=4
	Statistics	Chi^2=20.21, Df=4, **p=0.0005**				

2.	Ensuring intimacy and privacy during medical examinations	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	1.74% n=10	44.77% n=257	48.26% n=277	3.31% n=19	1.92% n=11
	Q2 2021	1.53% n=6	52.55% n=206	40.82% n=160	3.32% n=13	1.79% n=7
	Statistics	Chi^2=5.87, Df=4, **p=0.209**				
3.	Showing interest in patient's problems	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	2.26% n=13	45.99% n=264	42.68% n=245	5.92% n=34	3.14% n=18
	Q2 2021	2.81% n=11	51.02% n=200	40.05% n=157	3.83% n=15	2.3% n=9
	Statistics	Chi^2=4.49, Df=4, **p=0.343**				
4.	Understandable provision of information about current health condition. test results. planed treatment	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	3.66% n=21	45.12% n=259	44.43% n=255	4.88% n=28	1.92% n=11
	Q2 2021	4.34% n=17	48.21% n=189	40.05% n=157	4.85% n=19	2.55% n=10
	Statistics	Chi^2=2.23, Df=4, **p=0.694**				
E.	**NURSING/MATERNITY CARE – PLEASE EVALUATE**					
1.	Availability and ease of contact with a nurse/midwife if needed	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0.17% n=1	28.57% n=164	67.6% n=388	0.87% n=5	2.79% n=16
	Q2 2021	0.26% n=1	37.5% n=147	58.67% n=230	1.79% n=7	1.79% n=7
	Statistics	Chi^2=11.29, Df=4, **p=0.024**				
2.	Nurses/midwives' response to reported pain. worse well-being or other patient's discomfort	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0.17% n=1	27.35% n=157	65.68% n=377	2.79% n=16	4.01% n=23
	Q2 2021	0.51% n=2	34.95% n=137	59.44% n=233	2.55% n=10	2.55% n=10
	Statistics	Chi^2=8.19, Df=4, **p=0.085**				
3.	Assistance in daily activities (e.g. personal hygiene. moving. using the toilet. etc.)	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0.35% n=2	29.62% n=170	53.48% n=307	10.28% n=59	6.27% n=36
	Q2 2021	0.51% n=2	35.97% n=141	56.89% n=223	4.34% n=17	2.3% n=9
	Statistics	Chi^2=5.53, Df=4, **p=0.237**				
4.	Providing information on performed procedures and planned nursing/obstetric activities	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0.35% n=2	37.8% n=217	51.22% n=294	5.57% n=32	5.05% n=29
	Q2 2021	1.02% n=4	41.33% n=162	50.51% n=198	3.83% n=15	3.32% n=13
	Statistics	Chi^2=5.53, Df=4, **p=0.237**				
5.	Ensuring a sense of intimacy and privacy during nursing/ obstetric procedures	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0.35% n=2	36.93% n=212	53.31% n=306	5.4% n=31	4.01% n=23
	Q2 2021	0% n=0	43.88% n=172	48.98% n=192	4.08% n=16	3.06% n=12
	Statistics	Chi^2=6.45, Df=4, **p=0.168**				
F.	**REHABILITANS – PLEASE EVALUATE (if applicable)**					
1.	Attitude towards the patient (kindness. care and interest in patient. etc.)	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0% n=0	19.51% n=112	28.4% n=163	15.16% n=87	36.93% n=212
	Q2 2021	0% n=0	15.31% n=60	21.94% n=86	20.66% n=81	42.09% n=165
	Statistics	Chi^2=11.73, Df=4, **p=0.019**				

2.	Respect for dignity and intimacy during performed treatments	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0% n=0	17.94% n=103	29.44% n=169	14.81% n=85	37.8% n=217
	Q2 2021	0% n=0	15.56% n=61	19.9% n=78	20.66% n=81	43.88% n=172
	Statistics	Chi^2=15.86, Df=4, **p=0.003**				
3.	Understandable transfer of information about the improvement process (types of exercise. how to perform them. etc.)	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0% n=0	18.82% n=108	25.26% n=145	16.2% n=93	39.72% n=228
	Q2 2021	0% n=0	12.76% n=50	19.9% n=78	22.7% n=89	43.88% n=172
	Statistics	Chi^2=18.72, Df=4, **p=0.0001**				
G.	**DIAGNOSTIC TESTS – PLEASE EVALUATE (if applicable)**					
1.	Staff culture in diagnostic offices (e.g. X-ray. ultrasound. etc.)	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	1.39% n=8	37.63% n=216	43.38% n=249	6.1% n=35	11.5% n=66
	Q2 2021	0.77% n=3	34.69% n=136	39.03% n=153	10.46% n=41	15.05% n=59
	Statistics	Chi^2=10.32, Df=4, **p=0.035**				
2.	Respect for dignity and intimacy during performed treatments	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	1.05% n=6	36.41% n=209	44.77% n=257	7.32% n=42	10.45% n=60
	Q2 2021	0.26% n=1	34.95% n=137	40.31% n=158	7.4% n=29	17.09% n=67
	Statistics	Chi^2=11.04, Df=4, **p=0.026**				
H.	OVERALL EVALUATION					
1.	How do you evaluate (in general) the level of services provided at Masovian specialist hospital sp. z o. o. in Radom?	**badly**	**good**	**very good**	**no opinion**	**no answer**
	Q4 2019	0.52% n=3	50% n=287	45.47% n=261	1.05% n=6	2.96% n=17
	Q2 2021	0.77% n=3	42.35% n=166	49.49% n=194	3.83% n=15	3.57% n=15
	Statistics	Chi^2=12.48, Df=4, **p=0.014**				

I.	**SOCIODEMOGRAPHIC INFORMATION**		
1.	**Age:**	**Q4 2019**	**Q2 2021**
	up to 39	19.51% n=112	30.1% n=118
	40-65	39.02% n=224	39.03% n=153
	66 and above	35.71% n=205	28.83% n=113
	no answer	5.75% n=33	2.04% n=8
2.	**Gender:**	**Q4 2019**	**Q2 2021**
	woman	49.83% n=286	58.67% n=230
	man	43.9% n=252	37.5% n=147
	no answer	6.27% n=36	3.83% n=15
3.	**Time spent at the hospital:**	**Q4 2019**	**Q2 2021**
	up to 3 days	17.94% n=103	31.89% n=125
	from 3 to 7 days	28.75% n=165	33.93% n=133
	from 7 to 14 days	19.69% n=113	20.15% n=79
	above 14 days	28.57% n=164	8.93% n=35
	no answer	5.05% n=29	4.85% n=19

Discussion

A patient satisfaction survey on exercised hospital care is an integral criterion of the assessment of the quality of a health care unit. Its realization enables adjusting medical facility to the patient's needs and expectations, as well as creating a positive reputation on the market of healthcare providers Respecting the patient's requirements guarantee high quality of healthcare.[8] The periodic patient satisfaction assessment system at the Masovian Masovian Specialist Hospital, which is functioning in terms of The Quality Management System, is used to assess certain aspects of hospital care, enabling efficient identification of problems. It enables planning and implying corrective action, directed at improving the quality of medical services, thus raising the patient's satisfaction.

According to some authors, factors that influence the quality of the services provided and also affect the level of beneficiaries' satisfaction are inter alia: a way of patient's treatment, quantity and quality of provided information, attention and time dedicated to the patient by doctor and nurse, as well as safety and access to health services. It is being emphasized that doctors and nurses are the most relevant persons taking part in the prevention, diagnostics, and further treatment and nursing of the patient. Both patient treatment and nursing are complex and complementary processes.[3,9] In this research it was being analyzed amongst the other, availability and ease of contact with a doctor or a nurse, the way of patient's treatment, assurance of intimacy and privacy during medical examinations In this respect, over 80% of patients that had stayed at the Masovian Specialist Hospital in Radom showed contentment, although in comparison to the time before pandemic, mild decrease in ratings had been observed, thus from very good to good – approximately by 10%.

Other researches confirm that amongst medical staff, persons with the most personal contact with patients are primarily nurses, thus the patient's satisfaction is often perceived through the prism of nursing care. Per expectations of hospitalized patients on the surgical wards, in the exercise of preoperative care, it is necessary to take into consideration kindness, attention, understanding of needs, reaction speed to the problems and requests of the patient, along with providing intimacy during performed treatments. Those indicators are strongly affecting the development of the level of satisfaction directly associated with nursing care recipients.[9-11]

Time of the COVID-19 pandemic conveys several challenges in everyday nursing practice, such as fear of being infected, concerns about our loved ones, the unpredictability of events, tiredness, working under chronic stress, and feeling hopeless towards doing current professional duties. The pandemic situation revealed any weaknesses, highlighted the importance of communication, the necessity of procedures strictly being followed, and the creation of new ones adequate to the situation.

The first reports from foreign, particularly from Asia, but also from native research facilities reaffirms that in the present epidemiological situation, nurses managed to launch constructive strategy which includes coping with the consequences of the experienced psychological stress.[12,13]

Despite numerous concerns and emotional experiences, the nursing staff holds care for the patient by bearing in mind the bio-psycho-social needs, which finds confirmation in the own researches. Beyond 80% of survey participants pleasantly evaluated nursing interventions, notably staff reaction to the pain reported, worse wellness, help in self-reliant activities.

At the same time, as the survey indicates in the second quarter of 2021 (during the pandemic) 31% of patients showed discontent due to visiting restrictions. Implemented restrictions were designed to reduce SARS-CoV-2 virus epidemic risks as well as to improve the health and safety of patients and their families.

Profitable aspects of pandemic changes should be emphasized, including triage and organizing admission to the hospital, shortening the time of hospitalization, limiting the movement in the hospital, and introducing teleconference to an everyday work schedule.

The medical personnel's awareness has also increased in terms of obeying sanitary regimes and usage of personal protective equipment, which significantly reduced the number of nosocomial infections.[14] It also finds its acknowledgment in this research, since a substantial rise in very good opinions, regarding cleanness in wards (specifically tidiness of rooms, bed linens and sanitary facilities), has been observed compared to the time before pandemic.

What should be taken into particular consideration is respect for the patient's rights by medical personnel, being the statutory duty of everyone participating in providing healthcare services. The Ombudsman of Patient Rights is the guardian of the proper realization of the patient's rights, who at the request of the patient or his family, can initiate explanatory proceedings and undertake intervention measures.[7,15,16] According to the own research, above 80% of the surveyed confirmed being acknowledged with patient's rights. At the Masovian Specialist Hospital, a full version of the Patient's Statement of Rights and Responsibilities can be found in every ward at the nurses' station, and it is available upon the patient's request. Whereas Patient Rights Card is located in a widely available and conspicuous place in every ward. To meet the expectations and patients' rights during an epidemic emergency, medical facility managers should make it easier for the ill ones to have telephone contact with their relatives, provide information to families via phone, and where possible – allow per-

sonal visits while maintaining the sanitary regime. In the presented study valuable knowledge of patients' satisfaction with medical healthcare was gained. The analysis of indicators, which do not comply with patient's requirements, gives a possibility of introducing changes adapting healthcare entities to the needs and expectations of beneficiaries. Cyclical analysis of the satisfaction level helps to find many solutions and makes it possible to take actions leading to the constant improvement of medical healthcare.

Conclusion

The epidemic situation in most aspects of care did not reduce the level of patient satisfaction with medical services. Over 90% of patients expressed their contentment in hospital care. The biggest dissatisfaction of hospitalized patients during the COVID-19 pandemic involved certain restrictions on visitations and using pastoral services.

The high level of satisfaction of patients staying in the hospital during the pandemic applied to the widely understood medical and nursing care as well as sanitary conditions in wards, especially the cleanness of rooms, bed linens and sanitary facilities.

The Periodic assessment system outlining patients' satisfaction with medical services makes it possible to identify patients' needs, and enables performing detailed analysis as well as quick response to any imperfections. It is essential for succeeding in obtaining high-quality healthcare.

Acknowledgments

I express my gratitude to all the people who helped with the research but did not qualify for authorship. I would like to thank the Management Board of the Mazovian Specialist Hospital for making the tests possible, Ms. Joanna Brzezińska, Ms. Justyna Wasiak from the Department of Supervision and Marketing of the Hospital for the qualitative and quantitative analysis of the questionnaires from all departments of the hospital.

Declarations

Funding

The work is not financed from any source.

Author contributions

Conceptualization, R.W. and A.A.; Methodology, A.A. and E.G.; Validation, A.A., E.G. and R.W.; Formal Analysis, R.W.; Investigation, E.G. and A.A.; Resources, E.G.; Data Curation, E.G.; Writing – Original Draft Preparation, R.W. and A.A.; Writing – Review & Editing, A.A.; Visualization, A.A.; Supervision, R.W. and E.G.; Project Administration, R.W.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data that support the findings of this study are available from Masovian Specialist Hospital in Radom, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the management board of the Masovian Specialist Hospital in Radom.

Ethics approval

The retrospective study for the period 2019–2021 was conducted after obtaining the consent of the Mazovian Specialistic Hospital Management in Radom (approval no. 338 of January 10, 2022).

References

1. Smolińska A, Marciniak M, Ślusarz R, et al. Satisfaction level of health service on the neurological ward. *Udar Mózgu*. 2008;10(2):70–82.
2. Anrabi SA, Hamid S, Rohul J, et al. Measuring patient satisfaction: A cross- sectional study to improve quality of care at a tertiary care hospital. *Healthline*. 2012;3(1):59–62.
3. Kozimala M, Putowski L. The evaluation of patients satisfaction with nursery care in Medical Care Centre in Jaroslaw. *Ann Acad Med Siles*. 2009;63(1):20–27.
4. Trzcińska H. Patient satisfaction in selected aspects of the assessment of quality of medical services. *PWSZZ w Nowym Sączu*. 2019;1(3):119–136.
5. Szpringer M, Chmielewski J, Kosecka J, et al. Patients' satisfaction as one of the aspects of the quality of health care. *Med Og Nauk Zdr*. 2015;21(2):132–137.
6. Łukomska A, Szrajda J, Nowakowska I. The satisfaction of older people from family doctor's services. *Gerontol Pol*. 2012;20(2):68–72.
7. Commissioner for Patient' Rights. Patient problems in the face of the COVID-19 epidemic. *Report of the Commissioner for Patient' Rights*. W-wa 2020. https://www.gov.pl/web/rpp/raporty. Accessed February 20, 2022.
8. Maconko M, Kopański Z, Strychar J, et al. Patient satisfaction and the methods of its assessment. *JCHC*. 2016;3:14–19.
9. Żakowska A, Zera A., Krupienicz A. Quality of nursing care assessment by patients after surgical procedures performed under spinal anesthesia. *Prz Urol*. 2009;3(55):39–42.
10. Glińska J, Bednarska A, Lewandowska M. Analysis of the quality of nursing care in the opinion of nurses and patients. *Pielęg Chir Angiol*. 2012;4:151–160.
11. Delura M, Posłuszna-Owcarz M, Rezmerska L. Patients' satisfaction with preoperative nursing care. *Innowacje w Pielęgniarstwie i Naukach o Zdrowiu*. 2016;4(1):8–15.

12. Gniadek A, Nawara W, Padykuła M, et al. A Polish nurse during SARS-CoV-2 pandemic – various aspects of following a nursing profession. *Zdrowie Publiczne i Zarządzanie*. 2020;18(2):149–154.
13. Biegańska-Banaś J, Makara-Studzińska M. Coping strategies among nurses during the COVID-19 outbreak. *Probl Pielęg*. 2020;28(1):1–11.
14. Setyawan F.E.B, Supriyanto S, Tunjungsari F. Medical staff services quality to patients satisfaction based on SERVQUAL dimensions. *IJPHS*. 2019;8(1):51–57.
15. Dudzik-Urbaniak E, Kutaj-Wąsikowska H, Kutryba B, et al. Set of accreditation standards. Hospitals. *Centre for Quality Monitoring in Health Care*. Kraków 2009 (reissued 2016). https://www.cmj.org.pl/akredytacja/standardy.php. Accessed February 12, 2022.
16. Hatamizadeh M, Hosseini M, Bernstein C, et al. Health Care reform in Iran: Implications for nurses' moral distress, patient rights, satisfaction and turnover intention. *J Nurs Manag*. 2018;3:1–8.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.16

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 364–370

ORIGINAL PAPER

The relationship between the COVID-19 pandemic and the sexual life quality of nurses

Ebru Cirban Ekrem [1], Şeyma Kilci Erciyas [2]

[1] Department of Obstetrics, Gynecology Nursing, Bartın University, Bartın, Turkey
[2] Department of Obstetrics, Gynecology Nursing, Zonguldak Bülent Ecevit University, Zonguldak, Turkey

ABSTRACT

Introduction and aim. Sexuality is one of the basic human needs. The coronavirus has adversely affected every aspect of people's lives. The nurses who were in the closest contact with the patients were affected more negatively due to the many unknowns during the COVID-19 pandemic. Aim of this study is to determine the relationship between the COVID-19 pandemic and the sexual quality of life of nurses.

Material and methods. The sample of this descriptive-cross-sectional study consisted of 390 nurses who met the inclusion criteria. The data were collected over the social networks between November 2020-January 2021. The data were collected through social networks using the "Personal Information Form" and "Quality of Sexual Life Scale-Women" using the self-report method.

Results. The mean age of the nurses was 32.44±6.83, and 59.2% of them had a university level education. The mean score of the women's sexual quality of life scale was calculated as 87.37±20.83. It has been determined that there is a statistically significant difference between the scores obtained from the sexual life quality scale according to the status of the nurses having ($p<0.05$).

Conclusion. In the COVID-19 pandemic, it was determined that the sexual life quality of nurses was above the medium level and their sexual life was adversely affected.

Keywords. COVID-19, nurse, pandemic, sexuality

Introduction

The coronavirus disease (COVID-19), which emerged in Wuhan, China in late 2019, has affected almost all countries, especially starting from European countries such as Italy and Spain.[1] COVID-19, a member of the coronavirus family and discovered recently, is transmitted from the droplets of sick individuals or from contaminated surfaces by touching the mouth and throat mucosa. Due to its easy and rapid transmission, it has affected many people all around the world. More than 30 million people in the world and more than 314.000 people in Turkey have been infected with the coronavirus disease.[2,3]

Nurses have been playing a leading role in meeting the care needs of the society since the beginning of the COVID-19 pandemic, as in many wars, disasters and epidemics in history. Like all diseases, it is highly significant to determine the care priorities of patients diagnosed with COVID-19, to provide a holistic and individualized nursing care, to meet the psychosocial needs of the patients and to make them feel safe.[4]

Sexuality, which is stated to be one of the basic human needs and considered as a significant part that affects the lives of all individuals from birth to death, is an important factor that affects individuals biopsychosocially. Human behaviours and sexuality are influenced

Corresponding author: Ebru Cirban Ekrem, e-mail: cirban.ebru@gmail.com

Received: 14.06.2022 / Revised: 24.06.2022 / Accepted: 27.06.2022 / Published: 30.09.2022

Ekrem EC, Erciyas ŞK. *The relationship between the COVID-19 pandemic and the sexual life quality of nurses.* Eur J Clin Exp Med. 2022;20(3):364–370. doi: 10.15584/ejcem.2022.3.13.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license.

by physical, psychological and social conditions. Therefore, sexuality cannot be considered separately from the physical, socioeconomic and cultural factors in which an individual lives. Sexuality, a concept peculiar to the individual, is an integral part of human life. A healthy sexual life is one of the most important parameters for health and quality of life.[5-7]

Sexuality is affected by the factors such as trust of spouses/partners to each other, communication between partners, determination of the right place and time and providing a safe environment for sexual life, receiving and giving sexual pleasure, lack of education and knowledge on sexuality, false beliefs about sexual life, physical diseases, drugs, surgeries, alcohol, drug abuse, sexual abuse and rape, sexual orientation and gender identity problems, loss of interest to the partner, individuals growing up in extremely conservative and protective environments.[8,9] During the pandemic, many factors such as busy working pace of nurses in the field, inability to meet with their beloved ones for the fear of transmitting virus, stress, and the lack of an exact treatment for the virus may have had different effects on the sexual life of the individuals.

There have been many changes in almost every aspect of human life with the pandemic.[10] Sexuality is one of the basic needs of life and is affected by many biopsychosociocultural factors.[11] It will be an expected result that nurses' sexual quality of life will change during the pandemic period when considered that the nurses, who belong to a professional occupation group, have humanitarian needs as well. There have been no study in Turkey examining the nurses' sexual quality of life during COVID-19 pandemic.

Aim

This study, it was aimed to determine the relationship between the COVID-19 pandemic and the nurses' sexual quality of life.

Material and methods

Study design and participants

This research is a cross-sectional study. The population of the study was composed of female nurses working in health institutions. The minimum number of individuals that should be included in the sample of the study was calculated using the formula of sample whose population was unknown ($n=t^2.p.q/d^2$) and it was found to be 385 at a 95% confidence interval (d=0.05, t=1.96, p=0.5, q=0.5). This study consisted of 390 nurses who were female between the ages of 18-65 and married, who were working as a nurse, who were not pregnant and who volunteered to participate in the study.

Data collection tolls

In the data collection, the "Personal Information Form" prepared by the researchers and questioning socio-demographic characteristics, and the "Sexual Quality of Life Scale–Female", validity and reliability of which were performed by Tuğut and Gölbaşı, were used. In the Personal Information Form, there were 27 questions related to the socio-demographic characteristics (such as age, educational status, educational status of the spouse, region of residence, working shift, number of children, family type), medical and sexual life of the nurses.

The Sexual Quality of Life Scale–Female consists of 18 items and is in a 6-point Likert type (1 = I totally agree, 2 = I strongly agree, 3 = I partially agree, 4 = I partially disagree, 5 = I strongly disagree, 6 = I do not agree at all). The Cronbach α reliability coefficient of the scale is 0.83. In the original of the scale, it is stated that each item can be scored between 1–6 or 0–5. If the questions of the scale are calculated according to the 1-6 scoring system, the minimum score to be obtained from the scale is 18 and the maximum score is 108. The items numbered 1, 5, 9, 13, and 18 in the scale need to be scored by reversing their scores. In order to the total scale score's being turned into 100, (raw score obtained from the scale-18) x 100 / 90 formula need to be used. The high scores obtained from the scale indicate that the quality of sexual life is good.[12] The data were collected with self-report method by sharing the question form which was prepared using www.surveey.com between 24 November 2020 and 17 January 2021 via social networks (WhatsApp, Twitter, Facebook, Instagram, e-mail, etc.). The contact numbers of the researchers were shared for probable questions before the research. After the purpose and significance of the study had been explained, the participants were asked to approve their participation in the study. After confirming to participate in the study, the questions appeared on the screen. In addition, in order to prevent data loss, the participants were not allowed to see the following question before they answered a question and to complete the research. It took an average of 10 minutes to answer the questionnaire. The flowchart of the research is shown in Figure 1.

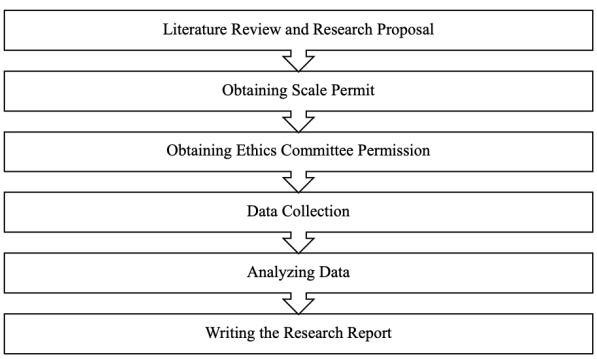

Fig. 1. Research flow chart

Ethical considerations

Written permission was obtained from the Bartın University Social and Human Sciences Ethics Committee

before starting the research (Date: 05.11.2020, No: 2020-SBB-0236). Research questions were uploaded to www.survey.com after obtaining permission from the ethics committee. The consent forms of participants were received to see whether they would participate in the questionnaire or not, and those who agreed to participate in the study were allowed to continue to fill out the forms.

Statistical analysis

Research data were analysed with IBM SPSS V23 program (IBM, New York, USA). Descriptive statistics such as frequency, percentage, arithmetic mean, and standard deviation were used in the evaluation of the data. With the Kolmogrov-Smirnov test, it was found that the data were not normally distributed. In the analysis of the data, Mann-Whitney U test was used to compare parameters that did not show a normal distribution. In the comparison of quantitative data, in case of more than two groups, the Kruskal-Wallis test was used for the comparison of parameters between groups, and the Mann-Whitney U test was used for the determination of the group that caused the difference. The reliability level of the scale was calculated with the Cronbach's alpha value, and $p<0.05$ level was considered significant in the comparisons.

Results

The mean age of the nurses is 32.44±6.83 (min.: 21, max.: 49) and education level of 59.2% is university. 25.9% of the nurses participating in the study live in the Aegean Region, 21.8% in the Central Anatolia Region and 95.4% have a nuclear family structure. 68.2% of nurses work both day and night (Table 1). 26.2% work in the intensive care unit, 9.5% in the emergency service, and the others in units such as inpatient services and polyclinics. 69.7% of the nurses' spouses are university graduates and have a regular job (74.4%). 67.2% of the nurses have children and 35.1% of them live separately from their children during the pandemic (Table 1).

31.8% of the nurses had a chronic illness and 27.9% of them took medicine continuously. 20.3% of the nurses were infected with COVID-19 and 16.2% of them took medicine during that period. Almost a quarter (23.3%) of the nurses received days-off/health report due to the pandemic (Table 2). The duration of days-off varied between 3 days and 5 months.

4.6% of the nurses stated that they were pregnant in the pre-pandemic period, 8.2% of them got pregnant during the pandemic, and 24.9% did not think of becoming pregnant during the pandemic. When the effect of COVID-19 disease on sexual life in the pandemic was examined, it was found that the sexual life of 17.9% of the nurses changed a lot and 35.1% changed slightly. The frequency of sexual intercourse of nurses decreased by 42.8% in the pandemic. In addition, 32.6% of the nurses and 25.9% of their husbands experienced a lack of sexual desire during this period. 22.8% of the nurses stated that they separated their beds or rooms with their spouses due to the pandemic (Table 2).

The total mean score of the nurses' sexual quality of life scale was calculated as 87.37±20.83 (min.:23, max.:105) in the study. The Cronbach alpha value of the scale for this study was found to be 0.964, and the scale is reliable for this sample. In Table 3, it is seen that the sexual quality of life scale score of nurses having a chronic illness, continuously taking medicine, infected with COVID-19 disease and taking medicine, receiving

Table 1. Distribution of the sociodemographic variables (n=390)*

Sociodemographic Variables		n (%)	±SD
Age	21–30	196 (50.3)	0.5
	31–49	194 (49.7)	
Educational Status	High School	52 (13.3)	0.868
	Two-year Degree	48 (12.3)	
	University	231 (59.2)	
	Postgraduate	59 (15.1)	
Educational Status of Spouses	Primary School	5 (1.3)	0.562
	High School	83 (21.3)	
	University	272 (69.7)	
	Postgraduate	30 (7.7)	
Region of Residence	Aegean Region	101 (25.9)	2.078
	Central Anatolian Region	85 (21.8)	
	Black Sea Region	68 (17.4)	
	Marmara Region	51 (13.1)	
	Mediterranean Region	43 (11)	
	South-East Anatolian Region	25 (6.4)	
	East Anatolian Region	17 (4.4)	
Working Shift	Day	121 (31)	0.925
	Night	3 (0.8)	
	Both day and night	266 (68.2)	
Family Type	Nuclear Family	372 (95.4)	0.21
	Extended family	18 (4.6)	
Status of having children	Yes	262 (67.2)	0.47
	1 child	116 (29.7)	
	2 children	114 (29.2)	
	3 children	32 (8.2)	
	No	128 (32.8)	

*Abbreviations: SD – standard deviation

Table 2. Distribution of information about medical and sexual life of nurses (n=390)*

Medical Information of Nurses	n (%)	±SD
Having a chronic illness		
Yes	124 (31.8)	0.466
No	266 (68.2)	
Taking medicine continuously		
Yes	109 (27.9)	0.449
No	281 (72.1)	
Getting through menopause		
Yes	30 (7.7)	0.267
No	360 (92.3)	
Getting infected with COVID-19		
Yes	79 (20.3)	0.402
No	311 (79.7)	
Taking medicine during the disease		
Yes	63 (16.2)	0.368
No	327 (83.8)	
Receiving days-off or health report during the disease		
Yes	91 (23.3)	0.423
No	299 (76.7)	
Information about the sexual life of nurses during the pandemic		
Status of getting pregnant during the pandemic		
Not pregnant	208 (53.3)	1.731
Getting pregnant in the pre-pandemic period	18 (4.6)	
Getting pregnant during the pandemic	32 (8.2)	
Thinking of getting pregnant	35 (9.0)	
Not thinking of getting pregnant	97 (24.9)	
Status of the pandemic's having changed the sexual life of nurses		
It has changed a lot	70 (17.9)	0.752
It has slightly changed	137 (35.1)	
It has not changed	183 (46.9)	
Frequency of sexual intercourse		
It has decreased compared to the pre-pandemic period	167 (42.8)	0.946
It has increased compared to the pre-pandemic period	41 (10.5)	
It has not been affected	182 (46.7)	
Experiencing low sexual desire		
Yes	127 (32.6)	0.469
No	263 (67.4)	
Sexual desire status of the spouse		
His sexual desire has increased	44 (11.3)	0.69
His sexual desire has decreased	101 (25.9)	
His sexual desire has not changed	245 (62.8)	
Bed/room separation status		
Yes	89 (22.8)	0.42
No	301 (77.2)	
Total	390 (100)	

*Abbreviations: SD – standard deviation

days-off/health report is lower and statistically significant ($p<0.05$).

According to the results of the Kruskal-Wallis H-test conducted to determine whether the nurses showed a significant difference in terms of sexual quality of life scale scores, the difference was found to be significant in terms of pandemic's having changed nurses' sexual life, frequency of sexual intercourse and frequency of sexual desire of the spouse ($p<0.05$). Mann-Whitney U test was performed to find which group caused the difference. According to the test, the sexual quality of life scale score was determined to be lower in the group with decreased sexual intercourse frequency and in the group with decreased sexual desire of the spouse ($p<0.05$) (Table 4).

Table 3. Sexual quality of life scale scores of the nurses according to their medical information*

Medical Information	n	$\bar{x}$	U	p
Having a chronic illness				
Yes	124	165.39	12758.000	<0.001
No	266	209.54		
Continuously taking medicine				
Yes	109	169.50	12480.000	0.005
No	281	205.59		
Getting through menopause				
Yes	30	196.87	5359.000	0.945
No	360	195.39		
Being infected with COVID-19				
Yes	79	166.54	9997.000	0.01
No	311	202.86		
Taking medicine during the disease				
Yes	63	150.52	7467.000	0.001
No	327	204.17		
Receiving days-off or health report				
Yes	91	163.39	10682.500	0.002
No	299	205.27		

*Abbreviations: $\bar{x}$ – mean rank, U – Mann-Whitney U test

Discussion

In this study, the level of nurses' sexual quality of life were found above the average level. The high score obtained from the sexual quality of life scale examined in the study indicates that the sexual quality of life of nurses is good. However, the presence of a chronic illness, continuous medication use, having COVID-19 disease, receiving COVID-19 treatment, receiving days-off/health report for COVID-19 negatively affected the sexual quality of life. In different studies examining the sex-

ual quality of life, it was observed that the sexual quality of life of women was affected depending on factors such as age period, current illness, and operation.[13-15]

Table 4. Sexual quality of life scale scores of the nurses according to their sexual life*

Information about Their Sexual Life	n	$\bar{x}$	KW	*p*
The status of getting pregnant in the pandemic				
Not pregnant	208	204.40	8.202	*0.084*
Getting pregnant in the pre-pandemic period	18	168.11		
Getting pregnant during the pandemic	32	185.23		
Thinking of getting pregnant	35	151.44		
Not thinking of getting pregnant	97	200.77		
Status of the pandemic's having changed the sexual life of nurses				
It has changed a lot	70	108.49	68.023	*0.000*
It has slightly changed	137	184.47		
It has not changed	183	237.04		
Frequency of sexual intercourse				
It has decreased compared to the pre-pandemic period	167	137.43	77.682	*0.001*
It has increased compared to the pre-pandemic period	41	240.99		
It has not been affected	182	238.53		
Sexual desire status of the spouse				
His sexual desire has increased	44	214.76	77.052	*0.000*
His sexual desire has decreased	101	111.07		
His sexual desire has not changed	245	226.84		

*Abbreviations: $\bar{x}$ – mean rank, KW – Kruskal-Wallis H test

More than half of the nurses participating in the study stated that their sexual life changed during the pandemic period. Approximately half of the participants stated that the frequency of sexual intercourse decreased, more than one third of them stated that the desire for sexual intercourse decreased and a small number of participants stated that they separated their bed or room with their spouse. Similarly, in a study conducted to determine the sexual attitudes of healthcare staff in the COVID-19 pandemic, it was found that there was a significant decrease in the sexual desire, frequency of sexual intercourse and duration of sexual intercourse compared to the pre-pandemic period.[16] In another study, it was reported that the COVID-19 pandemic led to a decrease in sexual desire and the frequency of sexual intercourse in Polish women.[17] In parallel with our research results, it was stated in international studies that the frequency of sexual intercourse, sexual desire and sexual functions between spouses / partners during the COVID-19 pandemic decreased compared to the pre-pandemic period and the sexual quality of life decreased.[18-21] However, Yüksel and Özgür (2020) found that women's sexual desire and frequency of sexual intercourse increased in the pandemic compared to the pre-pandemic period, but the sexual quality of life decreased.[22] Since there is not yet sufficient evidence on the fact whether the COVID-19 infection can be transmitted by sexual or genital tract secretions and the spouse/partners are in close contact with each other due to the nature of sexual intercourse, there have been changes in the sexual lives of individuals during the pandemic.[16] Some people may have decreased their sexual functions in this period due to the possibility of transmission whereas some may have increased sexual functions due to the increase in practices such as quarantine, long stay at home, and working distantly. However, it can be said that sexual functions and sexual quality of life of healthcare staff are more affected due to the fact that they are more likely to get infected with COVID-19 and contact with infected people.

The majority of the participants (82.8%) stated that they did not get pregnant during the pandemic and did not intend to become pregnant. Studies have also revealed that reasons such as future anxiety led by the pandemic, economic difficulties, and exposure of the fetus to the virus reduce the desire of women to become pregnant.[22,23] In the studies of Haung and Zhao, the stress and anxiety levels of healthcare staff during the pandemic were found to be quite high.[24] In another study conducted in Saudi Arabia, it was found that medical students experienced high levels of anxiety against the MERS virus.[25] Hamilton and Meston stated that high level of chronic stress caused a decrease in sexual desire. In this study, it is not surprising that sexual functions and desire for getting pregnant of the women working as nurses during the pandemic period have reduced due to the busy and stressful working conditions and social isolation measures.[26]

Limitations of the study

The study had several limitations. The women included in the study may have seen the questions about sexuality as a violation of their privacy and may not have answered fully and correctly. In addition, the online collection of the data of the study and the fact that the results of the study are based on the self-report of the participants can be considered as limitations.

Conclusion

The COVID-19 pandemic has negatively affected people's quality of life globally. Nurses have been more affected by the negative effects of the pandemic as they play an active role in the care and treatment of patients during the COVID-19 pandemic process. Since the nurses are afraid of transmitting the disease to their families and loved ones, restrictions have come with them in many areas of their lives. Although sexuality is not a fundamental factor for the continuation of life, it is one of the most important factors that increase the quality of life. In this study, it was found that the nurses' sexual quality of life during the COVID-19 pandemic was above the average level and their sexual life was negatively affected due to many reasons related to the pandemic. Since sexual life is a phenomenon affected by biopsychosocial and cultural factors, many factors such as the busy working pace of nurses in the field in the COVID-19 pandemic, inability to meet with beloved ones for fear of transmitting viruses, stress, the lack of an exact treatment for the virus, have different effects on the sexual life of individuals in this period. In order to clarify the unknowns about COVID-19, studies with large samples and comparing different parameters related to sexual life are required.

Acknowledgements

The authors thank all parents who participate in this study.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, E.C.E. and Ş.K.E.; Methodology, E.C.E. and Ş.K.E.; Software, E.C.E.; Validation, E.C.E. and Ş.K.E.; Formal Analysis, Ş.K.E.; Investigation, E.C.E. and Ş.K.E.; Resources, E.C.E. and Ş.K.E.; Data Curation, E.C.E. and Ş.K.E.; Writing – Original Draft Preparation, E.C.E.; Writing – Review & Editing, E.C.E. and Ş.K.E.; Visualization, E.C.E. and Ş.K.E.; Supervision, E.C.E. and Ş.K.E.; Project Administration, E.C.E. and Ş.K.E.; Funding Acquisition, E.C.E. and Ş.K.E.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Data availability

The data have not been made public, but are kept with the authors, if necessary.

Ethical approval

Ethical consent was obtained from Bartın University Social and Human Sciences Ethics Committee for the study, dated 05/11/2020 and numbered 0236.

References

1. World Health Organizatin (WHO). Coronavirus disease (COVID-19), Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/question-and--answers-hub/q-a-detail/coronavirus-disease-COVID-19. Accessed February 21, 2021.
2. WHO. Situation by region, country, territory & area. Available from: https://covid19.who.int/table. Accessed February 15, 2021.
3. Republic of Turkey Ministry of Health. COVID-19 information page. Available from: https://covid19.saglik.gov.tr/TR-66935/genel-koronavirus-tablosu.html Accessed February 10, 2021.
4. Göçmen Baykara Z, Eyüboğlu G. Nursing care in the COVID-19 pandemic. *GUHES.* 2020:9-17.
5. Bozdemir N, Özcan S. Overview of sexuality and sexual health. *TJFMPC.* 2011;5(4):37-46.
6. Kula S, Çakar B. The relationship between perceived life satisfaction and safety of people in society within the frame of maslow's hierarchical needs. *Journal of İ.İ.B.F. Bartin Unıversıty.* 2015;6(12):191-210.
7. Yılmaz Esencan T., Kızılkaya Beji N. Examination of the studies on sexuality until today. *Androloji Bülteni*. 2015;17(63):301-310.
8. Cirban E. Relationship of believing in sexual myths with the menopause attitudes and symptoms in postmenopausal women. T. C. Aydın Adnan Menderes University Health Sciences Institute. The Degree of Master Dissertation, Aydın; 2019.
9. Blanc AK, Glazer K, Ofomata Aderemi U, Akinfaderin Agarau F. Myths and misinformation: an analysis of text messages sent to a sexual and reproductive health Q&A service in Nigeria. *Stud Fam Plann.* 2016;47(1):39-53.
10. Nie S, Sun C, Wang L, Wang X. The Professional identity of nursing students and their intention to leave the nursing profession during the coronavirus disease (COVID-19) pandemic. *JNR.* 2021;29(2):e139.
11. Tseng YH, Weng CS, Kuo SH, Chou FH, Yang YH, Chiang LC. Gender differences? Internet use and parents-child communication about sex toward sexual attitudes among early adolescents in Taiwan. *JNR.* 2015;23(2):125-134.
12. Tuğut N, Gölbaşı Z. A validity and reliability study of Turkish version of the Sexual Quality of Life Questionnaire-Female. *CMJ.* 2010;32:172-180.
13. Aygün Topuz E. Evaluation of the effects of mastectomy on self-esteem and sexual life quality. T.C. Namık Kemal University Health Sciences Institute. The Degree of Master Dissertation, Tekirdağ; 2019.
14. Gölbaşı Z, Şentürk Erenel A. The quality of sexual life in women with gynaecological cancers. *Arch Gynecol Obstet.* 2012;285(6):1713-1717.
15. Küt D. Examination of young adult women's attachment styles, sexual life quality levels, sexual confidence and psychological symptoms. T. C. Maltepe University Social Sciences Institute. The Degree of Doktoral Thesis, İstanbul; 2018.

16. Çulha MG, Demir O, Sahin O, Altunrende F. Sexual attitudes of healthcare professionals during the COVID-19 outbreak. *IJIR.* 2021;33(1):102-109.
17. Fuchs A, Matonog A, Pilaska J, et al. The impact of COVID-19 on female sexual health. *Int J Environ Res Public Health.* 2020;17(19):7152-7160.
18. Lehmiller J, Garcia JR, Gesselman AN, Mark KP. Less sex, but more diversity: changes in sexual behavior during the COVID-19 coronavirüs pandemic. *Leisure Sciences.* 2020;43(1-2):295-304.
19. Li G, Tank D, Song B, et al. Impact of the COVID-19 pandemic on partner relationships and sexual and reproductive health: cross-sectional, online survey study. *JMIR.* 2020;22(8):e20961.
20. Mahanty C, Kumar R, Mishra BK. Analyses the efects of COVID-19 outbreak on human sexual behaviour using ordinary least-squares based multivariate logistic regression. *Quality & Quantity.* 2021;55(4):1239-1259.
21. Schiavi MC, Spina V, Zullo MA, et al. Love in the time of COVID-19: sexual function and quality of -life analysis during the social distancing measures in a group of Italian Reproductive age women. *J Sex Med.* 2020;17(8):1407-1413.
22. Yüksel B, Özgör F. Effect of the COVID-19 pandemic on female sexual behavior. *IJGO.* 2020;150(1):98-102.
23. Micelli E, Cito G, Cocci A, et al. Desire for parenthood at the time of COVID-19 pandemic: an insight into the Italian situation. *JPOG.* 2020;41(3):183-190.
24. Huang Y, Zhao N. Generalized anxiety disorder, depressive symptoms and sleep quality during COVID-19 epidemic in China: a web-based cross-sectional survey. *Psychiatry Res. 2020;288:112954.*
25. Al-Rabiaah A, Temsah MH, Al-Eyadhy AA, Hasan GM, Al-Zamil F, Al-Subaie S, et al. Middle East respiratory syndrome-corona virus (MERS-CoV) associated stress among medical students at a university teaching hospital in Saudi Arabia. *J Infect Public Health.* 2020;13(5):687-691.
26. Hamilton LD, Meston CM. Chronic stress and sexual function in women. *J Sex Med.* 2013;10(10):2443-2454.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 371–378

Instructions for Authors

About the Journal

The European Journal of Clinical and Experimental Medicine (Eur J Clin Exp Med) is an open access journal, and all articles are free to access, download, share, and re-use. The Eur J Clin Exp Med is a peer-reviewed, international scientific journal that publishes full-length articles on topics within medical science. The journal welcomes submissions of articles on current advances in life and health sciences, clinical and experimental medicine, and related disciplines.

Open access and creative commons

All articles are published with **free** open access under the CC-BY Creative Commons attribution license (the current version is CC-BY, version 4.0. If you submit your paper for publication by the Eur J Clin Exp Med, you agree to have the CC-BY license applied to your work. Under this Open Access license, you, as the author, agree that anyone may download and read the paper for free. In addition, the article may be reused and quoted provided that the original published version is cited. This facilitates freedom in re-use and also ensures that Eur J Clin Exp Med content can be mined without barriers for the research needs.

Article processing charges

The Eur J Clin Exp Med is an open access journal and does **not** levy an article processing charge. There are no submission, color, or page charges for all article types.

Copyright Statement

Authors of articles published in the Eur J Clin Exp Med retain copyright on their articles, except for any third-party images and other materials added by the Eur J Clin Exp Med which are subject to copyright of their respective owners. Authors are therefore free to disseminate and re-publish their articles, subject to any requirements of third-party copyright owners and subject to the original publication being fully cited. Visitors may also download and forward articles subject to the citation requirements. The ability to copy, download, forward or otherwise distribute any materials is always subject to any copyright notices displayed. Copyright notices must be displayed prominently and may not be obliterated, deleted or hidden, totally or partially.

Ethics in publishing

The Eur J Clin Exp Med takes responsibility of enforcing rigorous peer-review together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, inappropriate authorship credit, and the like, do arise. The Eur J Clin Exp Med takes such publishing ethics issues very seriously and our editors are trained to proceed in such cases with a zero tolerance policy. The Eur J Clin Exp Med is a member of and subscribes to the principles of the Committee on Publication Ethics (COPE).

Editorial and submission policies

When you submit a manuscript to the Eur J Clin Exp Med we will take it to imply that the manuscript has not already been published or submitted elsewhere. If similar or related work has been published or submitted elsewhere, then you must provide a copy of this work with the submitted manuscript. You may not submit your manuscript elsewhere while it is under consideration in the Eur J Clin Exp Med. If the manuscript includes personal communications, please provide a written statement of permission from any person who is quoted. Permission by email is acceptable.

We reserve the right to reject a paper even after it has been accepted if it becomes apparent that there are serious problems with its scientific content, or our publishing policies have been violated.

Author responsibilities

Authorship provides credit for a researcher's contributions to a study and carries accountability. Authors are expected to fulfil the criteria below (adapted from McNutt et al., Proceedings of the National Academy of Sciences, 2018, 201715374; DOI: 10.1073/pnas.1715374115):

— **Each author** is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it
— **AND** to have approved the submitted version (and any substantially modified version that involves the author's contribution to the study);
— **AND** to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

The Eur J Clin Exp Med does not require all authors of a research paper to sign the cover letter upon submission, nor do they impose an order on the list of authors. Submission to the Eur J Clin Exp Med is taken by the publication to mean that all the listed authors have agreed to all of the contents. The corresponding (submitting) author is responsible for having ensured that this agreement has been reached, and for managing all communication between the publication and all co-authors, before and after publication.

Author contributions statements

Authors are required to include a statement of responsibility in the manuscript (at the end of the main text, before the 'References' section) that specifies the contribution of every author. For articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; Methodology, X.X.; Software, X.X.; Validation, X.X., Y.Y. and Z.Z.; Formal Analysis, X.X.; Investigation, X.X.; Resources, X.X.; Data Curation, X.X.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, X.X.; Visualization, X.X.; Supervision, X.X.; Project Administration, X.X.; Funding Acquisition, Y.Y.".

Corresponding author – responsibilities

The corresponding (submitting) author is solely responsible for communicating with the Eur J Clin Exp Med and for managing communication between co-authors. Before submission, the corresponding author ensures that all authors are included in the author list, its order has been agreed by all authors, and that all authors are aware that the paper was submitted.

A confidential process

The Eur J Clin Exp Med treats the submitted manuscript and all communication with authors and referees as confidential. Authors must also treat communication with the Eur J Clin Exp Med as confidential: correspondence with the Eur J Clin Exp Med, referee reports and other confidential material must not be posted on any website or otherwise publicized without prior permission from the Eur J Clin Exp Med publishing team, regardless of whether or not the submission is eventually published. Our policies about posting preprints and post prints, and about previous communication of the work at conferences or as part of a personal blog or of an academic thesis, are described in the Confidentiality section.

Referee suggestions

During the submission process, please suggest three potential reviewers (names and institutional e-mail addresses) with the appropriate expertise to review the manuscript, but please keep in mind that we are not obliged to follow these recommendations. The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last five years. Proposed reviewers should be from different institutions to the authors. You may suggest reviewers from among the authors that you frequently cite in your paper. You may also name a limited number of scientists who should not review your paper (up to 3 named individuals or laboratories); these exclusions will be honored. The decision of the Editorial Board Member on the choice of referees is final.

Ethics, use of experimental animals, and human participants

For articles in the Eur J Clin Exp Med reporting experiments on live vertebrates and/or higher invertebrates, the methods section must include a statement: (i) identifying the institutional and/or licensing committee approving the experiments, including any relevant details; (ii) confirming that all experiments were performed in accordance with relevant guidelines and regulations.

For research involving human participants, authors must identify the committee that approved the research, confirm that all research was performed in accordance with relevant guidelines/regulations, and include in their manuscript a statement confirming that informed consent was obtained from all participants and/or their legal guardians.

Authors may be required to submit, on request, a statement from the research ethics committee or institutional review board indicating approval of the research.

Competing interests policy

In the interests of transparency and to help readers to form their own judgements of potential bias, authors must declare any competing financial and/or non-financial interests in relation to the work described. For the purposes of this policy, competing interests are defined as financial and non-financial interests that could directly undermine, or be perceived to undermine, the objectivity, integrity and value of a publication, through a potential influence on the judgements and actions of authors with regard to objective data presentation, analysis and interpretation. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

Competing interests statement format guidelines

The statement included in the article file must be explicit and unambiguous, describing any potential competing interest (or lack thereof) for EACH contributing author.

Examples of declarations are:

— Competing interests: The author(s) declare no competing interests.
— Competing interests: Dr X's work has been funded by A. He has received compensation as a member of the scientific advisory board of B and owns stock in the company. He also has consulted for C and received compensation. Dr Y and Dr Z declare no potential conflict of interest.
— Competing interests: "This work was supported by the [Funding Agency] under Grant [number]."

Peer-reviewers

The Eur J Clin Exp Med invites peer-reviewers to exclude themselves in cases where there is a significant conflict of interest, financial or otherwise. However, just as financial interests need not invalidate the conclusions of an article, nor do they automatically disqualify an individual from evaluating it. We ask peer-reviewers to inform the editors of any related interests, including financial interests as defined above that might be perceived as relevant. Editors will consider these statements when weighing peer-reviewers' recommendations.

Availability of materials and data

In order to maintain the integrity, transparency and reproducibility of research records, authors are encouraged to make their experimental and research data openly available either by depositing into data repositories or by publishing the data and files as supplementary information in this journal.

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of other repositories specialized in scientific and experimental data, please consult databib.org or re3data.org. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal Data also accepts submissions of data set papers.

Data availability statement format guidelines

The statement should be provided as a separate section (titled 'Data Availability') at the end of the main text, before the 'References' section. Data availability statements should include, where applicable, accession codes, other unique identifiers and associated web links for publicly available datasets, and any conditions for access of non-publicly available datasets. Where figure source data are provided, statements confirming this should be included in data availability statements. Depending on the data described in the manuscript, data availability statements commonly take one of the following forms, or can be a composite of the statements below:

— The datasets generated during and/or analyzed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS].
— The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.
— All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).
— The datasets generated during and/or analyzed during the current study are not publicly available due to [REASON(S) WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.
— No datasets were generated or analyzed during the current study.
— The data that support the findings of this study are available from [THIRD PARTY NAME] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [THIRD PARTY NAME].

Correction and retraction policy

The Eur J Clin Exp Med operates the following policy for making corrections to its peer-reviewed content.

Publishable amendments must be represented by a formal online notice because they affect the publication record and/or the scientific accuracy of published information. Where these amendments concern

peer-reviewed material, they fall into one of four categories: Publisher Correction (formerly Erratum), Author Correction (formerly Corrigendum), Retraction or Addendum.

Publisher Correction (formerly Erratum). Notification of an important error made by the journal that affects the publication record or the scientific integrity of the paper or the reputation of the authors or the journal.

Author Correction (formerly Corrigendum). Notification of an important error made by the author(s) that affects the publication record or the scientific integrity of the paper, or the reputation of the authors or the journal.

Retraction. Notification of invalid results. All co-authors must sign a Retraction specifying the error and stating briefly how the conclusions are affected, and submit it for publication. In cases where co-authors disagree, the in-house editors may seek advice from independent referees and impose the type of amendment that seems most appropriate, noting the dissenting author(s) in the text of the published version.

Addendum. Notification of additional information. Addenda are published when the in-house editors decide that the addendum is crucial to the reader's understanding of a significant part of the published contribution.

Peer-review process

Initial checks

Once submitted, your manuscript will be assigned to a member of our Editorial Board, who will read the paper and decide whether it is appropriate for the journal. Manuscripts that are within scope and seem, on initial assessment, to be technically sound and scientifically valid, will be sent to external reviewers. Copies of any papers containing similar or related work under consideration or in press at other journals must be included with the submission.

Manuscripts that do not fit the journal's ethics policy or do not meet the standards of the journal will be rejected before peer-review. Manuscripts that are not properly prepared will be returned to the authors for revision and resubmission.

Peer review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. Reviewers will be able to access your manuscript securely using our online system, whilst maintaining referee anonymity. A double-blind review is applied, where authors' identities are unknown to reviewers and vice versa. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

Editorial Decision

After considering the reviewer reports the Editorial Board Member will make one of the following decisions:

— Accept outright,
— Request a minor revision, where authors revise their manuscript to address specific concerns,
— Request a major revision, where authors revise their manuscript to address significant concerns and perhaps undertake additional work,
— Reject outright.

The final decision is made by the Editor-in-Chief.

Revisions

In cases where the referees or Editorial Board Member has requested changes to the manuscript, you will be invited to prepare a revision. The decision letter will specify a deadline for submission of a revised manuscript. Once resubmitted, the manuscript may then be sent back to the original referees or to new referees, at the Editorial Board Member's discretion.

A revised manuscript should be submitted via the revision link provided in the decision letter, and not as a new manuscript. The revision should also be accompanied by a point-by-point response to referees explaining how the manuscript has been changed. Please ensure that all issues raised have been addressed in the first round of revision. Where the authors disagree with a reviewer, they must provide a clear response.

Final submission and acceptance

When all editorial issues are resolved, your paper will be formally accepted for publication. Once accepted, the manuscript will undergo professional copy-editing, English editing, final corrections, pagination, and, publication on the http://www.ejcem.ur.edu.pl/. The Eur J Clin Exp Med reserves the right to make the final decision about matters of style and the size of figures.

Appeals

Even in cases where the Eur J Clin Exp Med does not invite resubmission of a manuscript, some authors may ask the Editorial Board to reconsider a rejection decision. These are considered appeals, which, by policy, must take second place to the normal workload. In practice, this means that decisions on appeals often take several weeks. Only one appeal is permitted for each manuscript, and appeals can only take place after peer review. Final decisions on appeals will be made by the Editorial Board Member handling the paper.

Decisions are reversed on appeal only if the relevant Editorial Board Member is convinced that the original decision was a serious mistake. Consideration of an appeal is merited if a referee made substantial errors of fact or showed evidence of bias, but only if a reversal of that referee's opinion would have changed the original decision. Similarly, disputes on factual issues need not be resolved unless they were critical to the outcome.

If an appeal merits further consideration, the Editorial Board Member may send the authors' response and the revised paper out for further peer review.

ORCID

The Eur J Clin Exp Med supports the use of ORCID. The Eur J Clin Exp Med mandates ORCID iDs for all submitting authors; this is published on the final article to promote discoverability and credit. Please provide the ORCID iDs of the authors in the title page.

Submission guidelines

Submission Process

Manuscripts for the Eur J Clin Exp Med should be submitted online at https://mc04.manuscriptcentral.com/pmur. The submitting author, who is generally the corresponding author, is responsible for the manuscript during the submission and peer-review process. The submitting author must ensure that all eligible co-authors have been included in the author list (read the criteria to qualify for authorship) and that they have all read and approved the submitted version of the manuscript. To submit your manuscript, register and log in to the submission website. All co-authors can see the manuscript details in the submission system, if they register and log in using the e-mail address provided during manuscript submission.

Cover letter

A cover letter must be included with each manuscript submission. It should be concise and explain why the content of the paper is significant, placing the findings in the context of existing work and why it fits the scope of the journal. Confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal. The names of proposed and excluded reviewers should be provided in the submission system, not in the cover letter.

Accepted File Formats

Authors must use Microsoft Word to prepare their manuscript. Please insert your tables, graphics (schemes, figures, etc.) in the main text after the paragraph of its first citation.

In most cases, we do not impose strict limits on word count or page number. However, we strongly recommend that you write concisely and stick to the following guidelines:

— We encourage not exceeding 20 pages for original and review papers, and 8 pages for case reports of standard computer text (1800 signs on a page).
— The main text should be no more than 4,500 words (not including Abstract, Methods, References and figure legends).
— The title should be no more than 20 words.
— The abstract should be no more than 250 words.
— Recommended font: Times New Roman, 12 points.
— Manuscript text should be double-spaced. Do not format text in multiple columns.

Types of Publications

Manuscripts submitted to the Eur J Clin Exp Med should neither be published previously nor be under consideration for publication in another journal. The main article types are as follows:

Original research manuscripts. The journal considers all original research manuscripts provided that the work reports scientifically sound experiments and provides a substantial amount of new information.

Reviews. These provide concise and precise updates on the latest progress made in a given area of research. Systematic reviews should follow the PRISMA guidelines.

The Eur J Clin Exp Med accepts also the following types of submissions: case reports, letters to the editor, commentaries, book reviews, and reports from scientific meetings and conferences.

Reporting guidelines

The guidelines listed below should be followed where appropriate. Please use these guidelines to structure your article. Completed applicable checklists, structured abstracts and flow diagrams should be uploaded with your submission; these will be published alongside the final version of your paper.

Please refer to existing guidelines for reporting methodology; e.g.:

— AGREE guidelines for clinical practice guidelines
— ARRIVE guidelines for *in vivo* animal studies
— CARE guidelines for clinical case reports
— CONSORT guidelines for clinical trials
— PRISMA guidelines for systematic reviews and meta-analyses
— SPIRIT for clinical trials
— STARD guidelines for studies of diagnostic accuracy
— STROBE guidelines for observational studies

Manuscript Preparation

Your paper should consist of the following parts. Title page should be supplied as a **separate** file.

Research manuscripts should comprise:

— Title page: Title, Author list, Affiliations, Abstract, Keywords.
— Research manuscript sections: Introduction, Aim, Materials and Methods, Results, Discussion, Conclusions.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions,

Conflicts of Interest, Data Availability, Ethics Approval, References.

Research manuscript sections:

— *Introduction*

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

— *Material and methods*

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

— *Results*

Results should be clear and concise. The section may be divided into subsections, each with a concise subheading. Tables and figures central to the study should be included in the main paper. Do not use the term "significant" unless p-values are provided. Show p-values to 2 or 3 decimal places. The Results section should be written in past tense.

— *Discussion*

This should explore the significance of the results of the work, not repeat them. Avoid extensive citations and discussion of published literature.

— *Conclusions*

Summarize the work's findings, state their importance, and possibly recommend further research.

Review manuscripts should comprise:

— Title page: Title, Author list, Affiliations.
— Abstract, Keywords, Literature review sections.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions, Conflicts of Interest, Data Availability, References.

Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the PRISMA guidelines.

Case reports should comprise:

— Title page: Title, Author list, Affiliations.
— Abstract, Keywords. Case reports should include a succinct introduction about the general medical condition or relevant symptoms that will be discussed in the case report; the case presentation including all of the relevant de-identified demographic and descriptive information about the patient(s), and a description of the symptoms, diagnosis, treatment, and outcome; a discussion providing context and any necessary explanation of specific treatment decisions; a conclusion briefly outlining the take-home message and the lessons learned.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions, Conflicts of Interest, Data Availability, Ethics Approval, References.

Requirements for case reports submitted to Eur J Clin Exp Med:

— Patient ethnicity must be included in the Abstract under the Case Presentation section.
— Consent for publication is a mandatory journal requirement for all case reports. Written informed consent for publication must be obtained from the patient (or their parent or legal guardian in the case of children under 18, or from the next of kin if the patient has died).

Language Style

Manuscripts must be submitted in English (American or British usage is accepted, but not a mixture of these).

Title page

These sections should appear in all manuscript types:

Title: The title of your manuscript should be concise and informative. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used.

Author List and Affiliations: Authors' full first and last names must be provided. For each affiliation provide the details in the following order: department, institution, city, country. If available, the e-mail address of each author should also be provided. At least one author should be designated as *corresponding author*, and his or her email address and other details should be included at the end of the affiliation section.

Abstract: The abstract should be a total of about 250 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts: *Introduction and aim:* Place the question addressed in a broad context and highlight the purpose of the study; *Material and methods*: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used. *Results:* Summarize the article's main findings; and *Conclusion*: Indicate the main conclusions or interpretations.

Keywords: Three to six pertinent keywords need to be added after the abstract in alphabetical order. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

Back Matter

Supplementary Materials: Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.

Acknowledgments: Thank all of the people who helped with the research but did not qualify for authorship. Acknowledge anyone who provided intellectual assistance, technical help, or special equipment or materials.

Funding Statement: All sources of funding of the study should be disclosed.

Author Contributions: Authors must supply an Author Contribution Statement as described in the *Author contributions statements* section.

Conflicts of Interest: Authors must supply a competing interests statement. For more details please see *Competing interests policy.*

Data Availability: Authors must include a Data Availability Statement in all submitted manuscripts; see *Availability of materials and data* section for more information.

Ethics approval: Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

References: References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, Reference Manager or Zotero to avoid typing mistakes and duplicated references.

References style

References should be prepared according to the American Medical Association style.

Examples: The degree of respiratory muscles fatigue depends on the applied exercise protocol and the research group's fitness level.[1,2] The greatest load with which a patient continues breathing for at least one minute is a measure of inspiratory muscles strength.[3] Diabetes mellitus is associated with a high risk of foot ulcers.[4-6]

A citation should contain a maximum of 6 authors. When an article has more than six authors, only the first three names should be given by adding 'et al.' If the source does not have any authors, the citation should begin with the title.

Journal titles should be given in brief according to the Index Medicus standard.

The following are examples of individual citations made according to the required rules of editing and punctuation:

— **Article from a journal, number of authors from 1 to 6**

Author AA, Author BB, Author CC. Title of article. *Abbreviated Journal Title*. Year;Volume(Issue):Page-Page.

Lee JC, Seo HG, Lee WH, Kim HC, Han TR, Oh BM. Computer-assisted detection of swallowing difficulty. *Comput Methods Programs Biomed*. 2016;134(2):72-78.

Wolf ZR. Nursing practice breakdowns: Good and bad nursing. *Medsurg Nursing*. 2012;21(1):16-36.

— **Article from a journal, number of authors more than 6**

Author AA, Author BB, Author CC, et al. Title of article. *Abbreviated Journal Title*. Year;Volume (Issue):Page-Page.

Gonzalez ME, Martin EE, Anwar T, et al. Mesenchymal stem cell-induced DDR2 mediates stromal-breast cancer interactions and metastasis growth. *Cell Rep*. 2017;18:1215-1228.

Jordan J, Toplak H, Grassi G, et al. Joint statement of the European Association for the Study of Obesity and the European Society of Hypertension: obesity and heart failure. *J Hypertens*. 2016;34:1678-1688.

— **Article from an online journal: DOI**

Author AA, Author BB. Title of article. *Abbreviated Journal Title*. Year;Volume(Issue):Page-Page. doi:xx.xxxx/xxxxxxxxxxxxxx

Coppinger T, Jeanes YM, Hardwick J, Reeves S. Body mass, frequency of eating and breakfast consumption in 9-13-year-olds. J Hum Nutr Diet. 2012;25:43-49. doi: 10.1111/j.1365-277X.2011.01184.x.

Cogulu O, Schoumans J, Toruner G, Demkow U, Karaca E, Durmaz AA. Laboratory Genetic Testing in Clinical Practice 2016. Biomed Res Int. 2017;2017:5798714. doi: 10.1155/2017/5798714.

— **Websites**

Webpage title. Name of Website. URL. Published or Updated date. Accessed date.

Cholera in Haiti. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/haiticholera/. Published October 22, 2010. Updated January 9, 2012. Accessed February 1, 2012.

Address double burden of malnutrition: WHO. World Health Organization site. http://www.searo.who.int/mediacentre/releases/2016/1636/en/. Accessed February 2, 2017.

— **Book**

Author AA, Author BB. *Title of Work*. Location: Publisher; Year:Page-Page

Doane GH, Varcoe C. *Family Nursing as Relational Inquiry: Developing Health– Promoting Practice*. Philadelphia, PA: Lippincott Williams & Wilkins; 2005:25-28.

London ML, Ladewig PW, Ball JW, et al. *Maternal & Child Nursing Care*. Upper Saddle River, NJ: Pearson Education; c2011:101-103.

— **Chapter in a book**

Author AA. Title of Work. Editor AA, Editor BB, eds. Location: Publisher; Year:PagePage.

Goodman LS, Brunton LL, Chabner B, Knollmann BC. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. Brunton LL, ed. New York, NY: McGraw-Hill; 2011:99.

NOTE: The editorial board requires consistent and carefully made references prepared according to the above-mentioned AMA standards. Otherwise, the work will be sent back to the authors.

Preparing Figures, Schemes and Tables

File for Figures and Schemes must be provided during submission and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.

All Figures, Schemes and Tables should be inserted into the main text close to their first citation and must be numbered following their number of appearance (Figure 1, Scheme I, Figure 2, Scheme II, Table 1, *etc.*).

All Figures, Schemes and Tables should have a short explanatory title (not on the figure itself) and caption. Tables should present new information rather than duplicating what is in the text. Readers should be able to interpret the table without reference to the text.

All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Please supply editable tables in appropriate place in the main text. Do not submit your tables in separate files.

Abbreviations

The journal requires using only standard abbreviations. Abbreviations should be defined in parentheses the first time they appear in the abstract, main text and in figure or table captions and used consistently thereafter. Ensure consistency of abbreviations throughout the article. Keep abbreviations to a minimum.

SI Units

SI Units (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible.