e-ISSN 2544-1361

European Journal of Clinical and Experimental Medicine

Formerly: Medical Review

Quarterly

Vol. 20, No. 2

Publication date: June 2022

Rzeszów, Poland 2022

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

ICV 2020: 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

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

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 (2): 140

Contents

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.1

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 141–145

ORIGINAL PAPER

Mehmet Ali Karaselek [1], Hasan Kapaklı [1], Şükrü Nail Güner [1], Ercan Kurar [2], Serkan Küççüktürk [3], Sevgi Keleş [1], İsmail Reisli [1]

A family screening of *CD19* gene mutation by PCR-RFLP

[1] Department of Pediatric Allergy and Immunology, Meram Medical Faculty, Necmettin Erbakan University, Konya, Turkey
[2] Department of Medical Biology, Meram Medical Faculty, Necmettin Erbakan University, Konya, Turkey
[3] Department of Medical Biology, Medical Faculty, Karamanoğlu Mehmetbey University, Karaman, Turkey

ABSTRACT

Introduction and aim. Mutation(s) in the gene encoding the CD19 molecule affect CD19 protein expression and primary immunodeficiency (PID) occurs. The PCR-RFLP method, which is faster and cheaper than other mutation detection methods, is rarely used in the diagnosis of PID. The study aimed to genetically identify CD19 deficiency, which is a PID, using the PCR-RFLP method.

Material and methods. A total of 8 patients and two healthy controls were included in the study and the relevant region genotypes in the CD19 gene were determined by performing PCR-RFLP analysis.

Results. The index case, newborn baby and mother were also included in the study. It was determined that the index case (P6) was homozygous mutant, the newborn baby (P7) and mother (P8) had heterozygous genotype. Based on this situation, one child (P1) was found to be homozygous mutant, mother (P2), father (P3) and other children (P4 and P5) had heterozygous genotype in the family, which was determined to be related to the first case.

Conclusion. In our study, it has been shown that PCR-RFLP is a method that can be used in the diagnosis of PID by determining genotypes using PCR-RFLP, and especially in terms of rapid genetic testing of family screenings.

Keywords. CD19, PID, RFLP

Introduction

Primary immunodeficiencies (PID) are a group of diseases that result from the development and/or dysfunction of various components of the immune system. In recent years, many genes that cause PID have been identified. Primary antibody deficiencies, mainly predisposed to bacterial infections, can be caused by mutations in genes involved in B cell differentiation.[1] Mutations in these genes inhibit immature B cell differentiation in bone marrow and cause agammaglobulinemia and low B cell in peripheral blood.[2] Although many genes (*CD20, CD21, CD81, CD225*) play a role in B cell development, one of the most important is the *CD19* gene.[3,4]

The *CD19* is a gene located in the p11.2 region of chromosome 16, and consists of 15 exons and 7.41 kb length (NC_000016.10).[5] This gene encodes the CD19 protein, a transmembrane glycoprotein of the immunoglobulin (Ig) superfamily. CD19 is also expressed in follicular dendritic cells as well as in normal and neoplastic B cells, which expression begins for the B cells in the bone marrow and continues until the plasma cell. In addition, CD19 forms a complex with CD21, CD81 and CD225 on the surfaces of mature B cells (Fig. 1).[6]

Corresponding author: Mehmet Ali Karaselek, e-mail: malikaraselek@gmail.com

Received: 15.09.2021 / Revised: 15.11.2021 / Accepted: 22.11.2021 / Published: 30.06.2022

Karaselek MA, Kapaklı H, Güner ŞN, Kurar E, et al. *A family screening of "CD19" gene mutation by PCR-RFLP*. Eur J Clin Exp Med. 2022;20(2):141–145. doi: 10.15584/ejcem.2022.2.1.

This complex co-operates with the B cell receptor (BCR) and stimulates both BCR-dependent and BCR-independent intrinsic B cell signaling pathways. Despite the mutation in the *CD19* gene, normal B cell differentiation occurs in the bone marrow, and the absolute number of B cells in the peripheral blood is normal. However, stimulation on BCR is impaired and the number of memory B cells in peripheral blood is decreased. This results in hypogammaglobulinemia and impaired antigen-specific humoral response.[7-8]

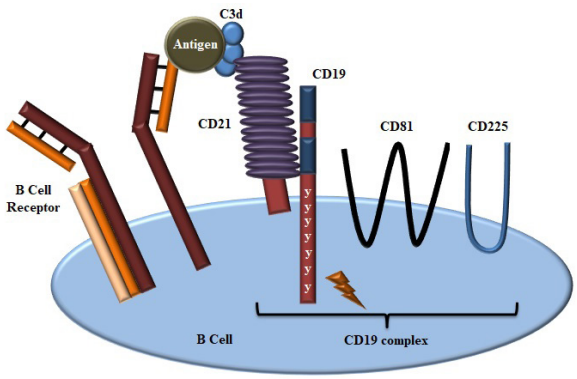

Fig. 1. B cell and CD19 complex

The first case of PID due to mutation in the *CD19* gene was described by us in a 10-year-old girl.[9] Following the identification of this case, a village screening of 208 cases was carried out and carrier was detected in 20 cases. Screening was performed by determining flow cytometric CD19 expressions.[10] She was admitted to us from the same village that was related to the previously described patient. According to the clinical and laboratory results of the patient, it was thought that *CD19* gene mutation might be present and genetic identification was aimed. In order to determine the mutation, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method which was used in *DCLRE1C* mutation detection.[11]

Aim

Therefore, in this study, it was aimed to determine *CD19* gene mutation by PCR-RFLP which is a cheap, reliable and fast method.

Material and methods

Patients

The index family has 4 children (Fig. 2). The patient was admitted to our clinic at 7 years old with complaints of frequent illness.

The history of the patient revealed that she had a fever 3-4 times a month and had a frequent fever since she started school and diagnosed at Idiopathic Thrombocytopenic Purpura (ITP) at 5 years old. Parents had a consanguineous marriage. It was learned that there was a baby who had intrauterine exitus at the age of 6 months in the family. We were learned that patient was related to the first case described by van Zelm et. al. in 2006.[11] As a result of this information and laboratory examination, it was thought that *CD19* gene mutation might be present. The region to be studied for mutation was selected as the previously defined region (c.973_973insA; p.Arg325AlafsX4; GenBank: AH005421.2). The patient (P1), mother (P2), father (P3), sister (P4), brother (P5), the patient identified in 2006 (P6), newborn baby of P6 (P7) and mother of P6 (P8) were included in the study. The studies were performed with 2 healthy controls (C1 and C2). Patient recruitment and the studies reported herein were approved by Institutional Review Board at the Necmettin Erbakan University Meram Medical Faculty. Written informed consent was obtained from participating patients' guardians and healthy controls.

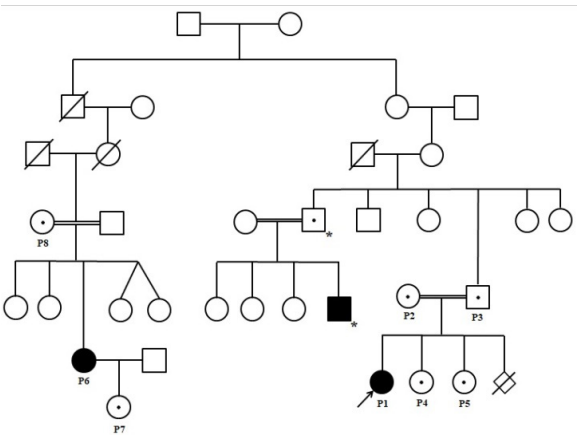

Fig. 2. Pedigree (*:Patients who had previously detected CD19 mutation and carriage)

Flow cytometry

Peripheral lymphocyte subgroup study of the patient and family members were performed. After the study, cell counts were performed with Becton Dickinson Canto II (BD Biosciences, Heidelberg, Germany) flow cytometry and their analysis were performed. In addition, CD19 expression was performed with monoclonal antibody from three different clones (SJ25C1, 4G7, HIB19).

PCR and PCR-RFLP

Polymerase chain reaction (PCR) primers covering mutation regions in *CD19* gene were designed (forward primer: 5'-CCTGAGGAGGAAAAGAAT-3' and reverse primer: 5'-GGAAACAGTAAGTGCAAGGCATA-3'). The mutation regions were amplified by PCR using these primers. A PCR protocol was used and cycling conditions were initial denaturation of 95°C for 10 minutes followed by 45 cycles of 95°C for 40 seconds, annealing beginning at 49,9°C and 72°C 15 seconds. A final extension of 72°C for 10 minutes was applied. Resulting PCR products were visualized by 1% agarose gel. DNA ampl-

icons of 118 bp were obtained after PCR amplification. PCR-RFLP method was applied to PCR products The PCR products were incubated for 1 hour at 65°C with *Bsm*I (New England Biolabs®Inc.). DNA fragments obtained after PCR-RFLP procedure were visualized on 3 % agarose gel and genotypes were determined as wild type, heterozygous and homozygous mutant.

DNA fragments of 118 bp were obtained after PCR with primers designed for *CD19* mutation site. *Bsm*I enzyme recognition region 5'…GAATGCN^…3'; 3'… CTTAC^GN…5' is selected according to the wild type allele. In the absence of mutation, enzyme digestion was performed in both alleles 94 and 24 bp DNA fragment was obtained after PCR-RFLP and these results were interpreted as normal homozygous genotype. If the patient is homozygous mutant genotype 118 bp DNA fragment was obtained. In the case of heterozygote, three DNA fragments will be obtained: 118 bp, 94 bp and 24 bp.

Results

In the peripheral lymphocyte subgroup analysis, CD3+ total T, CD4+ helper T, CD8+ cytotoxic T and CD16+CD56+ NK cell numbers were found to be normal but there was no CD19 expression on CD20+B cells. The patient had low IgG (347 mg/dl) and IgA (25 mg/dl) levels. In addition, the hepatitis B vaccine response was negative (0.74 mIU/ml; normal range 0-10 mIU/ml). Clinical and laboratory characteristics of the P1 are shown in Table 1.

Table 1. Laboratory and clinical features of the patient (P1)

Parameters	P1
Clinical features on admission	Frequent illness during school Fewer ITP
Age of diagnosis (years)	7
Lymphocytes (counts/mm^3)	3700
CD3+ (counts/mm^3)	2479
CD4+ (counts/mm^3)	1369
CD8+ (counts/mm^3)	814
CD19+ (counts/mm^3)	0*
CD20+ (counts/mm^3)	740
CD16+CD56+ (counts/mm^3)	222
IgG (mg/dl) (842-1943)	347*
IgA (mg/dl) (62-390)	25*
IgM (mg/dl) (54-392)	88

* below normal value

CD19 gene mutation analysis with PCR-RFLP was performed as a result of clinical and laboratory analysis. *Bsm*I enzyme was used for PCR-RFLP. 118 bp DNA fragment was obtained after PCR-RFLP due to lack of enzyme cleavage in P1 and P6 patients previously known to be mutations were determined to be homozygous mutant genotype. DNA fragments of 118 bp, 94 bp and 24 bp were obtained in P2, P3, P4, P5, P7 and P8 and evaluated as heterozygous. Because the enzyme cuts both alleles in healthy controls, 94 bp and 24 bp bands were obtained and genotypes were evaluated as homozygous normal. All results of PCR-RFLP are shown in Figure 3.

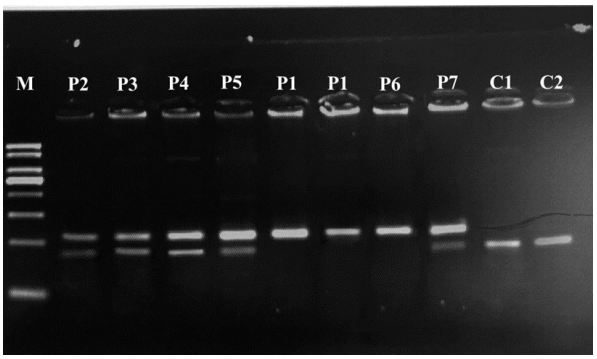

Fig. 3. PCR-RFLP results (M: Marker 50 bp; P1: Patient; P2: P1 mother; P3: P1 father; P4: P1 sister; P5; P1 brother; P6: Patient identified in 2006; P7: newborn baby of P6; C1 and C2: Control)

Discussion

Primary immunodeficiency caused by *CD19* gene mutation was first described in a 10-year-old girl by our team in 2006.[9] *CD19* gene mutation was identified in another patient who was living in the same village and was related to this patient. These two cases suggested that it might be in other cases living in this village and the village was screened for CD19 deficiency. As a result of this screening, 20 carrier individuals were identified.[10] Ten years after the village screening, a patient from the same village and related with index case applied to us. It was thought that *CD19* gene mutation was found in clinical and laboratory examinations. In this study, it was aimed to detect *CD19* gene mutation quickly, safely and inexpensively.

In our region where consanguineous marriages are high, the incidence of autosomal recessive diseases is higher. Therefore, in the light of clinical, laboratory and family history information, it is important to perform rapid mutation analyzes. Known mutations can also be detected by using the PCR-RFLP, which is less expensive, highly sensitive, requires basic laboratory instruments and is easier to implement. The PCR-RFLP is a reliable method and still used for the identification of some bacterial species in the laboratory.[12,13] Other than PCR-RFLP, polymerase chain reaction-single-stranded conformation polymorphism (PCR-SSCP) used for genotype determination after DNA post amplification are two independent methods used for post-amplification genotyping of DNA variations. Both techniques are used in a wide variety of screening applications to characterize single nucleotide polymorphisms (SNPs). Al-

though PCR-SSCP allows identification of a potentially causal unknown SNP that cannot be identified by PCR-RFLP, it has more complex steps than PCR-RFLP. However, it is used in many applications because it does not require complex steps to perform PCR-RFLP. On the other hand, PCR RFLP is easier to process in terms of time and interpretation experience.[15] Other of the reasons we prefer this method is that we have used it safely in the identification of *DCLRE1C* gene mutations. Therefore, the PCR-RFLP method was preferred in the study. That study also identified *DCLRE1C* gene mutation and carrier beers and the results were confirmed by DNA sequence analysis.[11] In our experience, the disadvantage of this method is that it can only be used to detect known mutations.

CD19 is a basic molecule that promotes the proliferation and survival of mature B cells. It forms a whole together with CD81 and CD225. The CD19 protein forms a whole with CD21, CD81 and CD225 on the surface of mature B lymphocytes. This complex works with the B cell receptor and plays an important role in the regulation of events after antigen stimulation.[15] To date, recurrent sinopulmonary infections, pyelonephritis, and gastritis have been reported in patients with CD19 gene mutations identified. [9,11,15,16] In our case, these findings were absent and there was a history of recurrent fever with bronchiolitis that had only been repeated once. Inadequate antibody responses, decreased memory B cell count and hypogammaglobunemia were seen in CD19 deficiency and in our case, it was laboratory compatible with the literature.

Conclusion

As with other diseases, the early detection of genes that cause primary immunodeficiency is important both for the course of the disease and for additional treatment options. In addition, identifying carriers and informing families about this issue is of critical importance. Although the PCR-RFLP method is a known method, it has been applied by us for the first time in the diagnosis of PID. Therefore, we believe that PCR-RFLP method can be used both in detection of known mutations and also in family screening.

Acknowledgments

We are grateful to all individuals participating in this study. We would like to thank to Necmettin Erbakan University Scientific Research Projects Coordinator for supporting this study with 182018008 research project numbers.

Declarations

Funding

The study was supported by Necmettin Erbakan University Scientific Research Projects Coordinator with project number 182018008.

Author contributions

Conceptualization, I.R., S.K. and Ş.N.G.; Methodology, E.K, MA.K. and S.K.; Software, S.K. and H.K.; Validation, M.A.K., H.K. and S.K.; Investigation, M.A.K., H.K., S.N.G., E.K., S.K., S.K. and I.R.; Resources, M.A.K. and H.K.; Data Curation, M.A.K.; Writing – Original Draft Preparation, M.A.K., H.K. and I.R.; Writing – Review & Editing, I.R., S.K., E.K. and Ş.N.G; Visualization, M.A.K., E.K. and S.K., Supervision, I.R. and S.K.; Project Administration, M.A.K., S.K., E.K., Ş.N.G. and I.R.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data used in the study and the data about the method can be accessed by contacting the corresponding author.

Ethics approval

The study was approved by Necmettin Erbakan University; protocol number (2017/818).

References

1. Picard C, Bobby Gaspar H, Al-Herz W, et. al. International Union of Immunological Societies:2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. *J Clin Immunol.* 2018;38(1):96-128.
2. Ballow M. Primary immunodeficiency disorders: antibody deficiency. *J Allergy Clin Immunol.* 2002;109:581-591.
3. Wang K, Wei G, Liu D. CD19: a biomarker for B cell development, lymphoma diagnosis and therapy. *Exp Hematol Oncol.* 2012;29(1):36.
4. Otero DC, Rickert RC. CD19 function in early and late B cell development. II. CD19 facilitates the pro-B/pre-B transition. *J Immunol.* 2003;171(11):5921-5930.
5. CD19 – CD19 molecule. https://ncbi.nlm.nih.gov./gene/?term=CD19. Accessed November 2, 2021.
6. Rich RR. *Clinical Immunology: Principles and Practice*. 5th Edition. Elsevier. Chine. 2019.
7. Rheingold SR, Brown VI, Fang J, Kim JM, Grupp SA. Role of the BCR complex in B cell development, activation, and leukemic transformation. *Immunol Res.* 2003;27(2-3):309-330.
8. Şekerel BE. Çocukluk Çağında Allerji Astım İmmünoloji. *Bilnet Publishing*. Turkey. 2015.
9. Reisli İ. Artaç H, Pekcan S, et. al. CD19 deficiency: A village screening. *Tur Arch Ped.* 2009;44:127-130.
10. Karaselek MA, Kapaklı H, Keleş S, et. al. Intrauterine detection of DCLRE1C (Artemis) mutation by restriction fragment length polymorphism. *Pediatr Allergy Immunol.* 2019;30(6):668-671.
11. van Zelm MC, Reisli I, van der Burg M, et. al. An antibody-deficiency syndrome due to mutations in the CD19 gene. *N Engl J Med.* 2006;354(18):1901-1912.

12. Dib C, Faure S, Fizames C, et. al. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature.* 1996;380(6570):152-154.
13. Ota M, Fukushima H, Kulski JK, Inoko H. Single nucleotidepolymorphism detection by polymerase chain reaction--restriction fragment lengthpolymorphism. *Nat Protocols.* 2007;2(11):2857-2864.
14. Hashim HO, Al-Shuhaib MB. Exploring the potential and limitations of PCR-RFLP and PCR-SSCP for SNP detection: A review. *J Appl Biotechnol Rep.* 2019: 5;6(4):137-144.
15. Rudd CE. Disabled receptor signaling and new primary immunodeficiency disorders. *N Eng J Med.* 2006:354;1874-1877.
16. Kanegane H, Agematsu K, Futatani T, et. al. Novel mutations in a Japanese patient with CD19 deficiency. *Genes Immun.* 2007;8(8):663-670.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.2

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 146–150

ORIGINAL PAPER

Mazlum Kılıç [1], Davut Tekyol [2]

The impact of the COVID-19 pandemic on critically ill patients with acute stroke and diabetic ketoacidosis

[1] Department of Emergency, University of Health Sciences, Fatih Sultan Mehmet Training and Research Hospital, İstanbul, Turkey
[2] Department of Emergency Medicine, University of Health Sciences, Haydarpasa Numune Training and Research Hospital, İstanbul, Turkey

ABSTRACT

Introduction and aim. This study aimed to compare the hospitalization rate, mortality rate and morbidity status of patients hospitalized with stroke and diabetic ketoacidosis (DKA) before and during the COVID-19 pandemic.

Material and methods. The data of 2522 patients who applied to the emergency department (ED) before and during the pandemic were evaluated. A Poisson regression analysis was used to examine the number of presentations between two different periods.

Results. Stroke cases during the pandemic era were compared to those during the pre-pandemic period, and it was shown that the mortality rate for stroke patients during the pandemic period was much higher. Treatment-related ED presentations decreased significantly during the pandemic period, particularly among patients aged 75–84 years. Rates of ED presentation decreased by 84 percent (IRR: 0.14, 95 percent CI: 0.03-0.59) in those with DKA and by 37 percent (IRR: 0.67, 95 percent CI: 0.53-0.75) in those with stroke during the pandemic period.

Conclusion. Conclusion: Stroke and DKA admissions decreased during the pandemic, but the rate of stroke mortality increased statistically 3.375 times. Getting emergency medical care increases their chances of survival. Even in a COVID-19 outbreak, treatment is critical.

Keywords. diabetic ketoacidosis, strokes, pandemics

Introduction

The first case of Coronavirus Disease-2019 (COVID-19) infection was discovered in Wuhan, China, in December of 2019. According to official sources, the World Health Organization (WHO) declared the illness a pandemic on March 11, 2020.[1] Because the early phases of the COVID-19 pandemic occurred during peak flu season, there was an increase in alert levels at ED throughout the world, which acted as the primary referral facilities for infected patients during this period.[2,3] As a consequence of the deployment of stringent measures to manage the pandemic, the number of visitors to the emergency room has dropped considerably in recent years.[4] There are a variety of probable explanations for this condition, including people's dread of infection and attempts to lessen the burden on the health-care delivery system.[5] Treatment that is delayed despite the development of symptoms may deteriorate the condition and increase the possibility of admission to an ED for more severe situations such as stroke or DKA to be treated.[6]

Following the revelation of the first COVID-19 case in Turkey on March 11, 2020, many steps were taken to contain the illness. These included halting foreign flights, closing schools and other meeting areas, and re-

Corresponding author: Davut Tekyol, e-mail: dtekyol34@hotmail.com

Received: 9.04.2022 / Revised: 5.05.2022 / Accepted: 5.05.2022 / Published: 30.06.2022

Kılıç M, Tekyol D. *The impact of the COVID-19 pandemic on critically ill patients with acute stroke and diabetic ketoacidosis.* Eur J Clin Exp Med. 2022;20(2):146–150. doi: 10.15584/ejcem.2022.2.2.

stricting access to the old and fragile.[7] Although these limits reduced ED visits, they delayed treatment of life-threatening conditions, increasing community mortality and morbidity.[8] It was necessary to compare the hospitalization rate, mortality rate, and morbidity status of patients coming to the ED with stroke and DKA during the COVID-19 pandemic to those who presented during the pre-pandemic era in order to establish these conclusions.

Aim

This study aimed to compare the hospitalization rate, mortality rate and morbidity status of patients hospitalized with stroke and diabetic ketoacidosis (DKA) before and during the COVID-19 pandemic.

Material and methods

This research was designed as a retrospective, observational and single-centered study. It was approved by the institutional review board, and a waiver of authorization was given (Ethics Committee decision no. 2021/514/20/28, date: 28.04.2021), allowing the study to proceed. The "pandemic period" is defined as the time period commencing March 11, 2020, and ending December 31, 2020, the date on which the first COVID-19 case was discovered in Turkey, and includes all subsequent cases. The "pre-pandemic period" is defined as the time period between January 1, 2019 and March 11, 2020 during which no COVID-19 illnesses were documented in Turkey, according to the World Health Organization. After their electronic records indicated ICD codes for stroke or DKA, patients diagnosed with stroke or DKA were invited to participate in the research (International Classification of Diseases and Related Health Problems). The study included 223 patients with DKA and 2299 stroke who applied to the ED in both periods. The researchers looked at factors such as age, gender, in-hospital mortality, and duration of hospital stay. They found that patients having diagnoses other than stroke or DKA were excluded from the study, as were those whose data could not be accessed for any reason.

Statistical analysis

The statistical analysis was carried out with the help of the StataCorp LLC (Texas, USA). In addition to the mean and standard deviation, the median and minimum-to-maximum values, and the percentage distribution, descriptive statistics were also provided for each variable. For the purpose of determining whether or not the data followed a normally distributed distribution, the Kolmogorov-Smirnov test was performed. To assess the number of patients with DKA and stroke who came to the ED before and throughout the pandemic, the LOWESS technique was employed before and during the pandemic period. The incidence rate ratio (IRR) was calculated by dividing the total number of ED visits be-

Table 1. Comparison of the distribution of the characteristics of patients who presented to the emergency department due to diabetic ketoacidosis and stroke before and during the pandemic

Variable	Diabetic ketoacidosis			p	Stroke			p
	Pre-pandemic n (%)	Pandemic n (%)	Total n (%)		Pre-pandemic n (%)	Pandemic n (%)	Total n (%)	
Gender								
Male	78 (45.3)	23 (45.1)	101 (45.3)	0.98	902 (57.9)	421 (56.9)	1323 (57.5)	0.66
Female	94 (54.7)	28 (54.9)	122 (54.7)		657 (42.1)	319 (43.1)	976 (42.5)	
Age group								
40-64 years	106 (61.6)	42 (82.4)	148 (66.4)	0.04	547 (35.1)	292 (39.5)	839 (36.5)	0.19
65-74 years	38 (22.1)	5 (9.8)	43 (19.39		421 (27.0)	191 (25.8)	612 (26.6)	
75-84 years	20 (11.6)	2 (3.9)	22 (9.9)		387 (24.8)	162 (21.9)	549 (23.9)	
85 years and above	8 (4.7)	2 (3.9)	10 (4.5)		204 (13.1)	95 (12.8)	299 (13)	
Survivor	170 (98.8)	50 (98)	220 (98.7)	0.66	1534 (98.4)	700 (94.6)	2234 (97.2)	0.001
Non-survivor	2 (1.2)	1 (2)	3 (1.3)		25 (1.6)	40 (5.4)	65 (2.8)	

chi-square analysis

Table 2. Comparison of the length of hospital stay of patients who presented to the emergency department due to diabetic ketoacidosis and stroke before and during the pandemic

Variable	Diabetic ketoacidosis		p	Stroke		P
	Pre-pandemic Mean ± SD	Pandemic Mean ± SD		Pre-pandemic Mean ± SD	Pandemic Mean ± SD	
Length of hospital stay (days)	6.9 ± 5.1	5.3 ± 5.5	0.05	5.4 ± 6.4	6.5 ± 10.6	0.002

SD, standard deviation

fore and during the pandemic by the total number of ED visits during the pandemic period. A Poisson regression analysis was used to examine the number of presentations between two different periods. The threshold of significance was set at 0.05, which was the lowest possible value that could be attained.

Results

The statistics below show the total mortality, gender, and age distribution of DKA and stroke patients admitted to the ED during pre- and post-pandemic periods. The study found no statistically significant change in DKA death rates between pre- and post-pandemic periods. The pandemic death rate for stroke patients rose considerably compared to pre-pandemic. The findings show that between pre- and post-pandemic periods, the mean duration of stay for patients with DKA and stroke dropped dramatically (Table 1).

A statistical analysis found that pre-pandemic DKA patients were hospitalized for substantially longer periods than pandemic patients, with some of the differences being statistically significant. When the time spent in hospitals for stroke presentations was compared between pre- and post-pandemic periods, the post-pandemic period was statistically significantly longer (Table 2).

Figure 1 illustrates smoothed data for ED presentations owing to DKA by age group before and during the pandemic period, as well as the total number of ED presentations.

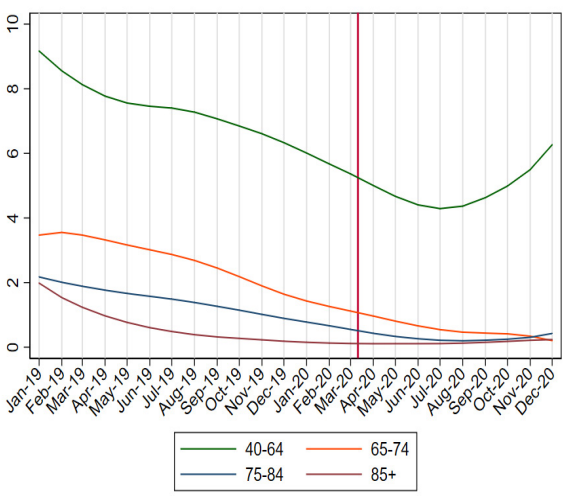

Fig. 1. Distribution of patients presenting to the emergency department with diabetic ketoacidosis according to age groups before and during the pandemic

After a thorough study, it was observed that the incidence of DKA presentations in the ED decreased progressively from pre-to pandemic levels during the course of the inquiry. The IRRs for changes in the number of diabetes-related ED presentations from the pre-to pandemic periods are shown in Figure 2.

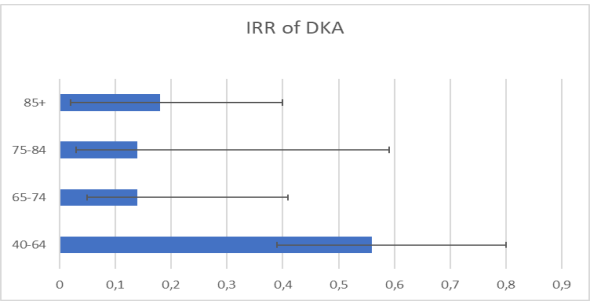

Fig. 2. IRR of DKA presentations before and during the pandemic

DKA presentations were 44% lower in the 40-64 age group [incidence rate ratio: 0.56, 95% confidence interval: 0.39-0.8], 84% lower in the 65-74 age group (incidence rate ratio: 0.14, 95% confidence interval: 0.05-0.41), 84% lower in the 75-84 age group [incidence rate ratio: 0.14, 95% confidence interval: 0.05-0.41], and 84% lower in the (IRR = 0.18; 95% confidence interval:

Figure 3 depicts the number of stroke-related ED presentations by age group before and during the pandemic, with smoothed data for each age group in each time period.

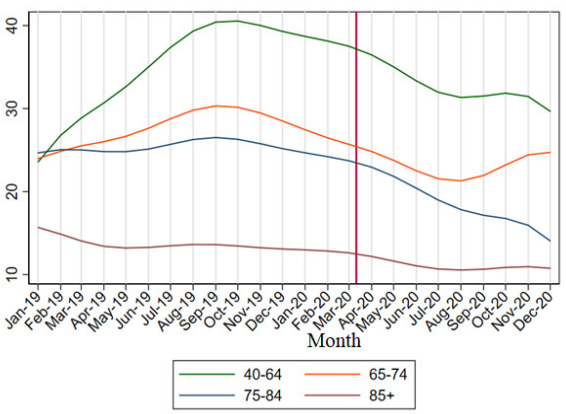

Fig. 3. Distribution of patients presenting to the emergency department with stroke according to age groups before and during the pandemic

After doing research, it was observed that the number of stroke presentations to ED decreased dramatically between the pre-and pandemic periods. During the period from 2019 to 2021, the IRRs for fluctuations in the number of stroke admissions were calculated for the pre- and pandemic periods. The rate of stroke cases presented to the ED decreased by 24 percent in the 40-64 year age group (IRR: 0.76, 95 percent CI: 0.67-0.89), 33 percent in the 65-74 year age group (IRR: 0.67, 95 percent CI: 0.57-0.79), 37 percent in the 75-84 year age group (IRR: 0.67, 95 percent CI: 0.57-0.75), and 31 percent in the 85-plus year age group. The IRR of stroke presentations before and during the pandemic is shown in Figure 4.

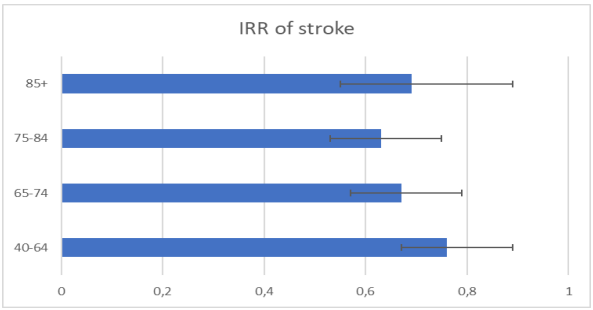

Fig. 4. IRR of stroke presentations before and during the pandemic

Discussion

Compared to the pre-pandemic period, the admissions to the ED of patients diagnosed with DKA and stroke decreased significantly during the pandemic period. This decrease was most observed among the elderly. The study also found that whereas DKA-related in-hospital mortality remained steady throughout the pandemic, stroke-related in-hospital mortality and length of stay rose. In addition to putting an enormous strain on the world's medical system, the fast spread of the COVID-19 pandemic has caused substantial disruption. As a consequence of the fast and extensive spread of viral pandemics, there has been an imbalance in the supply and demand of medical resources in many countries' healthcare systems. People have been afraid as a consequence of the uncertainty surrounding the situation and the possibility of a pandemic, resulting in medical visits being postponed even when serious diseases are present.[4,9,10] An Italian study conducted during the first 40 days of the pandemic revealed a large increase in out-of-hospital cardiac arrests when compared to the previous year's study conducted during the same time period, which indicated a panic situation.[11]

Untreated stroke and DKA may lead to increased mortality and morbidity. During the pandemic season, we saw a drop in both patient groups ED visits. A study that used neuroimaging data from over 850 institutions across the United States discovered that the number of patients tested for stroke-related symptoms decreased by 39% between 2005 and 2010.[12] According to statistics from China's Big Data Observatory Platform for Stroke, the overall number of instances of thrombolysis and thrombectomy was reduced by 26.7 percent and 25.3 percent, respectively, following the pandemic, according to Big Data Observatory Platform for Stroke data.[13] According to Canadian research, the number of code strokes that occur in ED is predicted to fall by 20 percent by the year 2020. In addition to other reasons, the authors mention a significant explanation for the decline in numbers as concerns about exposure to SARS-CoV-2.[14] Another study revealed that the number of patients coming to ED with ischemic stroke was reduced by 64 percent in 2020 when compared to the previous year, according to the researchers.[15] Diabetes mellitus (DM) is a chronic disease that affects around 34 million individuals in the United States. You may experience unusual blood sugar spikes if you have DKA or hyperosmolar hyperglycemia (HOH), two potentially life-threatening but treatable metabolic complications of diabetes.[16] Several weeks after COVID-19 was declared a national emergency in the United States on March 13, 2020, there was a 10% decrease in ED visits for hyperglycemic episodes.[18] When we analyzed the DKA-related death rates between the pre-and pandemic periods, we discovered that there was no statistically significant change. In light of the fact that patients with diabetes can frequently predict a DKA crisis using home glucose monitoring, this conclusion was not surprising.

A number of legal limitations apply to our inquiry. First, since this was a retrospective study at one institution, there is a possibility of selection bias. Second, the experiment only lasted a few months, and the participant fatality rate was modest considering the tiny sample size. As a result, future study should examine the pandemic's impact on stroke and DKA mortality.

Conclusion

While the research found that ED visits for life-threatening diseases including stroke and DKA reduced during the pandemic, stroke-related mortality increased. Getting them immediate medical attention increases their chances of survival. Even in a COVID-19 epidemic, prompt emergency treatment is crucial.

Declarations

Funding

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

Author contributions

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

Conflicts of interest

The authors declare no competing interests.

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. 2021/514/20/28, date: 28.04.2021).

References

1. Quah LJJ, Tan BKK, Fua TP, et al. Reorganising the emergency department to manage the COVID-19 outbreak. *Int J Emerg Med.* 2020;13(1):32.
2. Perlini S, Canevari F, Cortesi S, et al. Emergency Department and Out- of Hospital Emergency System (112-AREU 118) integrated response to Coronavirus Disease 2019 in a Northern Italy centre. *Intern Emerg Med*. 2020;15:825-833.
3. Ak R, Kurt E, Bahadirli S. Comparison of 2 Risk Prediction Models Specific for COVID-19: The Brescia-COVID Respiratory Severity Scale Versus the Quick COVID-19 Severity Index. *Disaster Med Public Health Prep*. 2021;15(4):e46-e50.
4. Hartnett KP, Kite-Powell A, DeVies J, et al. Impact of the COVID-19 Pandemic on Emergency Department Visits - United States, January 1, 2019-May 30, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(23):699-704.
5. Wong LE, Hawkins JE, Langness S, et al. Where are all the patients? Addressing COVID-19 fear to encourage sick patients to seek emergency care. *NEJM Catalyst*. 2020. doi: 10.1056/CAT.20.0193
6. Brick A, Walsh B, Keegan C, Lyons S. COVID-19 and emergency department attendances in Irish public hospitals. ESRI Series QEC Special Article Dublin. 2020. doi: 10.26504/qec2020may_SA_lyons
7. Timeline of the COVID-19 pandemic in Turkey. https://en.wikipedia.org. Accessed 3, August 2020.
8. Çıkrıkçı Işık G, Çevik Y. Impact of COVID-19 pandemic on visits of an urban emergency department. *Am J Emerg Med.* 2021;42:78-82.
9. Garcia S, Albaghdadi MS, Meraj PM, et al. Reduction in ST-Segment Elevation Cardiac Catheterization Laboratory Activations in the United States During COVID-19 Pandemic. *J Am Coll Cardiol.* 2020;75(22):2871-2872.
10. Gogia S, Newton-Dame R, Boudourakis L, et al. COVID-19 X-curves: illness hidden, illness deferred. *NEJM Catalyst*. 2020. doi: 10.1056/CAT.20.0231
11. Baldi E, Sechi GM, Mare C, et al. Out-of-Hospital Cardiac Arrest during the Covid-19 Outbreak in Italy. *N Engl J Med.* 2020;383(5):496-498.
12. Kansagra AP, Goyal MS, Hamilton S, Albers GW. Collateral Effect of Covid-19 on Stroke Evaluation in the United States. *N Engl J Med.* 2020;383(4):400-401.
13. Zhao J, Li H, Kung D, Fisher M, Shen Y, Liu R. Impact of the COVID-19 Epidemic on Stroke Care and Potential Solutions. *Stroke.* 2020;51(7):1996-2001.
14. Bullrich M, Fridman S, Mandzia JL, et al. COVID-19: Stroke Admissions, Emergency Department Visits, and Prevention Clinic Referrals. *Can J Neurol Sci*. 2020;47(5):693-696.
15. Pines JM, Zocchi MS, Black BS, et al. The effect of the COVID-19 pandemic on emergency department visits for serious cardiovascular conditions. *Am J Emerg Med*. 2021;47:42-51.
16. Benoit SR, Hora I, Pasquel FJ, Gregg EW, Albright AL, Imperatore G. Trends in Emergency Department Visits and Inpatient Admissions for Hyperglycemic Crises in Adults With Diabetes in the U.S., 2006-2015. *Diabetes Care.* 2020;43(5):1057-1064.
17. Lange SJ, Ritchey MD, Goodman AB, et al. Potential indirect effects of the COVID-19 pandemic on use of emergency departments for acute life-threatening conditions - United States, January-May 2020. *Am J Transplant.* 2020;20(9):2612-2617.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.3

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 151–158

ORIGINAL PAPER

Fadime Üstüner Top [1], Hasan Hüseyin Çam [2]

Complementary and alternative methods of increasing breast milk of mothers of children aged 0-24 months

[1] Department of Pediatric Nursing, Faculty of Health Sciences, Giresun University, Giresun, Turkey
[2] Department of Public Health Nursing, Yusuf Sereoglu Faculty of Health Sciences, Kilis 7 Aralık University, Kilis, Turkey

ABSTRACT

Introduction and aim. Some applications are made to increase the breast milk. This study has been conducted to determine the complementary and alternative methods to increase the breast milk of mothers with a child in the age of 0 to 24 months.
Material and methods. This study was conducted using the cross-sectional study design. The questionnaire included mothers' socio-demographic characteristics, obstetric histories, breastfeeding, and practices to increase breast milk.
Results. The mean age of the mothers was 29.75±5.97 years. 23.4% of the mothers stated that they did not continue breastfeeding; the mean duration of breastfeeding was 9.24±4.88 months. Mothers expressed to increase milk intake water/liquid food to increase (84.2%), frequently breastfed babies (43.3%), boiling greens to drink (34.6%), spiritual practices (12.8%). Mothers stated that the special drinks used to increase their milk were fennel (56.2%), instant milk enhancer (22.9%), and sage (8.9%). Mothers emphasized that the amount of water (85.1%) and sugar levels (50.0%) they consumed the most increased breast milk.
Conclusion. It was determined that children could not have enough breast milk until the age of two; the mean duration of breastfeeding was low. Mothers believe that their breast milk is not enough for their children, so they apply milk-increasing practices.
Keywords. galactogogues, lactation, mothers

Introduction

Breast milk is essential for laying the foundation of a healthy life, particularly when given exclusively during the first six months after birth and with supplementary food after that during the first two years.[1] Breast milk has been the most natural and healthiest resource of baby feeding since the beginning of time. Unfortunately, like all-natural life-supporting resources, breast milk is also wasted due to wrong information, attitude, and behaviors. The benefit of breast milk is affected by the duration of use and exclusive use. Stimuli or experienced trauma when life is the most critical or sensitive with regards to nutritional programming affect the rest of life in the long term.[2-5]

The 2015 report of the Centers for Disease Control and Prevention (CDC) reveals that 83.2% of babies start to get breastfeeding while 24.9% of them are exclusively breastfed during the first six months, and 17.2% of them are fed with formula in the first two days of their life.[6] In Turkey, 98% of the babies are breastfed for "a while," and 42% of them are fed with food other than breast milk. The median time for exclusive breastfeeding is 1.8 months; supplementary foods are started for babies in the early period.[7] These results indicate that babies are

Corresponding author: Fadime Üstüner Top, e-mail: fadime.ustuner@giresun.edu.tr

Received: 20.12.2021 / Revised: 2.02.2022 / Accepted: 6.02.2022 / Published: 30.06.2022
*The study has been accepted as the 2nd International 7th National Pediatric Nursing Congress 2019-Turkey oral presentation

Top FU, Çam HH. *Complementary and alternative methods of increasing breast milk of mothers of children aged 0-24 months*. Eur J Clin Exp Med. 2022;20(2):151–158. doi: 10.15584/ejcem.2022.2.3.

not at the desired level to create and maintain healthy nutrition.

Mothers' feeding methods are determined by various socio-economic, cultural, and personal factors. There are various factors with negative effects on the start of breastfeeding in the postnatal period and on the continuation of healthy nutrition period, including baby-related factors (crying, colic pain, breast refusal, etc.) and mother related factors (age, education, employment, attitude against breastfeeding, anxiety, breast problems, considering that milk is insufficient, traditional practice, etc.).[8,9] Providing plenty of breast milk and latching the baby on the breast during the first postnatal days are critical tasks. The reasons for cessation of breast milk in an early period include the concern of mothers that breast milk is not sufficient for the baby. Getting colostrum away from the breast through effective breastfeeding after birth and exclusive breastfeeding from the first day on may provide the production of breast milk to meet the baby's needs.[5,10-12] Although every mother's breast milk production at levels sufficient for her baby, researches shows that mothers believe that their milk is not sufficient and resort to complementary and alternative methods (CAM) to increase their milk.[13] The uses of complementary and alternative methods, including herbal medicines, are increasing dramatically in the general population worldwide.[14] Studies highlight that alternative methods considered galactagogues, including herbs, herbal teas/medicines, special food, massage, music, acupuncture, and heat, are popular among breastfeeding women despite the lack of data on their efficacy and safety.[8,15,16] Mothers are asked to carry out various practices to increase breast milk, and it is attempted to continue the nutrition of babies with supplementary food since birth. In this process, mothers' milk is reduced, babies cannot feed with breast milk, and both mothers and babies face various health problems.[17,18]

Health services to individuals and families to provide and maintain breastfeeding are essential to lay the foundations of healthy nutrition. Knowledge of beliefs and practices to increase breast milk will determine the priorities, particularly in this period.[19] The complementary and alternative methods applied to increase breast milk should be selected, and then their adequacy and efficacy should be investigated by evidence-based studies.[18,20] Ineffective, insufficient, harmful, or unnecessary practices that are believed to increase breast milk should be avoided.

Aim

This study has been conducted in order to determine the complementary and alternative methods that are believed to increase breast milk of mothers with a child in the age of 0 to 24 months.

Material and methods

Study design and participants

The present hospital-based cross-sectional study was conducted in pediatric wards of a tertiary care teaching institution from March 15, to June 15, 2019 in Turkey. The sample size was computed using the following formula: [$n=z^2pq/d^2$]. (n=sample size, $z=(1-\alpha)$, is the z-score corresponding to a 95% confidence interval and was computed as 1.96, p=0.50, q=(1−p)=0.50, d=desired margin of error or 0.05.) The estimated sample size was 384. However, to cover for possible dropouts due to missing information on crucial questions, a total of 436 participants were recruited for the study. Purposive sampling method was used in the study. The sample group consists of the mothers with a child in the age of 0 to 24 months in the institution where the study was conducted and 436 mothers were included in the study during the data collection dates (a period of 3 months). The study was conducted with the mothers with a child in the age of 0 to 24 months since the importance of breast milk for the first two years in baby nutrition is emphasized.

Data collection tools

In this study, a questionnaire form prepared in line with the literature was used as a data collection tool. Data collection tool consists of 47 questions including socio-demographic qualities of mothers (13 questions), obstetric histories (9 question), breastfeeding conditions (15 questions) and practices of mothers to increase breast milk (10 questions).[1,4,8,13,16] The researcher obtained the consent of mothers to collect data and their convenient times were determined. Data was collected by the researcher through face-to-face interview method within 30 to 40 minutes in average in an appropriate meeting room.

Ethics 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 the Ethics Committee of University of Giresun (Date: 19.12.2018/No: 08-16).

Statistical analysis

Statistical analyses were performed using the statistical software package SPSS version 20 for Windows (IBM, New York, USA). Means, standard deviations, percentages, and frequencies were used for descriptive analysis.

Results

The average age of mothers in the study was 29.75±5.97 years (18-45 years), and more than half of them (58.9%) are in the group of 30 years and younger. More than half

Table 1. Knowledge, attitudes and practices of mothers on breastfeeding (n=436)

	Mean ± standard deviation	n	%
Initiation of breastfeeding			
< 30 minute		299	68.6
30-60 minute		115	26.4
> 60 minute		22	5
Duration of feeding only breast milk	**6.60±2.84 months (1-24 months)**		
≤ 4 months		9	2.1
5 months		13	3
6 months		370	84.9
≥ 7 months		44	10.1
Food given first after birth			
Breast milk		398	91.3
Zamzam water		15	3.4
Water		10	2.3
Infant formula		9	2.1
Sugar water		4	0.9
Time they plan to breastfeed with complementary feeding	**20.76±6.10 months (6-36 months)**		
12 months and under		115	26.4
13-18 months		20	4.6
19-24 months		287	65.8
25 months and over		14	3.2
How many hours do they breastfeed their baby?			
Whenever it cried		224	51.4
Every 2-3 hours		210	48.2
When it comes to my mind		2	0.5
What the previous children fed in the first 6 months?			
Breast milk only		156	53.2
Breast milk and complementary feding		84	28.7
Breast milk and water		33	11.3
Breast milk and infant formula		19	6.5
Infant formula		1	0.3
Are you still breastfeeding your baby?			
Yes		334	76.6
No		102	23.4
If no, how many months did you breastfeed?	**9.24±4.88 months (2-24 months)**		
Reason for ending breastfeeding			
My milk is not enough/enough		69	15.8
Baby did not take the breast		29	6.7
I am separated from my baby		27	6.2
I did not want to breastfeed		20	4.6
It was the appropriate time to finish		10	2.3
My breasts were not suitable		9	2.1
I got pregnant		4	0.9
I was taking medication		2	0.5
Time to start complementary feeding	**5.56±1.00 months (3-12 months)**		
4 months and less		41	13.3
5 months		80	26.0
6 months		168	54.5
7 months and over		19	6.2
The reason for starting complementary feeding			
Additional food time has come		144	46.8
The baby was not getting enough		100	32.5
My milk didn't come		18	5.8
Midwife/nurse suggested		17	5.5
Doctor suggested		13	4.2
My baby couldn't suck		9	2.9
I was sick, I was taking medication		5	1.6
My milk didn't work		2	0.6
Do you think your milk is enough for your baby?			
Yes		174	39.9
No		262	60.1
How do you know your milk is not enough?			
Restless and not sleeping after sucking and changing diaper		169	38.8
By month low weight		62	14.2
It sleeps constantly, is inactive and cries very quietly		31	7.1
Why do you think your milk is not enough?			
I'm under extreme stress		100	38.2
I'm not fed well enough		82	31.3
I started to work		42	16
I drink little water		15	5.7
I've had breast problems		13	5
I have mental problems		4	1.5
Other		6	2.3

Table 2. Complementary and alternative methods of increasing breast milk supply for lactating mothers (n=436)

	n	%
Is there anything you do specifically to increase your milk?		
Yes	411	94.3
No	25	5.7
Which of the following did you do to increase your milk?		
I get plenty of water and juicy food	367	84.2
I breastfeed my baby often (8-10 times a day, every 2-3 hours)	189	43.3
I drink water by boiling greens	151	34.6
I wore the amulet	56	12.8
I breastfeed my baby for at least 4-5 minutes in a breast	51	11.7
Mixing spices with honey and eating on an empty stomach in the morning	36	8.3
I boil the barley and drink its water	29	6.7
Other	3	0.7
Who/who recommended practices to increase breast milk?		
Midwife/Nurse	253	58
Family member	180	41.3
Social media	125	28.7
Physician	70	16.1
Neighbors	46	10.6
Have you received training on breastfeeding and breast-enhancing measures / practices?		
Yes	314	72
No	122	28
Who did you get the training from?		
Midwife/Nurse	288	91.7
Physician	26	8.3
Are there particular foods you eat because you are breastfeeding?		
Milk and milk products	233	53.4
Milky desserts	120	27.5
Dumpling desserts	25	5.7
Juicy soups	250	57.3
Meat and meat products	59	13.5
Molasses/honey/jam/tahini	146	33.5
Lohusa sherbet	212	48.6
Rice pilaf/Bulgur pilaf	132	30.3
Herb teas	194	44.5
Dried legumes	68	15.6
Fruit	121	27.8
Vegetables, greens (parsley, dill)	229	52.5
Nuts, peanuts, walnuts	84	19.3
Onion, garlic	49	11.2
Raisins/figs	91	20.9
What are the special drinks you consume to increase your milk?		
Fennel	145	56.2
Milk enhancer teas/drinks	59	22.9
Sage	23	8.9
Nettle	21	8.1
Anise	7	2.7
Rosehip	3	1.2
What is your most used practice to increase your milk?		
Increasing water intake	371	85.1
Increasing green vegetable consumption	146	33.5
Increasing tea consumption	92	21.1
Increasing the frequency of breastfeeding	63	14.4
What foods and applications increased your milk the most?		
Sugary foods	218	50
Soup	206	47.2
Fruit	62	14.2
Milk	47	10.8
What drinks increased your milk the most?		
Liquid foods	224	54.4
Sugary drinks	97	23.5
Teas/Milk enhancer tea	91	22.1

of the mothers (52.5%) lives in urban areas, almost half of them (48.9%) are high school graduates, the majority of them (71.3%) are housewives or unemployed, majority of them (83.9) has a medium level of perceived socio-economic status and the majority of them (78%) have a nuclear family structure. In the study, it was determined that 17.4% of the mothers had an unintended pregnancy. One-fourth of mothers (75.7%) gave birth at state hospitals, and the birth ratio with cesarean section was 57.1%. The total average number of pregnancies is

2.14±1.1 (1-8), while the number of surviving babies is 1.87±0.85 (1-6). More than half of the babies were girls, 38.8% are between 0 to 6 months, 35.8% were between 7 to 12 months, and 25.5% were between 13 to 24 months.

In the study, most participants (68.6%) stated that they breastfed their babies in the first 30 minutes after giving birth, 26.4% in the first 30 to 60 minutes, 5.0% 1 hour later after giving birth. The majority of the mothers (91.3%) stated that they breastfed first as the first nutrition of the baby, 3.4% gave Zamzam water, 2.3% gave water, 2.1% showed a formula, and 0.9% gave sugared water (Table 1).

One-fourth of mothers (23.4%) said they were not currently breastfeeding, while the average breastfeeding duration was 9.24±4.88 months (2 to 24 months). The participants stated that the reason for ending breastfeeding was ablactation and insufficient breast milk (15.8%), breast refusal (6.7%), separation from baby (6.2%), unwillingness to breastfeed (4.6%) and other reasons. The time to start supplementary food is 5.56±1 months on average (3 months to 12 months). More than half of the participants (60.1%) stated that their milk was not sufficient due to factors including extreme stress (38.2%), insufficient nutrition (31.3%) and, resuming work (16%) (Table 1).

The participants stated that they mostly had plenty of water and liquid food (84.2%), frequently breastfed their babies (8 to 10 times a day, every 2 to 3 hours) (43.3%), drank boiled green juice (34.6%), and wore an amulet (12.8%). Almost three-fourths of the participants (72%) received breastfeeding and galactagogues measures/practices training. The majority of this training (91.7%) was carried out by midwives/nurses. The special drinks to increase breast milk included fennel (56.2%), instant galactagogues teas/drinks (22.9%), sage tea (8.9%), nettle (8.1%), aniseed (2.7%), and rosehip (1.2%). They stated that the most frequent methods to increase breast milk included the increase of water intake (85.1%), more consumption of green vegetables (33.5%), increased consumption of tea (21.1%), and increased frequency of breastfeeding (14.4%). They stated that their breast milk was increased most with foods such as sugary food (50%), soup (47.2%), fruit (14.2%), and milk (10.8%) (Table 2).

Discussion

Nutrition with breastfeeding is the first and the most step of healthy food. Breastfeeding is widespread and traditional in Turkey. However, exclusive breastfeeding for the first six months and breastfeeding is problematic. Breastfeeding mothers often believe that their milk is sufficient.[21] Breastfeeding babies frequently and through correct techniques, emptying the breasts, adequate sleep, and resting, and increasing mothers' self-confidence is reported to be effective practices in increasing breast milk. However, there are various cultural applications in practice.[22-24] This study helps determine complementary and alternative galactagogue methods.

The early start of breastfeeding is helpful for both mother and baby. Skipping this early postnatal period of the first half an hour harms the success and duration of breastfeeding.[25,26] The study revealed that mothers didn't breastfeed their babies in the first half an hour. Senarath et al. found that only 46.1% of mothers started breastfeeding in the first one hour.[27] Bergamaschi et al. reported that the breastfeeding ratio of mothers in the first one hour was 50% and that 61% of babies were not exclusively breastfed in the first three days.[26] Turkey Demographic and Health Survey (TDHS) 2018 data indicated that 71% of babies were breastfed in 1 hour.[7] In the present study, 53.2% of mothers stated that they exclusively breastfed their older child for the first six months. Exclusive breastfeeding for the first six months was reported in similar studies to be 30.7% by Senarath et al., 37.3% by Ukegbu et al., % ten by Mateus Solarte and Cabrera Arana and, 28.5 % by Osibogun et al.[27-30] The ratio of exclusively breastfed babies in the first six months in Turkey is 40.7%.[7] UNICEF emphasizes that early breastfeeding of babies after birth is helpful for mothers and babies and that babies need to be breastfed in the first 30 minutes after birth.[1] Pieces of training should continue on the necessity of early start of mother-baby contact, breastfeeding babies in the first half an hour, and having breast milk as babies' first food.

World Health Organization (WHO) recommends that babies continue to be breastfed until two years of age.[1] The present study found the average breastfeeding duration of babies to be low (9.24±4.88). Similarly, there are increases in the ratio of starting breastfeeding in several countries; however, data show that few women continue breastfeeding for the recommended duration, especially in developed countries.[31] In the present study, the reasons to end breastfeeding included primarily ablactation/insufficient breast milk followed by breast-related problems. The studies in this field indicate that most mothers believe that their milk is inadequate for their babies. Hence, they start supplementary food early (before six months) and primarily use formula and other liquid food as supplemental food.[5,10,21,32] While more than 50% of breastfeeding mothers perceive that their milk is insufficient, only five percent of them have physiological milk deficiency. The reason for mothers' perception of insufficient milk is their misinterpretation of infant behavior and their lack of confidence in their ability to breastfeed.[33] Mothers stated that they understood that their milk was not enough because 74.6% of their babies were restless, 23.7% of their babies did not gain enough weight and 1.7% of them were not active. Reliable signs of insufficient breast milk are insufficient weight gain and insufficient urination.[19,27]

The most critical problem at this stage is that mothers start different searches just on the assumption that their breast milk is insufficient without really finding out that their breast milk is inadequate. The study revealed that mothers intended to breastfeed their babies for the first six months exclusively; however, they started supplementary food early. This indicates that mothers have a positive attitude, but they fail in practice. Therefore, this demonstrates the need to support mothers concerning breastfeeding, breast milk, and its sufficiency.

Several studies reported that mothers applied traditional galactogogues methods rather than modern ones and that those methods need evidence.[13,24,34] The present study revealed that almost all mothers used complementary and alternative approaches to increase their milk. Sibeko et al. found that mothers used herbal tea by 56%, commercial galactogogues product by 13%, and ginger/beer by 3% to increase their milk.[32] In a qualitative study by Sim et al. with breastfeeding women on the use of herbal galactagogues, it was found that all women used fenugreek, three of them used a mixture of fenugreek and blessed thistle, and seven of them used "lactation tincture" including herbal components in their breastfeeding period.[20] Mothers stated that they were not against the idea of using herbal items and found them to be safer than chemicals and pharmacologic medicines.[34] In another study, it was reported that 69% of the lactation counselors heard about galactogogues herbal drugs, that 65% of the recommended one or more of these methods despite the lack of evidence on the methods and that they recommended most commonly fenugreek and blessed thistle as a galactogogues product.[4] Various herbs and foods are known to be used as galactagogue, including almond, aniseed, asparagus, cumin, chicken soup, coriander, coconut, dandelion, dill, fennel, fenugreek, garlic, hop, lettuce, radix althaea, millet, mushroom, stinging nettle, oat straw, daisy, rice, sage, sunflower seed, and thistle.[35-37] The studies conducted in Turkey revealed that the galactogogues practices are standard, including water and liquid foods, milk, and dairy products, herbs (fennel tea, stinging nettle, parsley, dill, onion, bulgur, cowpea, etc.), sugary foods (lohusa sherbet, tahini halva, milk puddings, boiled grape juice).[38,39] In the study by Dinc et al. determined the traditional galactogogues practices to be frequent breastfeeding by 29.7%, wearing blue bead and saying a prayer over it by 23.1%, consuming a lot of water by 23.1%, breastfeeding for a long time by 7.5%, having a rest by 7.1%, rubbing the breast by 6.6% and pouring lead by 2.8%.[39] It is noticed that the galactogogues practices are traditional rather than scientific. Therefore, it is evident that mothers need education and support for breastfeeding and lactation. Evidence-based studies should also be conducted for the effectiveness of the CAM used to increase milk.

Many mothers think that their breast milk is insufficient and employ various methods to increase breast milk within the traditional experience and beliefs. There is no standard information on the qualities of these methods, including use, frequency, dose, and composition.[16] There is limited literature on the complementary galactogogues methods. The complementary galactogogues methods vary among societies.

Limitations of the study

There are some limitations to our study. First, the study's design was cross-sectional, and the data did not account for assumptions concerning causation. Because cross-sectional studies involve some methodological limitations and conclusions should only be extrapolated to populations with similar characteristics. In a larger sample, multicenter and multidisciplinary studies can be planned. Secondly, our data came from self-reports. This may not avoid the subjective bias caused by individual recall.

Conclusion

The study revealed that children did not get sufficient breast milk until two years of age, average breastfeeding duration is low, and mothers start supplementary food early. Mothers believe that their breast milk is not sufficient and employ galactogogues practices. Mothers concentrate on consuming exceptional food and drinks during the lactation period, including mainly fennel tea, instant galactogogues teas, sugary foods, and water intake. It will be helpful to study the evidence-based effectiveness of the complementary galactogogues methods.

Since traditional methods are more common than modern methods to increase breast milk, training should be organized with consideration to cultural factors concerning breastfeeding, exclusively breastfeeding, and increasing breast milk. Lactation counseling should be mainstreamed; problems and solutions for the nutrition of babies should be defined and implemented to realize the breastfeeding recommendations of the World Health Organization and UNICEF. It should be remembered that lactation counseling is the most effective method to increase breast milk, and lactation problems of mothers should be solved timely with the homecare practices in the postnatal period recommending the continuation of breast milk which is necessary for babies.

What is the current knowledge?

The most crucial reason breastfeeding cannot be continued is perception of insufficient breast milk. Foods and drinks that increase breast milk (galactagogue) are used from past to present. Many foods and herbs can be counted as galactogogues. Many mothers around the world use herbs and foods to increase their milk. Although the mechanism of action of medicinal herbs and

foods used to increase breast milk is unknown, it is supported by traditional experiences and beliefs that they are effective. Many of the mechanisms are unknown.

What is new here?

Mothers believe that their breast milk is not sufficient and employ galactogogues practices. Mothers concentrate on consuming exceptional food and/or drinks during the lactation period, including mainly fennel tea, instant galactogogues teas, sugary foods, and water intake. It will be helpful to study the evidence-based effectiveness of the complementary galactogogues methods. Ineffective, insufficient, harmful, or unnecessary practices that are believed to increase breast milk should be avoided.

Acknowledgements

The authors thank all parents who participate in this study.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, F.Ü.T. and H.H.Ç.; Methodology, F.Ü.T.; Software, F.Ü.T.; Validation, F.Ü.T., H.H.Ç.; Formal Analysis, F.Ü.T., H.H.Ç.; Investigation, F.Ü.T.; Resources, F.Ü.T.; Data Curation, F.Ü.T.; Writing – Original Draft Preparation, F.Ü.T.; Writing – Review & Editing, F.Ü.T. and H.H.Ç.; Visualization, F.Ü.T. and H.H.Ç.; Supervision, F.Ü.T.; Project Administration, F.Ü.T.; Funding Acquisition, F.Ü.T. and H.H.Ç.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Data availability

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

Ethics approval

Ethical consent was obtained from Giresun University Ethics Committee for the study, dated 19/12/2018 and numbered 08/1.

References

1. World Health Organization. Infant and young child feeding: model chapter for text books for medical students and allied health professionals. 2009, Geneva: WHO.
2. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev.* 2002;(1):CD003517.
3. Cattaneo A,Yngve A, Koletzko B, Guzman LR. Protection, promotion and support of breast-feeding in europe: Currentsituation. *Public Health Nutr.* 2005;8(1):39-46.
4. Schaffir J, Czapla C. Survey of lactation instructors on folk traditions in breastfeeding. *Breastfeeding Med.* 2012;7(4):230-233.
5. Morton J, Lawrence RA. Nutrition - breast milk. In Rudolph CD, Rudolph AM, Lister GE, First LR, Gershon AA, Leventhal JM (Eds.), Rudolph's Pediatrics New York, McGraw-Hill; 2013.
6. Centers for Disease Control and Prevention. Breastfeedig Report Card United States. Available from: https://www.cdc.gov/breastfeeding/pdf/2018breastfeedingreportcard.pdf. Accessed 2020 Jan 13.
7. Turkey Demographic and Health Survey (TDHS) 2018. Hacettepe University Institute of Population Studies. Available from: http://www.hips.hacettepe.edu.tr/tnsa2018/rapor/tnsa2018. Accessed 2019 Nov 30.
8. Forinash AB, Yancey AM, Barnes KN, Myles TD. The use of galactogogues in the breastfeeding mother. *Ann Pharmacother.* 2012;46(10):1392-1404.
9. Thulier D, Mercer J. Variables associated with breastfeeding duration. *J Obstet Gynecol Neonatal Nurs.* 2009;38(3):259-268.
10. Lumbiganon P, Martis R, Laopaiboon M, et al. Antenatal breast feding education for increasing breastfeeding duration. *Cochrane Database Syst Rev*. 2012;9:Cd006425.
11. Renfrew MJ, McCormick FM, Wade A, et al. Support for health breastfeeding mothers with healthy term babies. *Cochrane Database Syst Rev.* 2012;5:Cd001141.
12. Sakha K, Behbahan AG. Training for perfect breastfeeding or metoclopramide: which one can promote lactation in nursing mothers? *Breastfeed Med.* 2008;3:120-123.
13. Zapantis A, Steinberg JG, Schilit L. Use of herbals as galactagogues. *J Pharm Pract.* 2012; 25:222-231.
14. Frass M, Strassl RP, Friehs H, et al. Use and acceptance of complementary and alternative medicine among the general population and medical personnel: a systematic review. *Ochsner J.* 2012;12:45-56.
15. Becker GE, Smith HA, Cooney F. Methods of milk expression for lactating women. *Cochrane Database Syst Rev.* 2016;9(CD006170).
16. Ayers JF. The use alternative therapies in the support of breastfeeding. *J Hum Lact.* 2000;16:52-56.
17. McCann MF, Bender DE. Perceived insufficient milk as a barrier to optimal infant feeding: examples from Bolivia. *J Biosoc Sci.* 2006;38:341-364.
18. Yamada R, Rasmussen KM, Felice JP. What is 'enough,' and how do ı make it?: A qualitative examination of questions mothers ask on social media about pumping and providing an adequate amount of milk for their infants. *Breastfeed Med.* 2019;14(1):17-21.
19. Hennessy VR. Nurse's role in breastfeeding promotion. The Faculty of the Department of Nursing of Gonzage Universty. The Degree of Master of Science in Nursing, Washington;2003.
20. Vargová Z, Kučerová JR. Herbs for increasing breast-milk production. *Ceska Slov Farm Spring.* 2018;66(5):208-219.

21. Neifert M, Bunik M. Overcoming clinical barriers to exclusive breastfeeding. *Pediatric Clinics of North America.* 2013;60(1):115-145.
22. Quigley MA. Increasing exclusive breastfeeding. *BMJ*. 2007;22:574-575.
23. Hussainy S, Dermele N. Knowledge, attitudes and practices of health professionals and women towards medication use in breastfeeding: A review. *Int Breastfeed J*. 2011;6:11.
24. Bazzano A, Hofer R, Thibeau S, et al. A review of herbal and pharmaceutical galactagogues for breast-feeding. *Ochsner J*. 2016;16:511-524.
25. Pollard M. Evidence-based care for breastfeeding mothers: a resource for midwives and allied healthcare professionals: Routledge;2012.
26. Bergamaschi N, Oakley L, Benova L. Is childbirth location associated with higher rates of favourable early breastfeeding practices in Sub-Saharan Africa? *J Glob Health.* 2019;9(1):010417.
27. Senarath U, Dibley MJ, Agho KE. Breastfeeding practices and associated factors among children under 24 months of age in Timor-Leste. *Eur J Clin Nutr*. 2007;61:387-397.
28. Ukegbu AU, Ebenebe EU, Ukegbu PO, Onyeonoro UU. Determinants of breastfeeding pattern among nursing mothers in Anambra State, Nigeria. *East Afr J Public Health*. 2011;8(3):226-231.
29. Mateus Solarte JC, Cabrera Arana GA. Factors associated with exclusive breastfeeding practice in a cohort of women from Cali, Colombia. *Colomb Med (Cali)*. 2019;50(1):22-29.
30. Osibogun OO, Olufunlayo TF, Oyibo SO. Knowledge, attitude and support for exclusive breastfeeding among bankers in Mainland Local Government in Lagos State, Nigeria. *Int Breastfeed J*. 2018;13:38.
31. Quigley MA, Carson C, Sacker A, Kelly Y. Exclusive breastfeeding duration and infant infection. *Eur J Clin Nutr*. 2016;30:1420-1427.
32. Sibeko L, Dhansay MA, Charlton KE, et al. Beliefs, attitudes, and practices of breastfeeding mothers from a periurban community in South Africa. *J Hum Lact*. 2005;21(1):31-40.
33. Wood NK, Sanders EA, Lewis FM, Woods NF, Blackburn ST. Pilot test of a home-based program to prevent perceived insufficient milk. *Women and Birth.* 2017;30(6):472-480.
34. Sim TF, Hattingh HL, Sherriff J, Tee LBG. Perspectives and attitudes of breastfeeding women using herbal galactagogues during breastfeeding: a qualitative study. *BMC Complement Altern Med.* 2014;14:216.
35. Nice FJ. Common herbs and foods used as galactogogues. Infant Child Adolesc Nutr. 2011;33:129-132.
36. Marasco L. Inside track: increasing your milk supply with galactogogues. *J Hum Lact.* 2008;24:455-456.
37. Humphrey S. Herbal therapies during lactation. In: Hale T, Hartmann P, eds. Textbook of Human Lactation. Amarillo, TX: Hale; 2007.
38. Tanrıverdi S, Koroglu AO, Kultursay N, Egemen A. Mothers' opinions and attitudes about the factors increasing breast milk. *J Ped Res.* 2014;1(2):84-86.
39. Dinc A, Dombaz I, Dinc D. Traditional practices related to breast milk and breastfeeding of mothers with babies of 6-18 months. *Balıkesir Health Sciences Journal*. 2015;4(3):125-130.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.4

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 159–166

ORIGINAL PAPER

Juliana Roncini Gomes da Costa, Alana Ludemila de Freitas Tavares, Gladson Ricardo Bertolini, Maria Luiza Serradourada Wutzke, Carolina De Toni Boaro, Diego Francis Saraiva Rodriguez, Rose Meire Costa, Lucinéia de Fátima Chasko Ribeiro

Histological aspects of whole-body vibration in the knee remobilization of Wistar rats

Universidade Estadual do Oeste do Paraná, Cascavel, Paraná, Brazil

ABSTRACT

Introduction and aim. The knee is one of the joints where immobilization is most used, however, it can cause morphological changes in the joint tissues and is a challenge to be overcome during rehabilitation. Whole-body vibration (WBV) is capable of generating repetitive oscillatory movements, which cause mechanical stimuli that interfere with tissue plasticity. The aim of this study was to analyze the knee morphology of Wistar rats submitted to remobilization with WBV.

Material and methods. 32 male rats were used, divided into four groups (n=8): Control Group (G1), Immobilization Group (G2), Immobilized Group and Free Remobilization (G3), Remobilized Group with WBV (G4). For immobilization, a plastered apparatus was used for 15 days. G3 and G4 carried out free remobilization or with WBV, respectively, for 2 weeks. The knee joints were processed for light microscopy.

Results. The WBV led to a reduction in the inflammatory infiltrate in the articular cavity and greater presence of adipocytes in the subintima of the synovial membrane.

Conclusion. Remobilization with WBV induced a better tissue response in the synovial membrane when compared to free remobilization.

Keywords. articular cartilage, knee, synovial membrane, vibration

Introduction

The practice of any sport is often associated with the development of musculoskeletal injuries, with great impact on the rehabilitation process for the return to the sport, recovery can take from weeks to months.[1] In many cases, as part of the initial treatment of these injuries, the first method used for protection of injured tissues and pain relief is complete immobilization of the joint, being a common practice for the treatment of orthopedic disorders such as sprains, ligament ruptures, muscle injuries, joints and fractures.[2–4]

Immobilization and associated movement restriction may affect bone, muscle and joint composition. Specifically for the joints, immobilization acts on the cartilage inducing its degeneration, including reduction of hydration, alteration of structure and reduction of proteoglycan and chondrocyte synthesis, and decrease of cartilage thickness, which reflects in the decrease of load absorption capacity, and may cause or worsen chondral lesions, leading in the long run to the development of osteoarthritis, some of these alterations being partially reverted to normal values after remobiliza-

Corresponding author: Gladson Ricardo Flor Bertolini, e-mail: gladsonricardo@gmail.com

Received: 4.03.2022 / Revised: 17.03.2022 / Accepted: 20.03.2022 / Published: 30.06.2022

da Costa JRG, de Freitas Tavares AL, Bertolini GR, et al. *Histological aspects of whole-body vibration in the knee remobilization of Wistar rats*. Eur J Clin Exp Med. 2022;20(2):159–166. doi: 10.15584/ejcem.2022.2.4.

tion.[5–9] The joint capsule is also affected by immobilization, which promotes morphological changes in the intima and subintima synovial, resulting in decreased synovial fluid production and reduced nutritional supply to the cartilage.[10]

Thus, early rehabilitation is extremely important to recover joint functionality.[2,3] There are numerous forms of remobilization, such as treadmill running, resistance and aquatic exercises.[10–12] However, the search for new therapies that accelerate the regenerative process in remobilization is of great value. In this sense, studies have shown that whole-body vibration (WBV) can act as an anabolic stimulus for various tissues, including the musculoskeletal system.[13–16] Despite their indications, there are still few studies evaluating WBV on joint tissue, as well as in the remobilization process.[17,18]

Aim

Thus, this study analyzed the morphological responses of the knee of immobilized and remobilized Wistar rats on a vibration platform.

Material and methods

Ethical approval

The study was approved by the Ethics Committee on the Use of Animals of the Universidade Estadual do Oeste do Paraná (UNIOESTE), 2729/2014-GRE.

Sample and experimental groups

The experiment was performed using 32 Wistar rats, with a mean age of 10 weeks and weight of 177.20±16.32 g, kept in an environment with a temperature of 23±1ºC, with a photoperiod of 12 hours, receiving water and feed ad libitum. The animals were randomly separated into four groups (n=8):

- Control Group (G1): without any kind of intervention;
- Immobilization Group (G2): submitted to the immobilization protocol;
- Group Immobilization/Free Remobilization (G3): submitted to the immobilization and free remobilization protocol;
- Group Immobilization/Remobilization WBV (G4): submitted to the immobilization and remobilization protocol with the use of the vibration platform.

Immobilization protocol

To perform the immobilization, the animals were anesthetized (xylazine hydrochloride 15 mg/kg and ketamine hydrochloride 80 mg/kg, intraperitoneally) and immobilized with a plaster bandage. The immobilized groups (G2, G3 and G4) had the orthosis molded from the abdominal region, just below the last ribs, following to the right pelvic limb of each animal, being placed in the whole extension of the limb so that it remained in complete extension of the knee joint as well as, complete plantar ankle flexion. The animals were kept in this position for a period of 15 consecutive days.

Remobilization protocol

For the remobilization was used a commercial platform of the brand Arktus professional, with frequency of 60 Hz and vibrations with amplitude of 2 mm, for 10 minutes, five days a week, with rest of two days at the end of the week, for 2 weeks, totaling 10 days of training with WBV. A support was used with the purpose of containing the animal during the vibration and enable the animals to exercise simultaneously, which was made of white MDF wood, and allowed to position 8 animals concomitantly, in stalls with 13 cm wide, 19 cm long and 25 cm high. To minimize a possible bias on the positioning of the animals on different areas of the vibratory platform that received less vibration amplitude, a rotation was performed between the stalls in which each day of treatment the animal was housed in a different place from the previous day.[19]

For G3, after the 15 days of immobilization and the removal of the cast bandage, the animals remained in the cage for 15 days receiving water and feed ad libitum.

Morphological analysis

The animals of the control group (G1) and the immobilization group (G2) were eutanasiated soon after the 15 days of intervention, while the animals of the free remobilization group (G3) and the platform remobilization group (G4) were eutanasiated after 2 weeks of remobilization. All the animals were weighed, duly anesthetized by intramuscular injection of 80 mg/kg ketamine and 8 mg/kg xylamine and decapitated in a guillotine. Then the right pelvic limb was dissected and the knee joints followed for histological processing, being fixed in a 10% formaldehyde solution for 24 hours, washed in running water for 1 hour and remaining immersed in 5% trichloroacetic acid for approximately 20 days, following the routine steps for inclusion in paraffin, where they were dehydrated in increasing series of alcohol and diaphanized in xylol. The histological paraffin embedding was performed, longitudinally sectioned in 7 μm thickness in a microtome (Olympus CUT 4055) and stained with hematoxylin and eosin.

The slides were photomicrographed under a light microscope (Olympus), with 200x magnification for morphology analysis. The morphological characteristics of the articular cartilage were observed, such as the distribution of chondrocytes, appearance of the articular surface and the presence of cracks in the extracellular matrix, floculation and cellular clones, as well as the subchondral bone and the synovial membrane, and the tissue aspects of the intima and subintima were observed.

Results

Morphological analysis of the synovial membrane of the control group (G1) revealed normal characteristics, that is, from two to three layers of synoviocytes (types A and B) in the intima synovialis, and subintima with a predominance of adipose cells and blood vessels within the normal range (Figure 1A). In the immobilized group (G2), the synovial membrane presented altered, with thickened and disorganized intima as to the distribution of the synoviocytes and subintima with substitution of adipocytes by connective tissue rich in collagen fibers, inflammatory infiltration and intense angiogenesis, with an increase in the number and caliber of blood vessels (Figure 1B). In the animals of the free remobilization group (G3) (Fig. 1C) and trained with WBV (G4) (Figure 1D) the morphological aspects were similar to the control (G1), with the intima organized in its characteristic layers, while the subintima presented tissue reorganization, with a small amount of connective tissue and inflammatory cells and vascularization within the normal range, when compared to G2, but with inflammatory infiltrate in the joint cavity in G3 (Fig. 1C) and larger amount of adipocytes in the subintima in G4 (Fig. 1D).

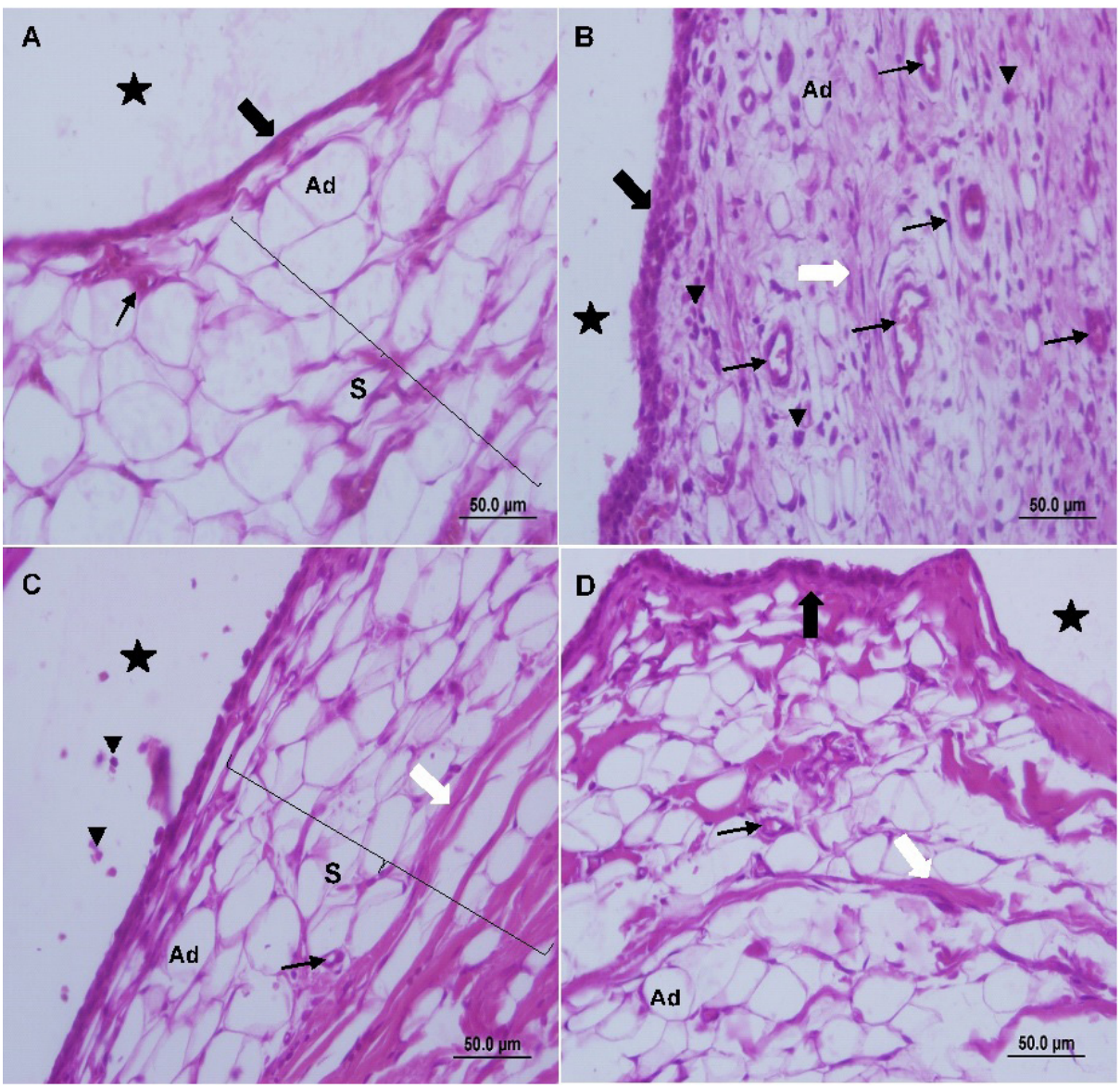

Fig. 1. Photomicrographs of the synovial membrane of the knee joint of Wistar rats, sagittal section, staining in hematoxylin and eosin. In A, control group (G1), with thin synovial intima (black arrow) and subintima (S), with predominance of adipose cells (Ad) and blood vessels within normal range (thin arrow). In B, immobilization group (G2), thick synovial intima and disorganization of synoviocytes, in subintima replacement of fat tissue by connective rich in collagen fibers (white arrow), presence of intense inflammatory infiltrate (▼) and large amount of blood vessels. In C, free remobilization group (G3) and D, WBV group (G4), intima and subintima returning to reorganization, with presence of inflammatory cells in the joint cavity of G3. Joint cavity (star)

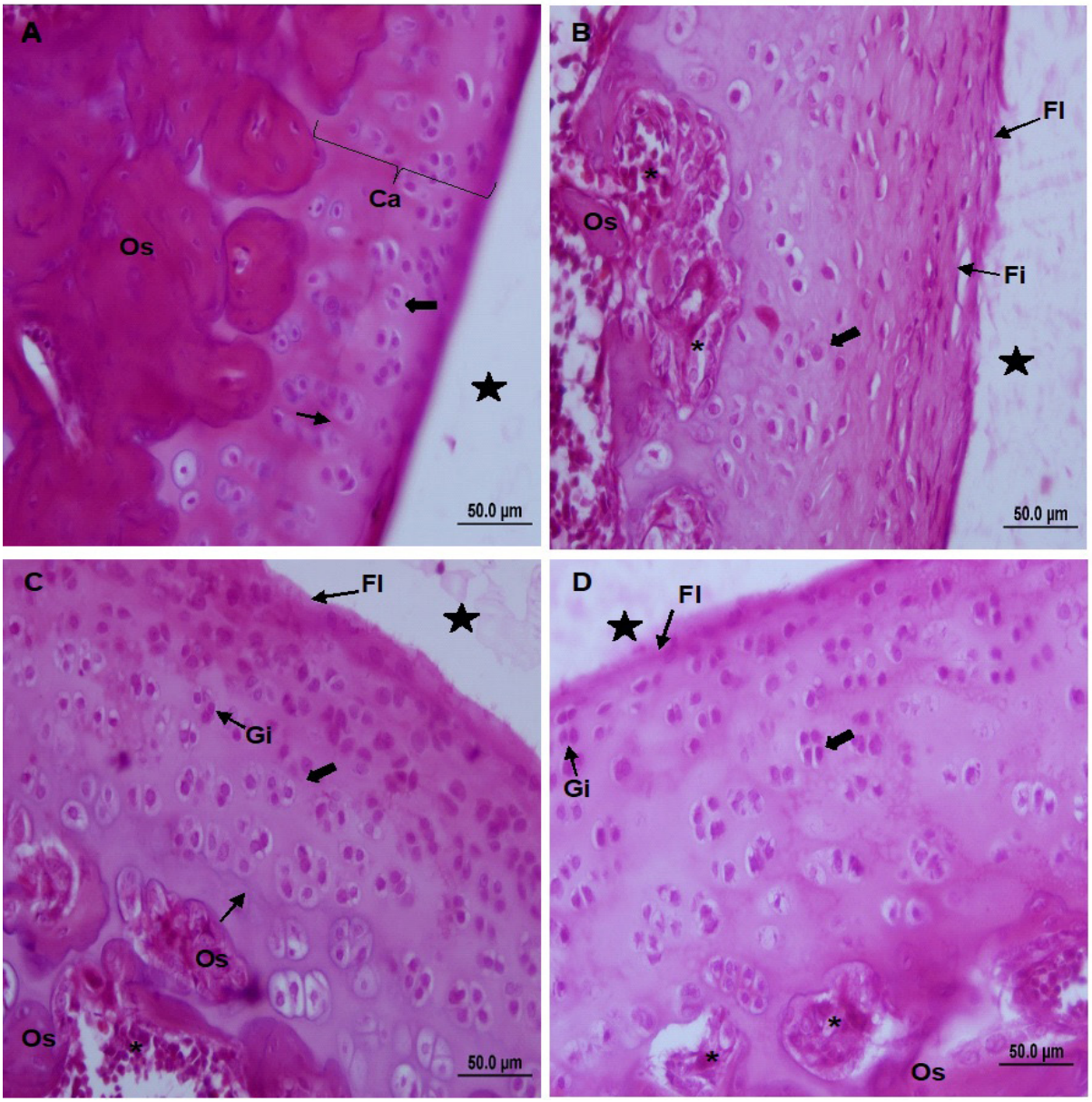

Fig. 2. Photomicrographs of femoral joint cartilage in the patellofemoral joint of Wistar rats, sagittal section, staining in hematoxylin and eosin. In A, control group (G1), normal joint cartilage organization (Ca), smooth surface, chondrocytes distributed in the characteristic zones (Black arrow), presence of tidemark (thin arrow), normal looking subchondral bone (OS) In B, immobilization group (G2), flocculations (Fl) on the surface of the cartilage, fissure (Fi) in the extracellular matrix, increase in the number of chondrocytes and disorganization of the zones, invaginations in the subchondral bone with presence of vascular connective tissue (asterisk) and absence of tidemark. In C, free remobilization group (G3) and D, platform remobilization group (G4), surface flocculation, with large number of isogenic groups (Gi) in the various layers, vascular connective tissue. Joint cavity (star)

In the morphological analysis of the knee joint, the cartilage of the femur and tibia, in G1, showed normal characteristics, where the surface was smooth and the chondrocytes organized in the four cell layers, and in the surface area, they were in horizontal clusters with a flattened appearance, in the intermediate more rounded, being isolated or in isogenic groups, in the deepest organized in gaps, being separated from the calcified area by a basophilic line, tidemark. Deep in the calcified zone, the subchondral bone had a normal aspect (Fig. 2A).

In G2, it was found that immobilization caused severe morphological changes, such as disorganization and flocculations on the joint cartilage surface, cracks in the extracellular matrix, increase in the number of chondrocytes that, lost the pattern of organization in areas with increased total thickness of the joint cartilage, absence of tidemark, besides invaginations in the subchondral bone with the presence of vascular connective tissue filling the medullar space immediately adjacent to the cartilage, where vascular tunnels appeared and pen-

etrated inside the deep layer of the cartilage, destroying the subchondral plateau. These findings were most evident in the femur in the patellofemoral joint region (Fig. 2B).

In animals of groups G3 (freely remobilized) and G4 (remobilized by WBV), the morphological results were similar. The cartilage still showed flocculation, but absence of fissures in the extracellular matrix, being possible to observe the presence of groups of cellular clones or isogenic groups in the superficial layer in great quantity, with beginning of the organization of the cellular zones and beginning of tidemark organization in some points. In the subchondral bone remained areas of invagination, but with a decrease in the vascular connective tissue that filled the medullary space (Fig. 2C and 2D).

Discussion

Morphological observations of the knee joint in the control group showed normality in the cellular organization of the synovial membrane and joint cartilage, expected in synovial joints.[20,21] On the other hand, the groups submitted to experimental immobilization (G2) presented morphological alterations of tissue disorganization in the synovial membrane and articular cartilage, corroborating with the findings of Kunz et al., or the free remobilization (G3) and WBV (G4) groups, there was absence of cracks in the extracellular matrix, presence of cellular clone and organization principle of the zones and tidemark, not differing between the forms of remobilization.[10]

Regarding the thickening of the synovial membrane, Del Carlo et al., found that immobilization degenerates the synoviotics, affecting the production of synovial fluid and the nutritional supply of the cartilage, making it rigid and causing its thickening, which is in line with what was observed in the present study, since the synovial membrane of the immobilization group (G2) proved to be totally disorganized in terms of its tissue structure, as well as the articular cartilage.[22]

The synovial membrane of G3 and G4 animals presented repair characteristics, with reorganization of the synoviotic layers in the intima and subintima, reduction of fibrous tissue, reappearance of adipocytes and reduction of angiogenesis. Besides these changes, a decrease in the amount of inflammatory infiltrate in the articular cavity of the remobilization group with vibratory platform was noted, while in the free remobilization group its presence was more expressive. These results corroborate the study by Vieira et al. [23], in which the animals performed an exercise protocol with ladder climbing, and also showed positive results in the acceleration of tissue regeneration, however, there was no presence of inflammatory infiltrate found in the joint cavity as was seen in this study. The inflammatory response that occurs in the synovial joints has principle in the production of interleukin-1 and tumor necrosis factor α (TNF-α), causing the inhibition of the production of collagen type II and proteoglycans and the stimulation of the synthesis of metalloproteinases, which act on the degradation of articular cartilage.[24]

In this study it was possible to verify that the immobilization protocol, for a time of 2 weeks, caused flocculations on the surface of the joint cartilage and disarrangements in the cartilaginous matrix, as already presented in the literature.[10,21] Synovial fluid diffusion in the articular cavity may be compromised by the immobilization procedure, hindering fluid diffusion and reducing nutrient supply to chondrocytes, leading to a reduction of its extracellular matrix and proteoglycan synthesis due to disuse.[5,20,22]

Del Carlo et al. found that cartilage lesions can be reversible with the use of remobilization programs.[22] In this study, the groups submitted to free remobilization and by WBV presented similar characteristics of tissue recovery, being observed the increased presence of isogenic groups that according to Melo et al., represent the hyperactivity of chondrocytes as an attempt of tissue repair after a period of immobilization.[25] Also, according to Ando et al. when a joint is submitted to its movement again, there will be a reduction in stiffness and an improvement in the synovial fluid flow in the joint cavity, thus promoting cartilage nutrition and consequent regeneration, which goes against the changes found in experimental groups submitted to free remobilization and IVC (G3 and G4).[26]

The use of WBV as a therapeutic method for the treatment of osteoporosis has been widely studied because of its mechanical stimulation mechanism characterized by active oscillations in bone tissue cells, as well as in muscle tissue, showing increased strength, balance, decreased risk of falls and morbidity, however there are still few studies that report its effects on joint tissues.[27-32] Van Heuvelen et al. point to the importance of presenting the parameters for use of the WBV, since only in this way is there a possibility of reproducibility of the results.[33] In this study, the WBV was used with a frequency of 60 Hz and vibrations with an amplitude of 2 millimeters, for 10 minutes, resulting in a recovery in the changes caused by the immobilization procedure similar to those found with free remobilization, with a decrease in connective tissue and infammatory infiltration of the articular cavity in the platform group. Conversely, Mierzwa et al. observed the joint degradation of the distal part of the femurs, infringed by the use of IVC, with a frequency of 60 Hz and amplitude of 0.6 mm, for 20 minutes, 5 days a week, with total duration of 3 months.[34] In Wistar ovariectomized rats, it may be inferred that the difference in the choice of vibration amplitude and the time of application of the experimental model,

in the case of hormonal deprivation, may have caused the divergences in the results found with this study, and no degenerative effect was observed in the animals treated with the platform, but a subtle improvement in the inflammatory process. Seeing studies that point to a reduction in the level of pain with the use of WBV, and being that this is a basis for neurogenic inflammation, it can be inferred as one of the means by which there was the tissue alteration found.[35,36]

Qin et al., and McCann et al., submitted rats and mice, respectively, to experimental procedures of osteoarthritis, using WBV at lower frequencies of 35 Hz with a wave amplitude of 0.3 millimeters, for 20 minutes, for 5 days a week, with total duration of 6, 12 and 18 weeks according to the groups, while in McCann's study the frequency used was 45 Hz, with a wave amplitude of 0.3 millimeters, for 30 minutes a day, for 5 days a week, with a total duration of 4 and 8 weeks and 4 weeks with another 4 weeks of recovery according to the group, obtaining in both studies the degradation of cartilage as a tissue response.[37,38]

In the present study, the tissue reorganization in the subintima and the decrease of inflammatory infiltrate in the articular cavity and the presence of cellular clones of the group treated with platform vibration, showed an initiation of more effective tissue recovery response in the platform group when compared to the free. Thus, it can be inferred that the application of the platform with other treatment parameters, could accelerate the recovery process, thus, new comparative research is needed regarding the increase in treatment time and the diversification of vibratory parameters to be used.

Conclusion

Use of WBV as a therapeutic method for remobilization, in the parameters used, showed better results in the tissue recovery of the synovial membrane of the knee joint of Wistar rats.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, J.C.R.G., G.R.F.B., R.M.C. and L.F.C.R.; Methodology, J.C.R.G., G.R.F.B., R.M.C. and L.F.C.R.; Formal Analysis, J.C.R.G., G.R.F.B., R.M.C. and L.F.C.R.; Investigation, J.C.R.G., A.L.F.T., M.L.S.W., C.D.T.B. and D.F.S.R.; Resources, J.C.R.G., G.R.F.B., R.M.C. and L.F.C.R.; Writing – Original Draft Preparation, J.C.R.G.; Writing – Review & Editing, A.L.F.T., G.R.F.B., M.L.S.W., C.D.T.B., D.F.S.R., R.M.C. and L.F.C.R.; Visualization, J.C.R.G., A.L.F.T., G.R.F.B., M.L.S.W., C.D.T.B., D.F.S.R., R.M.C. and L.F.C.R.; Supervision, G.R.F.B., R.M.C. and L.F.C.R.; Project Administration, G.R.F.B., R.M.C. and L.F.C.R.; Funding Acquisition, J.C.R.G., G.R.F.B., R.M.C. and L.F.C.R.

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.

Ethics approval

Study was approved by the Unioeste Animal Use Ethics Committee (2729/2014-GRE).

References

1. Andia I, Maffulli N. New biotechnologies for musculoskeletal injuries. *Surgeon*. 2019;17(4):244-255. doi:10.1016/j.surge.2018.08.004
2. Bączkowicz D, Skiba G, Falkowski K, Domaszewski P, Selkow N. Effects of immobilization and re-mobilization on knee joint arthrokinematic motion quality. *J Clin Med*. 2020;9(2):451. doi:10.3390/jcm9020451
3. Wahl EP, Richard MJ. Management of metacarpal and phalangeal fractures in the athlete. *Clin Sports Med*. 2020;39(2):401-422. doi:10.1016/j.csm.2019.12.002
4. Tomori Y, Nanno M, Takai S. Outcomes of nonsurgical treatment for transcondylar humeral fractures in adults: Clinical results of nonoperative management. *Medicine (Baltimore)*. 2019;98(46):e17973. doi:10.1097/MD.0000000000017973
5. Vanwanseele B, Lucchinetti E, Stüssi E. The effects of immobilization on the characteristics of articular cartilage: current concepts and future directions. *Osteoarthr Cartil*. 2002;10(5):408-419. doi:10.1053/joca.2002.0529
6. Nomura M, Sakitani N, Iwasawa H, et al. Thinning of articular cartilage after joint unloading or immobilization. An experimental investigation of the pathogenesis in mice. *Osteoarthr Cartil*. 2017;25(5):727-736. doi:10.1016/j.joca.2016.11.013
7. Lu W, Wang L, Yao J, Wo C, Chen Y. C5a aggravates dysfunction of the articular cartilage and synovial fluid in rats with knee joint immobilization. *Mol Med Rep*. 2018;18(2):2110-2116. doi:10.3892/mmr.2018.9208
8. Leroux MAM, Cheung HSH, Bau JL, Wang JY, Howell DSD, Setton LA LA. Altered mechanics and histomorphometry of canine tibial cartilage following joint immobilization. *Osteoarthr Cartil*. 2001;9(7):633-640. doi:10.1053/joca.2001.0432
9. Narmoneva DA, Cheung HS, Wang JY, Howell DS, Setton LA. Altered swelling behavior of femoral cartilage following joint immobilization in a canine model. *J Orthop Res*. 2002;20(1):83-91. doi:10.1016/S0736-0266(01)00076-6
10. Kunz RI, Silva LI, Costa JRG da, et al. Alterações histomorfométricas na articulação do joelho de ra-

tos Wistar após remobilização em meio aquático. *Fisioter e Pesqui*. 2015;22(3):317-324. doi:10.590/1809-2950/14234922032015

11. Morimoto A, Winaga H, Sakurai H, et al. Treadmill running and static stretching improve long-lasting hyperalgesia, joint limitation, and muscle atrophy induced by cast immobilization in rats. *Neurosci Lett*. 2013;534(1):295-300. doi:10.1016/j.neulet.2012.11.009
12. Ju Y-I, Sone T, Okamoto T, Fukunaga M. Jump exercise during remobilization restores integrity of the trabecular architecture after tail suspension in young rats. *J Appl Physiol*. 2008;104(6):1594-1600. doi:10.1152/japplphysiol.01004.2007
13. Luo X, Zhang J, Zhang C, He C, Wang P. The effect of whole-body vibration therapy on bone metabolism, motor function, and anthropometric parameters in women with postmenopausal osteoporosis. *Disabil Rehabil*. 2017;39(22):2315-2323. doi:10.1080/09638288.2016.1226417
14. Christiansen BA, Silva MJ. The effect of varying magnitudes of whole-body vibration on several skeletal sites in mice. *Ann Biomed Eng*. 2006;34(7):1149-1156. doi:10.1007/s10439-006-9133-5
15. Marín-Cascales E, Alcaraz PE, Ramos-Campo DJ, Martinez-Rodriguez A, Chung LH, Rubio-Arias JÁ. Whole-body vibration training and bone health in postmenopausal women: A systematic review and meta-analysis. *Medicine (Baltimore)*. 2018;97(34):e11918. doi:10.1097/MD.0000000000011918
16. Lin CI, Huang WC, Chen WC, et al. Effect of whole-body vibration training on body composition, exercise performance and biochemical responses in middle-aged mice. *Metabolism*. 2015;64(9):1146-1156. doi:10.1016/j.metabol.2015.05.007
17. Wang L, Wang Z, Liu Q, Su J, Wang T, Li T. Effect of whole body vibration on HIF-2α expression in SD rats with early knee osteoarthritis. *J Bone Miner Metab*. Published online 2020:Epub ahead of print. doi:10.1007/s00774-020-01092-3
18. Zazula MF, Wutzke MLS, da Costa JRG, et al. Morphological effects of whole-body vibration on remobilization of the tibialis anterior muscle of Wistar rats. *Anat Rec*. 2020;303(11):2857-2864. doi:10.1002/ar.24390
19. Kakihata CMM, Peretti AL, Wutzke MLS, et al. Morphological and nociceptive effects of mechanical vibration on the sciatic nerve of oophorectomized Wistar rats. *Motriz*. 2019;25(1):e101949. doi:10.1590/s1980-6574201900010005
20. Kojima S, Hoso M, Watanabe M, Matsuzaki T, Hibino I, Sasaki K. Experimental joint immobilization and remobilization in the rats. *J Phys Ther Sci*. 2014;26(6):865-871. doi:10.1589/jpts.26.865
21. Nagai M, Aoyama T, Ito A, et al. Alteration of cartilage surface collagen fibers differs locally after immobilization of knee joints in rats. *J Anat*. 2015;226(5):447-457. doi:10.1111/joa.12290
22. Del Carlo RJ, Galvão MR, Viloria MI V, et al. Imobilização prolongada e remobilização da articulação fêmoro-tíbio-patelar de ratos: estudo clínico e microscópico. *Arq Bras Med Vetrinária e Zootec*. 2007;59(2):363-370.
23. Vieira L, Lovison K, Kunz RI, et al. Resistance exercise recovers the structure of cartilage and synovial membrane of the ankle joint of rats after sciatic compression. *Mot Rev Educ Física*. 2017;23(3):e101707. doi:10.1590/s1980-6574201700030001
24. Elsaid KA, Jay GD, Chichester CO. Reduced expression and proteolytic susceptibility of lubricin/superficial zone protein may explain early elevation in the coefficient of friction in the joints of rats with antigen-induced arthritis. *Arthritis Rheum*. 2007;56(1):108-116. doi:10.1002/art.22321
25. Melo EG, Nunes VA, Rezende CMF, Gomes MG, Malm C, Gheller VA. Sulfato de condroitina e hialuronato de sódio no tratamento da doença articular degenerativa em cães. Estudo histológico da cartilagem articular e membrana sinovial. *Arq Bras Med Vet e Zootec*. 2008;60(1):83-92. doi:10.1590/S0102-09352008000100013
26. Ando A, Hagiwara Y, Chimoto E, Hatori K, Onoda Y, Itoi E. Intra-articular injection of hyaluronan diminishes loss of chondrocytes in a rat immobilized-knee model. *Tohoku J Exp Med*. 2008;215(4):321-331. doi:10.1620/tjem.215.321
27. Weber-Rajek M, Mieszkowski J, Niespodziński B, Ciechanowska K. Whole-body vibration exercise in postmenopausal osteoporosis. *Prz Menopauzalny*. 2015;14(1):41-47. doi:10.5114/pm.2015.48679
28. Gnyubkin V, Guignandon A, Laroche N, Vanden-Bossche A, Malaval L, Vico L. High-acceleration whole body vibration stimulates cortical bone accrual and increases bone mineral content in growing mice. *J Biomech*. 2016;49(9):1899-1908. doi:10.1016/j.jbiomech.2016.04.031
29. Xu J, Lombardi G, Jiao W, Banfi G. Effects of exercise on bone status in female subjects, from young girls to postmenopausal women: an overview of systematic reviews and meta-analyses. *Sport Med*. 2016;46(8):1165-1182. doi:10.1007/s40279-016-0494-0
30. Park S-Y, Son W-M, Kwon O-S. Effects of whole body vibration training on body composition, skeletal muscle strength, and cardiovascular health. *J Exerc Rehabil*. 2015;11(6):289-295. doi:10.12965/jer.150254
31. Bemben D, Stark C, Taiar R, Bernardo-Filho M. Relevance of whole-body vibration exercises on muscle strength/power and bone of elderly individuals. *Dose-Response*. 2018;16(4):155932581881306. doi:10.1177/1559325818813066
32. Sá-Caputo D, Paineiras-Domingos LL, Francisca-Santos A, et al. Whole-body vibration improves the functional parameters of individuals with metabolic syndrome: An exploratory study. *BMC Endocr Disord*. 2019;19(1):6. doi:10.1186/s12902-018-0329-0
33. Van Heuvelen MJG, Rittweger J, Judex S, et al. Reporting guidelines for whole-body vibration studies in hu-

mans, animals and cell cultures: A consensus statement from an international group of experts. *Biology (Basel)*. 2021;10:965. doi:10.3390/biology10100965

34. Mierzwa AGH, Campos JF, Jesus MF, Nader HB, Lazaretti-Castro M, Reginato RD. Different doses of strontium ranelate and mechanical vibration modulate distinct responses in the articular cartilage of ovariectomized rats. *Osteoarthr Cartil*. 2017;25(7):1179-1188. doi:10.1016/j.joca.2017.02.793
35. Sá-Caputo DC, Paineiras-Domingos LL, Oliveira R, et al. Acute effects of whole-body vibration on the pain level, flexibility, and cardiovascular responses in individuals with metabolic syndrome. *Dose-Response*. 2018;16(4):1-9. doi:10.1177/1559325818802139
36. Kelly S, Dunham JP, Donaldson LF. Sensory nerves have altered function contralateral to a monoarthritis and may contribute to the symmetrical spread of inflammation. *Eur J Neurosci*. 2007;26(4):935-942. doi:10.1111/j.1460-9568.2007.05737.x
37. Qin J, Chow SKH, Guo A, Wong WN, Leung KS, Cheung WH. Low magnitude high frequency vibration accelerated cartilage degeneration but improved epiphyseal bone formation in anterior cruciate ligament transect induced osteoarthritis rat model. *Osteoarthr Cartil*. 2014;22(7):1061-1067. doi:10.1016/j.joca.2014.05.004
38. McCann MR, Yeung C, Pest MA, et al. Whole-body vibration of mice induces articular cartilage degeneration with minimal changes in subchondral bone. *Osteoarthr Cartil*. 2017;25(5):770-778. doi:10.1016/j.joca.2016.11.001

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.5

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 167–175

ORIGINAL PAPER

Nesrin Elif Ortakaş [1], Özlem Öztürk Şahin [2]

The effect of foot reflexology applied to neonates before oro/nasopharyngeal suctioning on procedural pain and comfort in the neonatal intensive care unit

[1] Neonatal Intensive Care Unit, Cebeci Research and Application Hospital, Ankara University, Ankara, Turkey
[2] Department of Pediatric Nursing, Faculty of Health Sciences, Karabuk University, Karabuk, Turkey

ABSTRACT

Introduction and aim. This study was performed to examine the effect of foot reflexology applied to the neonates on the level of pain and discomfort developed due to suctioning procedure.

Material and methods. The study was conducted experimentally by taking pretest and repeated measurements on reflexology and control groups determined by simple randomization. The study was carried out with 66 neonates (reflexology applied: 33 and control group: 33). Neonatal Information Form, Neonatal Infant Pain Scale (NIPS) and Newborn Comfort Behavior Scale (NCBS) were used as the data collection tools.

Results. The during ($p<0.001$) and after suctioning ($p<0.001$), the NIPS scores of the neonates in the intervention group was statistically lower than the control group. The NCBS scores of the neonates in the intervention group during ($p<0.001$), and after suctioning ($p<0.001$), were statistically significantly lower than the control group.

Conclusion. It was concluded that foot reflexology applied to neonates was effective both in reducing pain during and after the suctioning and in increasing comfort during and after the suctioning.

Keywords. comfort, foot reflexology, neonates, procedural pain, suctioning procedure

Introduction

Many painful procedures are performed every day for neonates admitted to hospitals and this situation is repeated in intensive care units.[1] Neonates requiring intensive care undergo on average between 7.5 to 17.3 painful procedures per day during their hospital stay, with the most common procedures being heel lancing, venipuncture and suctioning.[2]

Pain management among infants at neonatal intensive care units (NICUs) has received increased attention over the last years, and it is well known that inadequate pain management is linked to negative shortand long-term consequences for the infant.[3] Besides, exposure to untreated procedural pain has been linked to adverse effects on neonatal outcomes, most notably brain development, neurodevelopment, immune system, regulation of stress systems, and, later, pain perception and sensitivity.[1,2,4] Procedures performed on neonates can cause pain as well as impairment the comfort of neonates.[5–7]

In addition to pharmacological methods, non-pharmacological methods can also be used in reducing pain and increasing comfort during invasive procedures in neonates.[6,7] Pharmacological methods are generally not recommended for use in neonates due to serious side effects such as apnea and respiratory depression.[8] Non-pharmacological methods are generally preferred

Corresponding author: Özlem Öztürk Şahin, e-mail: ozlem.ozturk@karabuk.edu.tr

Received: 10.01.2022 / Revised: 26.02.2022 / Accepted: 30.03.2022 / Published: 30.06.2022

Ortakaş NE, Şahin ÖÖ. *The effect of foot reflexology applied to neonates before oro/nasopharyngeal suctioning on procedural pain and comfort in the neonatal intensive care unit.* Eur J Clin Exp Med. 2022;20(2):167–175. doi: 10.15584/ejcem.2022.2.5

for the most common repeated painful procedures as a results of their apparently superior efficacy and favorable safety profile.[2] Also, evidence suggests that neonate, could benefice of non-pharmacological interventions to relive mild to moderate pain and discomfort from minör invasive procedures.[9,10] Massage, therapeutic touch, acupressure, acupuncture and reflexology, which are among the non-pharmacological methods applied to neonates and infants[9,11,12], are also defined as Complementary and Alternative Medicine (CAM) Methods or Complementary Health Approaches (CHAs).[13-15]

Reflexology enables endorphin and encephalin release by stimulating the pituitary gland through the pressure and massage performed on reflex points on hands, feet and ears. It resolves problems in organs and body parts that correspond to these points, reduces pain, and relieves discomfort.[16,17] Foot reflexology is among the most popular forms of reflexology. It's an easy, safe, and non-invasive method which can be utilized without the need for special equipment.[18]

There are studies examining the effects of foot reflexology applied to the neonates on meconium passage, neonatal jaundice, neonatal abstinence syndrome, colic symptoms, and physiological index.[15,18-21] However, while a limited number of studies exists examining the effect of foot reflexology on neonatal pain[17,21,22], no studies examining its effect on comfort were found. Whereas, nurses are consistent caregivers for neonates, and their assessment of pain and pain practice is invaluable.[23] In addition, in recent years, nursing practices in NICUs have attracted attention with the aspect of increasing the comfort of neonates.[5]

Foot reflexology, which we used in this study, is one of the methods nurses use to manage symptoms in neonates.[15,17,22] Non-pharmacological methods such as reflexology generally advocate considering the individual as a body, soul, and mind. In the Watson model regarding postmodern nursing, it is recommended to use touch consciously (practices such as therapeutic massage, and reflexology). Therefore, CHAs that contribute to nursing are important for providing holistic care.[7] Also, nurses frequently exhibit a positive attitude toward the use of CAM because its philosophy complies with a holistic nursing care approach.[13] The fact that the foot reflexology used in this study was performed with an invasive procedure such as suctioning, which has not been studied before, is thought to make an important contribution to the literature.

Aim

This study was performed to examine the effect of foot reflexology applied to the neonates on the level of pain and discomfort developed due to suctioning procedure. Hypotheses of the study; foot reflexology applied to neonatal;

- Is effective in reducing pain that develops during suctioning procedure.
- Is effective in reducing pain that continues after suctioning procedure.
- Is effective in increasing the comfort of the neonatal during suctioning procedure
- Is effective in increasing the comfort of the neonatal after suctioning procedure.

Material and methods

Design, setting and sample size

The study was conducted experimentally by taking pretest and repeated measurements on reflexology and control groups determined by simple randomization. The universe of this study consisted of 154 neonates admitted to the NICU at a university hospital in Ankara, Turkey between 1 July to 30 September 2017. A power analysis was performed using the G*Power 3.1 program to determine the size of research sample with two study groups. The results of research were used conducted on infants and neonates that determine the effect of foot reflexology before vaccination on acute pain; the effect of foot reflexology on meconium passage; the effect of foot massage on pain responses to heel stick.[17,19,24] The sample size was determined to include a total 52 neonates, 26 in each group, at a power of 80%, a significance level of 5%, and an effect size of 0.80 (t=2.009; Effect size d=0.80). Due to possible data loss, the sample consisted of 66 neonates (intervention group: 33 and control group: 33) who met the study inclusion criteria.

Data collection tools

Neonatal information form (NIF)

In this form prepared by the researchers; there are a total of 10 questions, 8 of which are open-ended, questioning descriptive features such as sex, gestational age, postnatal age, anthropometric measurements, medical diagnosis and medicines used.

Neonatal infant pain scale (NIPS)

Developed by Lawrence et al., and adapted to Turkish by Akdovan.[25,26] This scale is used to evaluate the pain associated with the procedures applied in term and premature neonates. It includes 6 behavioral parts that include facial expression, cry, breathing patterns, state of arousal, arm and leg movements. The total score ranges from 0-7. The high score indicates that the severity of pain increased. Lawrence et al., determined the cronbach alpha value of the scale is between .92-.97. and Akdovan as .83-.86.[25,26] In this study, it was found to be .91.

Newborn comfort behavior scale (NCBS)

"Comfort Scale", Ambuel et al. evaluates the distress of pediatric patients who were followed up in the NICU with mechanical ventilator support.[27] Van Dijk et al.

revised this scale as "COMFORTneo scale" for neonates and Kahraman et al. adapted to Turkish.[28,29] This scale is composed of 7 behavioral dimensions. These are alertness, calmness/agitation, facial tension, muscle tone, body movement, respiratory response (only in mechanically ventilated neonates) and crying (only in spontaneously breathing neonates). In this study, the dimension of "crying" was evaluated because neonates had spontaneous breathing and 6 dimensions in total were rated. As responses are on a 1 to 5 Likert scale, total scores range from 6 to 30. Scoring 13 points and below on the scale indicates that the neonate is comfortable, and scoring between 14-30 indicate that the neonate has pain or distress. Kahraman et al., found the scale's cronbach alpha value as .85 before medical care.[29] In this study, it was found as .85. In addition, a positive and high level relationship was found between NIPS and NCBS medians during and after suctioning (Table 1).

Table 1. Correlation between NIPS and NCBS*

		NCBS			
		Control group		Intervention group	
NIPS	During suctioning	r_s 0.728*	p<0.001	r_s 0.775*	p<0.001
	After suctioning	r_s 0.728*	p<0.001	r_s 0.834*	p<0.001

*Both scales showed a correlation above .70

Intervention phases

Pre-intervention

NIF, NIPS and NCBS were applied to 154 neonates hospitalized in NICU. Neonates, who got 0 points from NIPS (that shows they had no pain) and under 13 points from NCBS (that shows they were comfortable), included in the study. Other inclusion criteria were as follows: being born between the 37 and 42 weeks, not receiving analgesic and sedative medicine treatment, not being diagnosed with neurological or congenital heart disease in the prenatal and postnatal period, being on noninvasive respiratory support (Noninvasive Continuous Positive Airway Pressure "NCPAP"), and no skin problem that would prevent the application of reflexology. Eighty-eight neonates, who did not meet these inclusion criteria, were found to have pain according to NIPS and were found to be uncomfortable by NCBS, were excluded from the study. 66 neonates who met the inclusion criteria constituted the sample of the study. Randomization was performed to determine the intervention and control groups in the study. The method of randomization was based on patient admission order. A protocol number (ie, 1 through 66) was assigned and recorded to each neonate in order of admission to the NICU. Odd numbers were assigned to the intervention group and even numbers to the control group.

Intervention

Foot reflexology was applied to the neonates in the intervention group. No intervention was made to the control group. One of the researchers (N.E.O.) applied foot reflexology to neonates. N.E.O. is working as a nurse at NICU where the study is conducted, and also received a reflexology practitioner training certificate after three months of training. N.E.O. applied foot reflexology in the NICU. None of the parents of the neonates were present at the time of reflexology. Neonates lying naked in their incubators with only their diapers were placed in open beds in the NICU so that reflexology could be applied comfortably. While the neonates were lying in the infant incubator or reflexology was applied to the neonates in open beds, the neonates were connected to the bed warmer by means of a prop. Thus, instantaneous body temperature of neonates was seen and body temperature was followed. There was no risk of hypothermia in any of the neonates. Neonates were in the supine position during reflexology. Foot reflexology was performed to both feet with clean and non-cold bare hands for neonates. Reflexology time (minutes): The recommended reflexology session for infants is (10-15 min for each foot) 20-30 min total for both feet.[15,17,18,22,30] In this study, it was applied to both feet for a total of 30 min, 15 min to one foot of each neonates (Fig. 1).

Post-intervention

Reflexology applied neonates were taken from their open beds where reflexology was applied and placed in their incubators. The neonates in the control group, on the other hand, were never placed in an open bed because reflexology was not applied to them, they were in their incubators.

Before suctioning: Preparations and arrangements were made in both groups for situations that may affect the pain and comfort levels of neonates. These are; diaper replacement of the neonatal, feeding at least one hour before, keeping away from stimulating sounds such as ventilator, monitor and giving supine position to the neonatal. NIPS and NCBS were applied to neonates in both groups just before the suctioning. It was determined that all neonates in the intervention and control group did not have pain by getting "0" points from NIPS and were comfortable by getting under "13" points from NCBS. The vital signs of neonates were measured before the suctioning procedure. There was no statistically significant difference between the intervention and control groups in terms of median fever value (p=0.375); mean pulse value (p=0.483); median oxygen saturation value (p=0.151); and median blood pressure value (p=0.832). In addition, there was no obstacle to suctioning procedure in terms of vital signs.

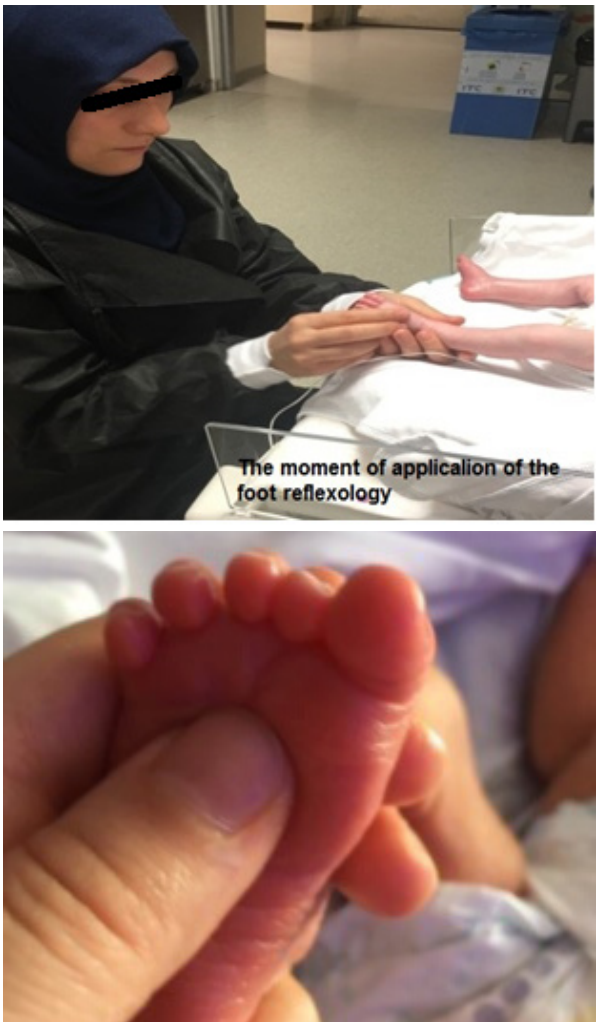

Fig. 1. The moment of application of the foot reflexology and the foot reflexology pressure points

During suctioning: Suctioning procedure for all neonates was done by N.E.O. The mean time of suctioning was 16.29±2.665 (min-max 8-22) seconds. The same brand 8 Fr disposable suctioning probe was used for each neonatal. The suctioning was performed with a pressure in the range of 80-100 mmHg, first being the oropharyngeal and then the nasopharyngeal. While performing the suctioning, NIPS and NCBS were applied to the neonates. The vital signs of neonates were measured during the suctioning procedure. There was no statistically significant difference between the intervention and control groups in terms of median fever value (p=0.989); mean pulse value (p=0.431); median oxygen saturation value (p=0.110); and median blood pressure value (p=0.390). In addition, there was no situation requiring early termination of the suctioning.

After suctioning: NIPS and NCBS were not administered to the neonates immediately after suctioning. Because neonates cry after suctioning, the crying time of neonates was measured. The mean time of crying was 12.50±12.953 (min-max 0-55) seconds. During crying, neonates in both groups were not held during crying. Each neonates was in his own incubator. After measuring the crying times of the neonates, their vital signs were measured. There was no statistically significant difference between the intervention and control groups in terms of median fever value (p=0.439); median oxygen saturation value (p=0.462); and median blood pressure value (p=0.059). However, while the mean pulse value of the intervention group was 138.67±14.59, it was 162.03±15.615 in the control group, and there was a statistically significant difference between the two groups (p<0.001). A 10-minute period was defined for completion of neonates crying times and vital signs measurements and, the nurse other than the researcher, to be ready to administer the scales. NIPS and NCBS were applied to all neonates in the intervention and control groups 10 minutes after the suctioning (Fig. 2).

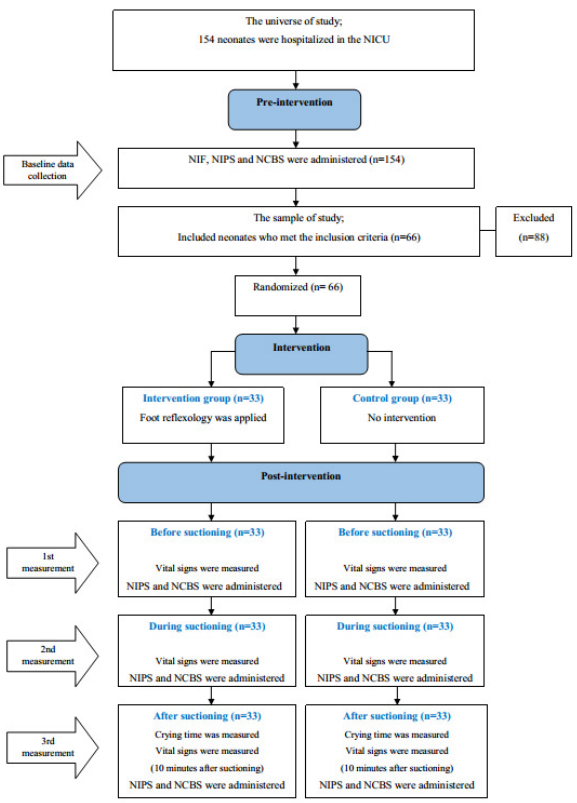

Fig. 2. Intervention phases diagram of the study

Data analysis

The IBM SPSS Statistics 23 (IBM Corp, Armonk, New York, USA) was used to evaluate the data. Kolmogorov-Smirnov test was used to determine whether the variables showed normal distribution. The difference between categorical variables with two groups; evaluated with independent t test (for normally distributed variables) and Mann Whitney U test (for non-normally distributed variables). Wilcoxon test was used to determine the difference in measurements made at two different times. Repeated measures analysis of variance (for normally distributed variables) was used to examine

the difference in measurements made at different three times, and the Friedman test (for non-normally distributed variables) to determine the difference in measurements made at different three times.

The Chi-square test was used to analyze the relationship between two categorical variables. In addition, the Sepearman Correlation coefficient was used to analyze the relationship between two numerical variables.

Results

The study was conducted with a total of 66 neonates. When neonates in the intervention and control groups are compared according to their descriptive characteristics; there was no significant difference between groups in terms of sex (p=0.805), mode of delivery (p=0.592), and medical diagnosis (p=1) (Table 2). Also, when age and physical characteristics of neonates are compared according to the groups; there was no statistically significant difference between groups in terms of gestational age (p=0.44), postnatal age (p=0.646), birth weight (p=0.837), length (p=0.157) and head circumference (p=0.577) (Table 3).

As a result of the applied Mann Whitney U test; during (p<0.001) and after suctioning (p<0.001), the median pain (NIPS) scores of the neonates in the intervention group were statistically lower than the control group. As a result of the Friedman test applied, there was a statistically significant difference between the times in terms of median level of pain in the intervention (p<0.001) and control groups (p<0.001). When time differences (significance between measurements) were examined; In the intervention group, the decrease occurred after suctioning according to the during suctioning was found to be statistically significant (p<0.001). However, in the control group, the decrease occured after suctioning according to the during suctioning was not statistically significant (p=0.419) (Table 4).

As a result of the applied Mann Whitney U test, there was no statistically significant difference between the intervention and control groups in terms of median NCBS scores before suctioning (p=0.429), while there was a statistically significant difference during (p<0.001) and after (p<0.001) suctioning. Accordingly, the NCBS score medians of the neonates in the intervention group during and after suctioning were statistically significantly lower than the control group. As a result of the Friedman test applied, there was a statistically significant difference the times in terms of median level of NCBS intervention (p<0.001) and control groups (p<0.001). When time differences (significance between measurements) were examined; In the intervention group, the increase in NCBS score medians during (p<0.001) and after suctioning (p=0.004) was found to be statistically significant according to before suctioning, while the decrease that occurred after suctioning was statistically significant according to during suctioning (p<0.001). In the control group, the increase in NCBS score medians during (p<0.001) and after suctioning (p<0.001) was found to be statistically significant according to before suctioning, while the decrease that occurred after suctioning was statistically significant according to during suctioning (p=0.003) (Table 5).

Table 2. The descriptive characteristics of neonates*

Characteristics	Intervention group (n=33)		Control group (n=33)		Total (n=66)		x^2	P
	n	%	n	%	n	%		
Sex								
Female	15	45.5	16	48.5	31	47	0.061	0.805
Male	18	54.5	17	51.5	35	53		
Mode of delivery								
Caesarean	22	66.7	24	72.7	46	69.7	0.287	0.592
Normal	11	33.3	9	27.3	20	30.3		
Medical diagnosis								
Respiratory system diseases	28	84.8	28	84.8	56	84.8	<0.001	1
Other*	5	15.2	5	15.2	10	15.2		

*Intrauterine growth retardation (4), Meconium aspiration syndrome (6)

Table 3. Comparison of age and physical characteristics of neonates by groups*

Characteristics	Intervention group median (min-max)	Control group Median (min-max)	MW*	P
Gestational age	38.30 (37-40.2)	38.30 (37.0-40.2)	0.773	0.44
Postnatal age	1.00 (1-30)	1 (1-30)	0.459	0.646
Birth weight (g)	3.26 (1.54-4.3)	3.26 (1.54-4.3)	-0.205	0.837
Birt length (cm)	48 (36-53)	50 (36-54)	1.141	0.157
Birt head circumference (cm)	35 (30-38)	35(30-38)	0.557	0.577

*Mann Whitney-U test

Table 4. Examination of the NIPS score differences according to the group and time*

Groups	Before suctioning (1) median (min-max)	During suctioning (2) median (min-max)	After suctioning (3) median (min-max)	F**	p	Difference
Intervention group	0 (0-0)	4 (1-7)	2 (0-4)	61.443	<0.001	2 > 3
Control group	0 (0-0)	7 (3-7)	6 (0-7)	58.308	<0.001	
MW*	<0.001	4.845	6.725			
P	1	<0.001	<0.001			

*Mann Whitney-U test, **Friedman test

Table 5. Examination of the NCBS score differences according to the group and time*

Groups	Before suctioning (1) median (min-max)	During suctioning (2) median (min-max)	After suctioning (3) median (min-max)	F**	P	Difference
Intervention group	6 (6-10)	16 (10-28)	13 (6-19)	60.452	<0.001	1 < 2 > 3
Control group	6 (6-9)	23 (16-30)	19 (15-25)	61.477	<0.001	1 < 2 > 3
MW*	-0.79	5.371	6.483			
P	0.429	<0.001	<0.001			

* Mann Whitney-U test, **Friedman test

Discussion

Some factors are effective in sensing pain and creating a response to pain in neonates. These factors include sex, gestational age, birth weight, age, mode of delivery, neonatal illness severity, procedural pain exposure.[31–33] In this study, features such as sex, mode of delivery, medical diagnosis, gestational and postnatal age, birth weight, length and head circumference were homogeneous in the intervention and control groups. Therefore, while evaluating the effectiveness of applied foot reflexology on pain and comfort level, the possibility of being affected by these features was eliminated.

The hypotheses which are created in the planning stage of our study that "the foot reflexology applied to the neonatal is effective in reducing the pain that develops during the suctioning procedure and continues after the suctioning procedure" have been confirmed. When we look at the difference of pain scores over time; the median NIPS score decreased significantly over time only in the intervention group.

In this study, while evaluating the effectiveness of foot reflexology applied to neonates in reducing pain during and after the procedure; in the literature, the effects of reflexology on pain after the procedure have been examined. In these studies, they have been reported that foot reflexology applied to neonates and infants before procedure is effective in reducing acute pain after procedure.[17,21] In another study; while there was no statistically significant difference between the reflexology and control groups in terms of NIPS scores during the heel lance procedure, it has been reported that there was a statistically significant difference between the two groups in favor of the reflexology group in the NIPS scores after the heel lance procedure.[22] In our study, it was found that there was a statistically significant difference in favor of the intervention group (reflexology) between the two groups in terms of NIPS scores both during and after the suctioning procedure, and it was not consistent with this study. Reflexology based on touching skills is a form of massage that regulates body functions.[34] Foot massage and reflexology are some of the pain management procedures for neonates.[35] In studies evaluating the effectiveness of foot massage on pain, in accordance with our study, foot massage applied to neonates was found to be effective in reducing the pain of neonates.[24,36]

Painful and stressful interventions in the neonatal accompany the deterioration of the comfort of the neonatal.[37] In addition, physical structure, medical care and invasive procedures in NICU can cause loss of comfort in the neonatal.[29] In our study, the state of affecting comfort due to only the suctioning procedure performed on neonates was examined. The hypotheses which are created in the planning stage of our study that "the foot reflexology applied to the neonatal is effective in increasing the comfort of the neonatal during and after the suctioning procedure" have been confirmed. When we look at the difference of comfort scores over time, the comfort score medians decreased significantly after suctioning compared to during suctioning in both the intervention and control groups. The comfort level of neonates in the intervention group after suctioning is 13 points, and it can be said that these neonates are comfortable. However, the comfort level of neonates in the control group after suctioning is 19 points (14-30 points on the scale indicates that the neonate has pain and distress), and it can be said

that the distress of these neonates still continues even if the suctioning procedure is completed. Although there is no study in the literature examining the effect of reflexology directly on comfort; in one study, reflexology was reported to have positive effects in infantile colic, which is thought to negatively affect the comfort of infants.[34] In addition, the application of non-pharmacological methods other than reflexology has been reported to be effective in reducing the pain of the neonatal as well as increasing comfort, as seen in our study.[5,37,38]

Strengths and limitations

In order to fill the scales used objectively, the scales were administered by another nurse working at NICU, other than the researchers. While the nurse was administering the scales, it was ensured that the nurse did not know the neonates who underwent reflexology. The fact that the person evaluating the scales (the pain and comfort scores) is not one of the researchers, being a competent nurse working in this NICU and does not know which group the neonates are in (intervention or control?) is one of the strengths of the research. However, one of the weaknesses of the study is that the person evaluating the scales was the only person and the video recording could not be taken.

Conclusion

It was concluded that foot reflexology applied to neonates was effective in reducing pain that developed during the suctioning procedure and continued afterwards. It was concluded that the applied reflexology was effective both during and after the suctioning procedure in increasing the comforts that are directly related to the pain felt by neonates.

It was concluded that foot reflexology can be used in the management of procedural pain caused by suctioning in neonates. Based on this, nurses can use foot reflexology before the suctioning procedure for the pain sensation and comfort deterioration that may develop due to the suctioning in the neonates. In-service trainings on CHAs such as reflexology and massage, which can be used in the management of procedural pain in neonates, can be organized for nurses.

Acknowledgements

We thank the neonatal nurse for her assistance in administering the scales.

Declarations

Funding

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

Author contributions

Conceptualization, Ö.Ö.Ş. and N.E.O.; Methodology, Ö.Ö.Ş. and N.E.O; Software, N.E.O.; Validation, N.E.O.; Formal Analysis, Ö.Ö.Ş. and N.E.O. ; Investigation, N.E.O.; Resources, Ö.Ö.Ş and N.E.O.; Data Curation, Ö.Ö.Ş and N.E.O; Writing – Original Draft Preparation, N.E.O.; Writing – Review & Editing, Ö.Ö.Ş.; Visualization, Ö.Ö.Ş.; Supervision, Ö.Ö.Ş.

Conflicts of interest

The authors declare no conflict 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 parents of all neonates were interviewed with information about the study. Verbal and written consent was obtained from the parents who agreed to participate in the study based on volunteering. The study was conducted in accordance with the ethical standards in the Helsinki Declaration of 1975, asrevised in 2008. Ethics approval for the study was taken from Republic of Turkey Ministry of Health Dr. Zekai Tahir Burak Women's Health Education and Research Hospital, the Clinical Research Ethics Committee (Date: 28/02/2017, Decision No. 35/2017), and an intitutional permission was obtained from the Ankara University Faculty of Medicine, Department of Child Health and Diseases (Number: 69545805-806.01.03-H.29765). Scale permissions for NIPS and NCBS uses used in the study were obtained via e-mail.

References

1. Tarjoman A, Vasigh A, Pouy S, Safari S, Borji M. Pain management in neonatal intensive care units: A cross sectional study of neonatal nurses in Ilam City. *J Neonatal Nurs*. 2019;25(3):136-138. doi:10.1016/j.jnn.2018.08.006
2. Orovec A, Disher T, Caddell K, Campbell-Yeo M. Assessment and management of procedural pain during the entire neonatal intensive care unit hospitalization. *Pain Manag Nurs*. 2019;20(5):503-511. doi:10.1016/j.pmn.2018.11.061
3. Blomqvist YT, Gradin M, Olsson E. Pain assessment and management in Swedish neonatal intensive care units. *Pain Manag Nurs*. 2020;21(4):354-359. doi:10.1016/j.pmn.2019.11.001
4. Doesburg SM, Chau CM, Cheung TPL, et al. Neonatal pain-related stress, functional cortical activity and visual-perceptual abilities in school-age children born at extremely low gestational age. *Pain*. 2013;154(10):1946-1952. doi:10.1016/j.pain.2013.04.009
5. Kahraman A, Gümüş M, Akar M, Sipahi M, Bal Yılmaz H, Başbakkal Z. The effects of auditory interventions on pain and comfort in premature newborns in the neonatal intensive care unit; a randomised controlled trial. *Intensive Crit Care Nurs*. 2020;61:102904. doi:10.1016/j.iccn.2020.102904

6. Tauzin M, Durrmeyer X. Managing neonatal pain in the era of non-invasive respiratory support. *Semin Fetal Neonatal Med*. 2019;24(4):101004. doi:10.1016/j.siny.2019.04.004
7. Yavaş S, Bülbül T, Topçu Gavas H. The effect on pain level and comfort of foot massages given by mothers to newborns before heel lancing: Double-blind randomized controlled study. *Jpn J Nurs Sci*. 2021;18(4):e12421. doi:10.1111/jjns.12421
8. Tansky C, Lindberg CE. Breastfeeding as a pain intervention when immunizing infants. *J Nurse Pract*. 2010;6(4):287-295. doi:10.1016/j.nurpra.2009.09.014
9. Lago P, Garetti E, Pirelli A, et al. Non-pharmacological intervention for neonatal pain control. *Ital J Pediatr*. 2014;40(S2):1-2. doi:10.1186/1824-7288-40-s2-a52
10. da Motta G de CP, da Cunha MLC. Prevention and non-pharmacological management of pain in newborns. *Rev Bras Enferm*. 2015;68(1):123-127. doi:10.1590/0034-7167.2015680118p
11. Johnston CC, Fernandes AM, Campbell-Yeo M. Pain in neonates is different. *Pain*. 2011;152(3 Suppl):65-73. doi:10.1016/j.pain.2010.10.008
12. Oğul T, Yılmaz Kurt F. Effect of acupressure on procedural pain before heel lancing in neonates. *J Tradit Chin Med*. 2021;41(02):331-337. doi:10.19852/j.cnki.jtcm.2021.02.011
13. Ustuner Top F, Ekim A, Ozdemir Alkanat H. Use of complementary and alternative medicine in pediatric respiratory diseases. *Holist Nurs Pract*. 2021;35(2):92-97. doi:10.1097/HNP.0000000000000434
14. Samarehfekri A, Dehghan M, Arab M, Ebadzadeh MR. Effect of foot reflexology on pain, fatigue, and quality of sleep after kidney transplantation surgery: A parallel randomized controlled trial. *Evid Based Complement Alternat Med*. 2020;2020:e5095071. doi:10.1155/2020/5095071
15. Karatas N, Isler Dalgic A. Is foot reflexology effective in reducing colic symptoms in infants: A randomized placebo-controlled trial. *Complement Ther Med*. 2021;59:102732. doi:10.1016/j.ctim.2021.102732
16. Bahrami T, Rejeh N, Heravi-Karimooi M, Vaismoradi M, Tadrisi SD, Sieloff CL. Aromatherapy massage versus reflexology on female elderly with acute coronary syndrome. *Nurs Crit Care*. 2018;23(5):229-236. doi:10.1111/nicc.12302
17. Koç T, Gözen D. The effect of foot reflexology on acute pain in infants: A randomized controlled trial. *Worldviews Evid Based Nurs*. 2015;12(5):289-296. doi:10.1111/wvn.12099
18. Basiri Moghadam M, Esmaeili M, Khosravan S, Mojtabavi SJ. Effects of foot reflexology on neonatal jaundice: A randomized sham-controlled trial. *Eur J Integr Med*. 2020;38:101173. doi:10.1016/j.eujim.2020.101173
19. Basiri Moghadam M, Khosravan S, Mojtabavi SJ, Esmaeili M. Effect of foot reflexology on meconium passage in healthy infants. *Complement Med J*. 2016;6(2):1522-1534.
20. Sajadi S, Kazemi M, Bakhtar B, Ostadebrahimi H. Comparing the effects of auricular seed acupressure and foot reflexology on neonatal abstinence syndrome: A modified double blind clinical trial. *Complement Ther Clin Pract*. 2019;36:72-76. doi:10.1016/j.ctcp.2019.06.002
21. Samadi N, Allahyari I, Mazaheri E, et al. Effect of foot reflexology on physiologic index of neonates. *Iran J Neonatol*. 2014;5(1):19-22. doi:10.22038/IJN.2014.2352
22. Yilmaz D, Yilmaz Kurt F. The effect of foot reflexology on procedural pain before heel lancing in neonates. *Arch Pediatr*. 2021;(Available. doi:10.1016/j.arcped.2021.02.015
23. Cong X, Delaney C, Vazquez V. Neonatal nurses' perceptions of pain assessment and management in NICUs: A national survey. *Adv Neonatal Care*. 2013;13(5):353-360. doi:10.1097/ANC.0b013e31829d62e8
24. Ibrahim EM, Shadia, El-Giundy R, Rashad HM, Mebed MH. Effect of foot massage on pain responses to heel stick in preterm infants. *Med J Cairo Univ*. 2016;84(2):25-31.
25. Lawrence J, Alcock D, McGrath P, Kay J, MacMurray SB, Dulberg C. The development of a tool to assess neonatal pain. *Neonatal Netw NN*. 1993;12(6):59-66. doi:10.1016/0885-3924(91)91127-u
26. Akdovan T. Assessment of pain in healthy neonates investigation of the effects of pacifying and holding in the arms (master's thesis). 1999.
27. Ambuel B, Hamlett KW, Marx CM, Blumer JL. Assessing distress in pediatric intensive care environments: The comfort scale. *J Pediatr Psychol*. 1992;17(1):95-109. doi:10.1093/jpepsy/17.1.95
28. Van Dijk M, Roofthooft DWE, Anand KJS, et al. Taking up the challenge of measuring prolonged pain in (premature) neonates the COMFORTneo scale seems promising. *Clin J Pain*. 2009;25(7):607-616. doi:10.1097/AJP.0b013e3181a5b52a
29. Kahraman A, Başbakkal Z, Yalaz M. Turkish validity and reliability of COMFORTneo Scale. *Int Refereed J Nurs Res*. 2014;1(2):1-11. doi:10.17371/uhd.2014210143
30. Jazayeri Z, Sajadi M, Dalvand H, Zolfaghari M. Comparison of the effect of foot reflexology and body massage on physiological indicators and bilirubin levels in neonates under phototherapy. *Complement Ther Med*. 2021;59:102684. doi:10.1016/j.ctim.2021.102684
31. Walker SM. Long-term effects of neonatal pain. *Semin Fetal Neonatal Med*. 2019;24(4):101005. doi:10.1016/j.siny.2019.04.005
32. Wang Y, Li Y, Sun J, et al. Factors influencing the occurrence of neonatal procedural pain. *J Spec Pediatr Nurs*. 2020;25(2):e12281. doi:10.1111/jspn.12281
33. Valeri BO, Linhares MBM. Pain in preterm infants: Effects of sex, gestational age, and neonatal illness severity. *Psychol Neurosci*. 2012;5(1):11-19. doi:10.3922/j.psns.2012.1.03
34. Icke S, Genc R. Effect of reflexology on infantile colic. *J Altern Complement Med*. 2018;24(6):584-588. doi:10.1089/acm.2017.0315

35. Fitri SYR, Wardhani V, Rakhmawati W, Pahria T, Hendrawati S. Culturally based practice in neonatal procedural pain management: A mini review. *Front Pediatr*. 2020;8:540. doi:10.3389/fped.2020.00540
36. Koç Özkan T, Şimşek Küçükkelepçe D, Aydin Özkan S. The effects of acupressure and foot massage on pain during heel lancing in neonates: A randomized controlled trial. *Complement Ther Med*. 2019;46:103-108. doi:10.1016/j.ctim.2019.08.004
37. Küçük Alemdar D, Güdücü Tüfekci F. Effects of maternal heart sounds on pain and comfort during aspiration in preterm infants. *Jpn J Nurs Sci*. 2018;15(4):330-339. doi:10.1111/jjns.12202
38. Gao H, Xu G, Gao H, et al. Effect of repeated Kangaroo Mother Care on repeated procedural pain in preterm infants: A randomized controlled trial. *Int J Nurs Stud*. 2015;52(7):1157-1165. doi:10.1016/j.ijnurstu.2015.04.006

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.6

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 176–184

ORIGINAL PAPER

Zeynep Aközlü [1], Özlem Öztürk Şahin [2]

The effects of mother's voice and white noise on APGAR scores of newborns and attachment processes – a randomized controlled trial

[1] Medical Services and Techniques Department, Vocational School, Maltepe University, Istanbul, Turkey
[2] Department of Pediatric Nursing, Faculty of Health Sciences, Karabük University, Karabük, Turkey

ABSTRACT

Introduction and aim. The present study aims to determine the effects of mother's voice and white noise on newborns' APGAR scores and attachment processes.

Material and methods. The current study was a randomized controlled trial and concluded with 87 newborns and their mothers who had given elective cesarean section (mother voice=29; white noise=28; and control group=30). The mother voice and white noise groups were exposed to recordings, and the broadcast continued for five minutes. The APGAR scores and attachment indicators of newborns (eye contact, rooting, and latch-on) of all groups were examined by the Newborn Attachment Indicators Observation Form.

Results. The 1st and 5th minute APGAR scores in control group were lower than mother voice (1st p=0.05; 5th p=0.001) and white noise (1st p=0.015; 5th p=0.002) groups. The rooting ratio was higher in mother voice and white noise than in the control group (p=0.004). The newborns in the control group had lower latching on rates than mother voice and white noise (p=0.002) groups. Both mother voice and white noise positively affected APGAR scores, rooting, and latching. However, only mother voice had a positive effect on all attachment indicators.

Conclusion. Mother voice and white noise listened to by the newborns born with a cesarean section right after birth in their early-period care positively affect APGAR scores; furthermore, mother voice positively affects attachment indicators as first successful sucking time and eye-to-eye contact.

Keywords. APGAR score, attachment, breastfeeding, newborn, white noise

Introduction

When newborns breathe for the first time, various physiological changes commence adapting to the world outside the uterus.[1] The first few minutes after birth are significant in the adaptation processes of mothers and newborns and their relationship with each other.[2] Initiation of mutual gaze, skin-to-skin contact, and breastfeeding in the first minutes after birth is crucial for the cardiopulmonary stabilization of the infant and initiation of mother-infant bonding.[3] Early initiation of the mother-infant relationship facilitates the newborn's adaptation to extrauterine life and accelerates the attachment process.[1] The first 60-90 minutes after birth is suitable for initiating mother-infant interaction.[4] However, the increase in births given with cesarean section (CS) negatively affects mother-infant attachment.[5] The negative effect is particularly noticeable in cesarean deliveries performed under general anesthesia.[6]

Corresponding author: Zeynep Aközlü, e-mail: zeynepakozlu@maltepe.edu.tr

Received: 4.03.2022 / Revised: 18.03.2022 / Accepted: 20.03.2022 / Published: 30.06.2022

Aközlü Z, Şahin ÖÖ. *The effects of mother's voice and white noise on APGAR scores of newborns and attachment processes – a randomized controlled trial.* Eur J Clin Exp Med. 2022;20(2):176–184. doi: 10.15584/ejcem.2022.2.6.

Attachment is defined as attaching in two-way between the baby and the parent; on the contrary, bonding is a one-way emotional bonding of the parents to the baby; it is expected in the first days after birth.[7] In a positive mother-infant interaction, a mother makes eye-to-eye contact with the baby, touches, talks, and smiles. She further wants to breastfeed the baby and holds the baby in her arms except for caring. The baby also responds to the mother by rooting, latch-on, sucking, swallowing, and eye contact.[1]

The first acoustic stimuli for the fetus to be exposed before birth are the mother's voice and the sounds of the mother's heartbeat.[8] In the postpartum period, the mother's voice (MV) is an exclusive line of communication between the mother and the infant, and it is the primary auditory stimulus for newborns.[9] Moreover, in preterm newborns, MV has a positive effect on decreasing apnea and bradycardia attack frequencies, improving sleep quality, stabilizing vital signs, and promoting early discharge from neonatal intensive care units.[10]

White noise (WN) consists of a mixture of various frequencies coming from the environment and is a constant, monotonous noise that covers all disruptive noises coming from the external environment.[11] It is created in a sound laboratory environment by digitally mixing equal amounts of sounds of different frequencies and reducing the unpleasant frequencies in these sounds.[12] Because of its monotonous humming characteristics, WN resembles the noises during fetal stages.[8] The fetus is affected by the mother's heartbeat while in intrauterine life. Hearing these familiar sounds and rhythms once more in the postpartum period has a calming effect on the newborn.[12]

In the literature, postpartum MV is generally used for cardiopulmonary stabilization of preterm newborns.[13–15] WN is mainly used during painful procedures applied to term newborns.[12,16] However, no prior study is found about the effects MV or WN has on newborns' APGAR scores and attachment indicators.

Aim

The current study aims to establish the effects of MV and WN on the APGAR scores and attachment processes of newborns born with elective CS.

The hypotheses of the study were:

H_1. Mother's voice introduced right after birth positively affects APGAR scores.

H_2. White noise introduced right after birth has a positive effect on APGAR scores.

H_3. Mother's voice introduced right after birth positively affects attachment processes.

H_4. White noise introduced right after birth has a positive effect on attachment processes.

Material and methods

The setting, sample size, and randomization

This randomized controlled trial population consisted of women who had given elective CS and their newborns residing at a research and training hospital in Turkey between January and June 2017. In the study, primary assessments of newborns were made in the operating room, where the CS procedure takes place in an adjacent room located inside the operating room. In the hospital where this study took place, the room used for newborn care was located inside the operating room, and it was a 12 square-meter room dedicated to this purpose only. Having a dedicated newborn care room was significant in choosing this hospital for the study.

Literature was taken as a reference to determine the sample size.[17] Additionally, posthoc analysis was used to confirm whether or not a sufficient sample size was reached at the end of the study with G*Power 3.1.9.2 (Heinrich Heine University Düsseldorf, Germany). As a result of the analysis, the effect size was 0.4359, and the power calculated by posthoc analysis as a result of the study conducted with 87 people was 0.95. For posthoc analysis, the minimum power value to be obtained is 0.67. In this case, the power of the study was on an acceptable level.

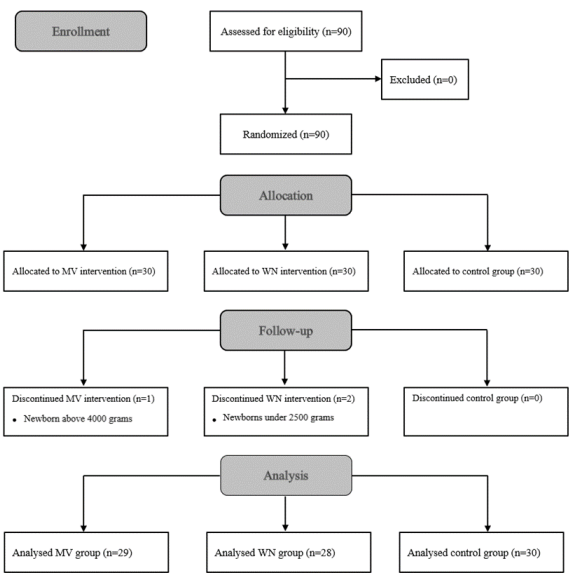

Fig. 1. CONSORT flow diagram of study

Since CS planned the deliveries, the hospitalization of the pregnant women took place the day before the surgery. The mothers were separated into groups using simple randomization (90 sealed envelopes (MV: 30, WN: 30, CG: 30) when they were hospitalized. After the randomization, the Mother Information Form (MIF) was applied to the 90 mothers by the implementer researcher (IR). The inclusion criteria for mothers were determined as having a healthy, single pregnancy, not

taking any medication during pregnancy, not having a background of auditory or speech impediments, having a CS under general anesthesia, and voluntarily taking part in the current study. The inclusion criteria for newborns were obtained as being born at term, being single live birth, having a birth weight between 2500 and 4000 grams, and not having any congenital anomalies. Exclusion criteria were intraoperative complications for mothers and the need for postnatal resuscitation for newborns. The current study was concluded with 87 newborns (MV: 29, WN: 28, CG: 30) in accounts of a newborn in the MV having a birth weight over 4000 grams and two newborns in the WN having a birth weight under 2500 grams (Fig. 1).

Tools

Mother information form (MIF)

The researchers prepared the form, and included questions on the mother's sociodemographic characteristics, number of pregnancies, and prenatal care status. The form included questions on stroking the belly, talking to the fetus, and whether or not the pregnancy was planned, which have evidence of affecting attachment in the literature.[18,19] It also questioned whether or not there was any hearing/speech problem in the mother and whether she had experienced any health problem during her pregnancy.[20] The IR used MIF in the pre-intervention phase of the study.

Newborn information form (NIF)

The researchers prepared the form, and it contained questions on the date-hour of birth and gestational week of the newborn, sex, postpartum anthropometric measurements and, 1st and 5th minute APGAR scores.[21] The IR used NIF in the intervention phase of the study.

Newborn attachment indicators observation form (NAIOF)

The form was prepared by the researchers and used by the OR in the post-intervention phase of the study. Various attachment scales in the literature may be applied before or after delivery.[22] However, these scales investigate the bonding of the mother to the newborn. This form is on the indicators of newborns in the postpartum period as crying, making eye contact, rooting, latch-on breast, starting active sucking, and on which minute after delivery this indicator is displayed.[23,24] The NAIOF recorded the newborns' crying state and at what stages of their interaction with their mother they stopped crying.

Intervention phases

Pre-intervention

The IR conducted face-to-face interviews with the mothers one night before the procedure. MIF was filled for 90 participants who had voluntarily accepted to partake in the current study.

Mother voice group: First, it was explained to each mother in the MV group that the noise they would be broadcasting to their infants would not harm them, and the noise levels would be kept in check with a decibel meter (BENETECH GM1351, Shenzhen, China). Instructions on using the voice recorder (SONY ICD-PX440, Tokyo, Japan) were given by the IR to the mothers in the MV group. A handheld voice recorder, which works with an external battery and can record for up to 72 hours without interruptions, recorded the mothers' voices. A 5-minute improvisational monologue was demanded from the mothers, consisting of the first words they wanted their infants to hear. For the mothers to express their emotions freely, they were left alone in the room. The voice recordings were transferred to the computer (LENOVO G510, Peking, China). Every mother should have her baby listen to the recording. The mothers listened to the audio recordings before the surgery and confirmed to avoid accidents with these recordings. Copies were made onto a compact disc for the mothers who preferred to keep these recordings as a memento.

White noise group: A 5-minute recording prepared by a producer in a digital sound laboratory was used as the WN demo. For the production of the WN, reFX Nexus VSTi v2.4, a sound synthesizer in the FL Studio Producer Edition V12.0.1 Digital Audio Workstation software (Gent, Belgium), was used. By utilizing the White Noise Cut-off filter included in the software, the sharpness of the noise was reduced to -75; thus, it was attempted to acquire a sound similar to womb sounds. Studies on the effects of white noise in the scientific literature were examined to establish that these recordings had melodic tones in them.[11,12,25] The sound recording used in this study was produced exclusively to eliminate melodic tones, acquiring a sound much similar to the womb sounds and not have any problems over royalties. A demo recording of white noise was played (with PHILIPS SPA2201, Amsterdam, Netherlands) to the mothers in the WN group. It was notified that the sound is very similar to the baby's sounds in the womb, and WN they would be broadcasting to their infants would not harm them, and the noise levels would be kept in check with a decibel meter (BENETECH GM1351, Shenzhen, China). LENOVO G510 laptop was used to store the records and broadcast the WN. All newborns in the WN group listened to the same demo. Copies were made onto a compact disc for the mothers who preferred to keep this demo as a memento.

Control group: No specific preliminary preparations were made for the mothers in CG.

All mothers were instructed not to tell the OR which sounds their newborns listened to after they were taken

to the room. The OR was blind to the sound listened to by the newborns while filling out the NAIOF.

When the mothers were taken into the operating room, the preparation of the neonatal care room was achieved by the neonatal nurse accompanied by the IR. Before each birth, equipment and the room cleanliness were checked for malfunctions, test measurements were taken with the decibel meter, and the radiant heater that was used was set constant at 26°C.

Intervention

The newborns were taken to the care room by the neonatal nurse immediately after their birth. The CS in this study took place under general anesthesia; the newborn was brought to the care room immediately after birth without skin-to-skin contact with its mother. As the neonatal care room was next to the operating room where the deliveries took place, it took 3-5 seconds on average to bring the newborns to the neonatal care room. The doors were kept closed to block any noise that could infiltrate the room. Only the neonatal nurse and the IR were present during the procedures in the room. The decibel meter was placed 5 cm above their top lines to assess the noise levels reaching the newborns' ears. Battery operated, handheld decibel meter was used to measure noises between 30-130 dB with a precision of ± 1.5 dB and can take up to 2 measurements every second (BENETECH GM1351, Shenzhen, China).

The newborns in the intervention groups (MV, WN) were exposed to the recordings as soon as they arrived at the room, and the voice broadcast continued for five minutes. For the broadcastings of the sounds, PHILIPS SPA2201 stereo speakers, with analog control for the noise levels and a power indicator, were used. Following the suggestions made by the AAP on the maximum noise levels suitable for newborns, the noise level was limited at 45 dB.[26] The newborns in CG did not receive any auditory interventions and were only exposed to background noise.

During the listening of the sounds to all groups, the newborns' ankle IDs were worn by the newborn nurse; umbilical care was performed, anthropometric measurements were taken, Hepatitis B vaccines and vitamin K were administered (A calibrated SECA354 digital baby scale with an accuracy of ± 5-10 grams was used for weighing newborns. An inflexible tape measure with 0.1 cm intervals was used to measure the head circumference and height of the newborns). At this stage, the same researcher always made the 1st and the 5th minute APGAR score measurements (IR). In contrast, the routine newborn care procedures were always carried out by the same neonatal nurse working at the institution who was not among the researchers of this study. The researcher only kept time and scored the APGAR. The neonatal nurse constantly measures the APGAR score to record it on the hospital's forms. The neonatal nurse and IR wrote down each newborn's APGAR score on separate papers and then showed them simultaneously to avoid bias in APGAR score measurement. All responses showed consistency. Calibrated NELLCOR N560 console type pulse oximeter was utilized to establish the heart rates of the neonates to determine their APGAR scores. The total APGAR scores and information about the newborns were recorded on the NIF.

Post-intervention

As the hospital where the study was carried out is a baby-friendly hospital, although deliveries occur by CS, it is ensured that the newborns and mothers meet within the first 30 minutes. When the mothers are taken from the operating room to the recovery room, they are monitored for 15 minutes and then taken to their rooms. It takes 10 minutes on average to take the mother to her bed in her room and apply her post-op care. All mothers had skin-to-skin contact with their babies within 30 minutes. At this moment, in the neonatal care room, the first examination of the newborn is made by a pediatric doctor. As soon as the mothers left the recovery room and were brought into the private rooms, the OR started applying the NAIOF. The mothers' postpartum readiness to receive their newborns was assessed by obstetric nurses, such as not having pains and stable conditions. According to the Delivery Room Management Guide of the Turkish Neonatal Society, only newborns with the 1st and 5th minute APGAR scores of 7-10 are those that do not require further monitoring and can be given to their mothers.[27] Obstetric nurses informed the neonatal nurse that the mother was ready to receive the newborn, and the newborns were then carted into their mothers' rooms by the neonatal nurse in a wheeled baby cart. The newborns were given to their mothers in a cradling position. The mothers were encouraged about the initialization of breastfeeding to start the attachment. Neonatal nurses carried out these procedures, whereas the OR continued to observe. The time elapsed for breastfeeding to be initiated by the newborns was calculated following the scientific literature. Effective breastfeeding was described as one where a mother can hear her infant swallow and feel her infant's temporalis muscle movements.[28] While the temporalis muscle movements of the newborns were being observed, the mothers were instructed to alert the OR when they heard their infants swallow. Thus, the exact time that elapsed for breastfeeding to begin was recorded in a controlled manner. The mothers were also instructed to alert the OR when they made eye contact with their infants. As newborns' initial period of reactivity lasts for about 60 minutes on average, the OR completed the observations and left the room on the 90th minute.[29]

Data analysis

The IBM SPSS Statistics 22 (IBM Corp, Armonk, New York, USA) was used to evaluate the data. The level of statistical significance was set at $p<0.05$. The descriptive results are presented with the person count, percentage, standard deviation, and mean values. Comparisons of the discrete variables were carried out with Chi-squared Test, and the normality assessments of the distribution for the continuous variables were conducted using Shapiro-Wilk Test. In the comparison between more than two groups, when the distribution of the variables was normal, One-Way ANOVA, when the distribution was abnormal, Kruskal-Wallis H test was utilized. In order to assess the normality of the dependent variable distributions (APGAR score), Kolmogorov-Smirnov and Shapiro Wilk tests were utilized. The variable was established to show a normal distribution. A newborn in the MV group and two newborns in the WN group were excluded from data analysis because of incomplete and missing data.

Results

A total of 87 newborns and mothers who met the criteria participated in the current study (MV: 29, WN: 28, CG: 30). There was no significant difference among the three groups when the mothers and their newborns in the study were analyzed based on their descriptive characteristics (Table 1).

When the 1st minute APGAR scores of the newborns were compared to each other, the difference between the mean scores was statistically significant (p=0.009). The scores in CG were lower than those in the MV (Z=-2.836; p=0.05) and WN groups (Z=-2.432; p=0.015). In comparing the 5th minute APGAR scores of the newborns, the difference between the mean scores of the groups was statistically significant (p=0.001). The scores in CG were lower than those in the MV (Z=-3.363; p=0.001) and WN groups (Z=-3.080; p=0.002). The 1st and 5th minute APGAR scores of the MV group were higher than those in the WN group. However, the difference was not statistically significant in both cases (p>0.05) (Table 2). These findings support the current study's first and second hypotheses (H_1-H_2).

There was no significant difference among the groups concerning the crying status of the newborns when they were first brought into the room (p=0.442) and the crying statuses of the same newborns after they were cradled by their mothers (p=0.109). Every newborn in each group was cradled and breastfed by their mother, establishing eye contact. The rooting ratio was higher in the MV and the WN groups than the newborns in CG (p=0.004). The newborns in CG had lower latching on rates than the MV and WN groups (p=0.002) (Table 3).

In the duration of attachment indicators of the newborns' comparison, in the mean times of starting to suck and having eye contact, CG had the highest mean scores, while MV had the lowest ones. These mean scores of CG were also higher than those in WN; however, the differences were not significant in any of the three parameters (p>0.05). A statistically significant difference was determined in the elapsed mean time between the groups for the first sucking to occur in the newborns (p=0.002). CG in comparison to the MV group (Bonferroni Mean Difference (BMD)=-6.174; p=0.001) and the WN group in comparison to the MV group (BMD=-4.350; p=0.041) had higher scores. A statistically significant difference was determined between the groups when examining the elapsed meantime for eye contact between the mothers and their newborns (p=0.002). CG in comparison to the MV group (BMD=-3.440; p=0.001) and the WN

Table 1. Descriptive characteristics of newborns and mothers

Variables	CG (*n*=30) Mean ± SD n	%	MV (*n*=29) Mean ± SD n	%	WN (*n*=28) Mean ± SD n	%	Total (*N*=87) Mean ± SD n	%	*Test value*	*p*
Gestational age	38.77 ± 0.68		38.69 ± 0.71		38.86 ± 0.76		38.77 ± 0.71		0.859	0.651[b]
Height (cm)	50.3 ± 1.15		49.93 ± 1.12		49.25 ± 1.56		49.84 ± 1.38		1.804	0.17[b]
Weight (g)	3296.33 ± 346.34		3288.62 ± 301.51		3252.68 ± 366.38		3279.71 ± 346.35		0.479	0.787[b]
Head circumference (cm)	35.07 ± .58		35.59 ± 1.05		35.36 ± 0.99		35.33 ± 0.91		3.943	0.139[b]
Mothers' age	29.78 ± 5.64		29.69 ± 4.38		30.5 ± 3.83		29.98 ± 4.66		0.252	0.778[c]
Pregnancy status										
Planned	25	83.3	27	93.1	21	75	73	83.9	3.688	0.158[a]
Unplanned	5	16.7	2	6.9	7	25	14	16.1		
Sex										
Female	15	50	14	48.3	14	50	43	49.4	0.023	0.989[a]
Male	15	50	15	51.7	14	50	44	50.6		
Talking with fetus and stroking belly										
Yes	24	80	22	75.9	23	82.1	69	79.3	0.356	0.837[a]
No	6	20	7	24.1	5	17.9	18	20.7		

Abbreviations: SD – Standard deviation; a – Chi-squared test; b – Kruskal-Wallis H test; c – One-Way ANOVA test

group in comparison to the MV group (BMD=-2.384; p=0.017) had higher scores (Table 4). These findings support the third hypothesis (H_3) and reject the study's fourth hypothesis (H_4).

Discussion

In the present study, only the 1st and 5th minute APGAR scores were checked, and all the newborns had higher scores than 7. The 1st minute and the 5th minute mean APGAR scores were significantly higher with the newborns exposed to their MV or WN than the CG. There was no significant difference between them when it was checked which MV and WN had more positive effects on the APGAR scores. Although there are some studies in the literature examining the effects of MV and WN on newborns, the current study is the first to examine the effects of these two sounds on the APGAR score. Still, preterm newborns who were not exposed to their MV had significantly lower heartbeat rate than those exposed to their MV.[30] Sajjadian et al. reported in a study conducted with newborns that the ones exposed to their MV had a more stable pulse reading, respiration, and oxygen saturation than the control.[15] In these two studies, MV positively affected the APGAR parameters of pulse and respiration, which was in agreement with the present study. Further, considering that oxygen satu-

Table 2. Comparison of APGAR scores of newborns

APGAR scores	CG (*n*=30) Mean ± SD (Min-Max)	MV (*n*=29) Mean ± SD (Min-Max)	WN (*n*=28) Mean ± SD (Min-Max)	Total (*N*=87) Mean ± SD (Min-Max)	*Test value*	*p*	*difference*
1st min	7.7 ± 0.6 (7-9)	8.17 ± 0.6 (7-9)	8.14 ± 0.71 (7-9)	8 ± 0.67 (7-9)	9.355	0.009[a*]	CG < MV CG < WN
5th min	8.87 ± 0.68 (8-10)	9.48 ± 0.57 (8-10)	9.43 ± 0.57 (8-10)	9.25 ± 0.67 (8-10)	14.331	0.001[a*]	CG < MV CG < WN

Abbreviations: SD – Standard deviation; a – Kruskal-Wallis H test; * = p<0.05

Table 3. Comparison of attachment indicators of newborns

Variables	CG (*n*=30) n	%	MV (*n*=29) n	%	WN (*n*=28) n	%	Total (*N*=87) n	%	*Test value*	*p*
Crying status of newborns when they were first brought into the room										
Crying	9	30	13	44.8	9	32.1	31	35.6	1.633	0.442[a]
Not crying	21	70	16	55.2	19	67.9	56	64.4		
Crying status of newborns who continue to cry when brought to the room after cradled by their mother (*n*=31)**										
Still crying	5	55.6	2	15.4	4	44.4	11	35.5	4.431	0.109[a]
Not crying	4	44.4	11	84.6	5	55.6	20	64.5		
Rooting										
Yes	13	43.3	23	79.3	22	78.6	58	66.7	11.222	0.004[a*]
No	17	56.7	6	20.7	6	21.4	29	33.3		
Latch-on										
Yes	6	20	17	58.6	17	60.7	40	46	12.465	0.002[a*]
No	24	80	12	41.4	11	39.3	47	54		

Abbreviations: a – Chi-squared test; * = p<0.05; ** Newborns that did not cry when brought into the room were not included in the analysis

Table 4. Comparison of attachment indicator durations of newborns

Variables	CG (*n* = 30) Mean ± SD	MV (*n* = 29) Mean ± SD	WN (*n* = 28) Mean ± SD	Total (*N* = 87) Mean ± SD	*Test value*	*p*	*difference*
Elapsed time for first sucking (minute)	66.97 ± 5.95	60.79 ± 7.5	65.14 ± 6	64.32 ± 6.96	6.937	0.002[a*]	CG > MV WN > MV
Elapsed time for first eye contact with mother (minute)	79.80 ± 8.31	71.24 ± 9.65	77.25 ± 9.24	76.13 ± 9.67	12.605	0.002[b*]	CG > MV WN > MV

Abbreviations: SD – Standard deviation; a – One-Way ANOVA test; b – Kruskal Wallis test; * = p<0.05

ration is directly related to the blood circulation in the body.[31] It may be stated that MV could positively affect appearance, which is another APGAR parameter. The current study agreed with the results reported by Sajjadian et al. in this matter.[15]

In the early postpartum period, keeping the baby in the same room with the mother, skin-to-skin contact, eye contact, and breastfeeding are essential factors that lead to mother-infant attachment.[32] Nonetheless, CS is the potential for delays in establishing breastfeeding and skin-to-skin contact.[5] In a report published by WHO, it is noted that Turkey is among the first five countries where more CS is performed than normal birth all over the world.[33] In the present study, since the study was conducted was a baby-friendly hospital, skin-to-skin contact was initiated in all newborns within 30 minutes and started sucking in the first hour on average. In two other studies, breastfeeding rates in CS were significantly lower in the first 24 hours.[34,35] In the present study, breastfeeding initiation in the first 24 hours was a positive result. The early start of breastfeeding in the study in all groups may explain why the institution that carried out the study had received the Baby-Friendly Hospital Certificate from the Turkish Ministry of Health. New mothers were provided support to breastfeeding their infants in the first hour by neonatal nurses.

Breastfeeding is a parenting factor that has been associated with the attachment of the infant and the mother. There is an enduring link between breastfed babies and infant secure attachment.[36] In the present study, the newborns that listened to MV started sucking in a shorter time than the WN and CG. The result showed that MV provided to newborns with CS might positively affect breastfeeding, which was a significant parameter of the attachment process. Akca and Aytekin found the sucking success of newborns that listened to WN higher than those that did not.[25] In the current study, although higher rates of rooting and latch-on were determined in the newborns that listened to WN and MV compared to the CG, the difference between WN and CG in starting sucking was not significant.

The time to make eye contact, which is a crucial component of the attachment process, was evaluated in the study. It was observed that initiation of eye contact took more than an hour for every newborn in each group. However, making eye contact after birth was not more than 80 minutes. A study conducted with preterm newborns portrayed that eye contact behavior was rarely found since most newborns were in a sleep state that hindered their mother's ability to make eye contact.[37] Upon delivery, the newborn stays awake for approximately two hours. The eyes are wide open with usually large pupils.[38]

Moreover, the sensitive period of 60 to 90 minutes after birth may be crucial for mothers to contact their infants for infant-mother attachment to occur.[4] In the present study, the onset of eye contact in less than 90 minutes for all groups may be interpreted as a positive result for the attachment process in line with the literature. Besides, the study also has established that the exposed to MV are faster in initiating eye contact than the other two groups. Newborns who stay with their mothers and hear her voice early are more inclined to initiate eye contact. The eye contact of newborns with their mothers allows mothers to develop positive motherhood feelings and behaviors towards their newborns; hence, the mother-infant attachment process is supported.[1] Accordingly, the exposure of newborns to the MV positively affects the attachment process in the current study.

Study limitations

The current study had some limitations. The IR was not blinded when measuring the APGAR score since she had to be exposed to sounds played to newborns. Since newborns were term, the APGAR score and the newborns' responses to sounds may have been positively affected. The generalizability of the findings in this study was limited to term newborns. Further, the generalization of the study was limited because of the small sample size.

Conclusion

In line with the current study's findings, both sounds positively have affected the APGAR scores. However, when one of the sounds is more effective in increasing the APGAR scores are questioned, there is no difference between mother voice and white noise. In examining the effects mother voice and white noise played on newborns on the attachment process, positive effects of both sounds on the newborn's rooting and latch-on were found. Only mother voice positively affects attachment indicators as first successful sucking time and eye-to-eye contact. In line with these results, in countries like Turkey, where there is an increase in surgical deliveries, listening to the mother voice to the newborns immediately after CS can be used as a breastfeeding and attachment-supportive practice.

Acknowledgments

We would like to thank the producer C.A. who contributed to the preparation of the white noise demo used in the study. We would like to thank the mothers and newborns for participation in the study.

Declarations

Funding

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

Author contributions

Conceptualization, Z.A. and ÖÖŞ; Methodology, Z.A. and Ö.Ö.Ş.; Formal Analysis, Z.A. and Ö.Ö.Ş.; Investi-

gation, Z.A.; Resources, Z.A.; Writing – Original Draft Preparation, ZA and Ö.Ö.Ş.; Writing – Review & Editing, Z.A. and Ö.Ö.Ş.; Visualization, Z.A.; Supervision, Ö.Ö.Ş.

Conflicts of interest

The authors declare no conflict 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 present study protocol was carried out following the Helsinki Declaration of 1975 as revised in 2008. Ethics approval for the study was taken from Bülent Ecevit University Clinical Research Ethics Committee (Protocol no: 2015-93-21/10). The clinical research ethics committee, whose permission was obtained before the study, reviewed and approved the study's design and flow charts, informed consent forms, and data collection forms. After that - with the permission of the clinical research ethics committee - permission of the intervention was obtained from the Turkish Ministry of Health, Karabük Public Hospitals Association General Secretariat (88919140-663-08-08-E.293) and Karabük University Training and Research Hospital (34771223-663.08/00480), where the research conducted. It was explained to the participants that any information would be kept confidential, would only be used for scientific purposes, and would be notified about the study results. Participants were informed that they could leave the study at any stage. Written and verbal consents were taken from the participants who had volunteered to partake in the current study.

References

1. Widström AM, Brimdyr K, Svensson K, Cadwell K, Nissen E. Skin-to-skin contact the first hour after birth, underlying implications and clinical practice. *Acta Paediatr*. 2019;108(7):1192-1204. doi:10.1111/apa.14754
2. Phuma-Ngaiyaye E, Welcome Kalembo F. Supporting mothers to bond with their newborn babies: Strategies used in a neonatal intensive care unit at a tertiary hospital in Malawi. *Int J Nurs Sci*. 2016;3(4):362-366. doi:10.1016/j.ijnss.2016.10.001
3. Zaichkin J, Fraser D. The Healthy Newborn. In: Evans RJ, Brown YM, Evans MK, eds. *Canadian Maternity, Newborn & Women's Health Nursing*. 2st ed. Lippincott Williams&Wilkins; 2014.
4. Mutlu C, Yorbik O, Tanju I, Celikel F, Sezer R. Association of prenatal, natal, and postnatal factors with maternal attachment. *Anatol J Psychiatry*. 2015;16(6):440. doi:10.5455/apd.172669
5. Cetisli NE, Arkan G, Top ED. Maternal attachment and breastfeeding behaviors according to type of delivery in the immediate postpartum period. *Rev Assoc Med Bras*. 2018;64(2):164-169. doi:10.1590/1806-9282.64.02.164
6. Sak S, Peker N, Uyanıkoğlu H, Binici O, İncebıyık A, Sak ME. Which should be performed; General or spinal anesthesia in elective cesarean section? *Zeynep Kamil Tip Bül*. 2018;49(1). doi:10.16948/zktipb.348924
7. Bowlby J. Attachment and loss: retrospect and prospect. *Am J Orthopsychiatry*. 1982;52(4):664-678. doi:10.1111/j.1939-0025.1982.tb01456.x
8. Webb AR, Heller HT, Benson CB, Lahav A. Mother's voice and heartbeat sounds elicit auditory plasticity in the human brain before full gestation. *Proc Natl Acad Sci USA*. 2015;112(10):3152-3157. doi:10.1073/pnas.1414924112
9. Persico G, Antolini L, Vergani P, Costantini W, Nardi MT, Bellotti L. Maternal singing of lullabies during pregnancy and after birth: Effects on mother–infant bonding and on newborns' behaviour. Concurrent Cohort Study. *Women Birth*. 2017;30(4):e214-e220. doi:10.1016/j.wombi.2017.01.007
10. Provenzi L, Broso S, Montirosso R. Do mothers sound good? A systematic review of the effects of maternal voice exposure on preterm infants' development. *Neurosci Biobehav Rev*. 2018;88:42-50. doi:10.1016/j.neubiorev.2018.03.009
11. Karakoç A, Türker F. Effects of white noise and holding on pain perception in newborns. *Pain Manag Nurs*. 2014;15(4):864-870. doi:10.1016/j.pmn.2014.01.002
12. Kucukoglu S, Aytekin A, Celebioglu A, Celebi A, Caner I, Maden R. Effect of white noise in relieving vaccination pain in premature infants. *Pain Manag Nurs*. 2016;17(6):392-400. doi:10.1016/j.pmn.2016.08.006
13. Filippa M, Panza C, Ferrari F, et al. Systematic review of maternal voice interventions demonstrates increased stability in preterm infants. *Acta Paediatr*. 2017;106(8):1220-1229. doi:10.1111/apa.13832
14. Lee H, White-Traut R. Physiologic responses of preterm infants to the male and female voice in the NICU. *J Pediatr Nurs*. 2014;29(1):e3-5. doi:10.1016/j.pedn.2013.04.007
15. Sajjadian N, Mohammadzadeh M, Alizadeh Taheri P, Shariat M. Positive effects of low intensity recorded maternal voice on physiologic reactions in premature infants. *Infant Behav Dev*. 2017;46:59-66. doi:10.1016/j.infbeh.2016.11.009
16. Karakoc A, Turker F. Effects of white noise and holding on pain perception in newborns. *Pain Manag Nurs*. 2014;15(4):864-870.
17. Robiquet P, Zamiara PE, Rakza T, et al. Observation of skin-to-skin contact and analysis of factors linked to failure to breastfeed within 2 hours after birth. *Breastfeed Med*. 2016;11(3):126-132. doi:10.1089/bfm.2015.0160
18. Maddahi MS, Dolatian M, Khoramabadi M, Talebi A. Correlation of maternal-fetal attachment and health practices during pregnancy with neonatal outcomes. *Electron Physician*. 2016;8(7):2639-2644. doi:10.19082/2639
19. Nishikawa M, Sakakibara H. Effect of nursing intervention program using abdominal palpation of Leopold's

maneuvers on maternal-fetal attachment. *Reprod Health*. 2013;10(1):12. doi:10.1186/1742-4755-10-12

20. Korver AMH, Smith RJH, Van Camp G, et al. Congenital hearing loss. *Nat Rev Dis Primer*. 2017;3. doi:10.1038/nrdp.2016.94
21. Apgar V. A proposal for a new method of evaluation of the newborn infant. *Anesth Analg*. 1953;32(4):250-259. doi:10.1213/ANE.0b013e31829bdc5c
22. Perrelli JGA, Zambaldi CF, Cantilino A, Sougey EB. Mother-child bonding assessment tools. *Rev Paul Pediatr*. 2014;32(3):257-265. doi:10.1590/0103-0582201432318
23. Als H. The Newborn Communicates. *J Commun*. 1977;27(2):66-73. doi:10.1111/j.1460-2466.1977.tb01828.x
24. Essa RM, Abdel Aziz Ismail NI. Effect of early maternal/newborn skin-to-skin contact after birth on the duration of third stage of labor and initiation of breastfeeding. *J Nurs Educ Pract*. 2015;5(4):98. doi:10.5430/jnep.v5n4p98
25. Akca K, Aytekin A. Effect of soothing noise on sucking success of newborns. *Breastfeed Med Off J Acad Breastfeed Med*. 2014;9(10):538-542. doi:10.1089/bfm.2014.0131
26. Parra J, de Suremain A, Berne Audeoud F, Ego A, Debillon T. Sound levels in a neonatal intensive care unit significantly exceeded recommendations, especially inside incubators. *Acta Paediatr*. 2017;106(12):1909-1914. doi:10.1111/apa.13906
27. Oygür N, Önal E, Zenciroğlu A. *Delivery Room Management Guide*. Turkish Neonatal Society; 2016. http://neonatology.org.tr/wp-content/uploads/2016/12/dogum_odasi_yonetimi.pdf Accessed: 2021-01-21
28. França EC, Sousa CB, Aragão LC, Costa LR. Electromyographic analysis of masseter muscle in newborns during suction in breast, bottle or cup feeding. *BMC Pregnancy Childbirth*. 2014;14(1):154. doi:10.1186/1471-2393-14-154
29. Korotchikova I, Stevenson NJ, Livingstone V, Ryan CA, Boylan GB. Sleep–wake cycle of the healthy term newborn infant in the immediate postnatal period. *Clin Neurophysiol*. 2016;127(4):2095-2101. doi:10.1016/j.clinph.2015.12.015
30. Filippa M, Devouche E, Arioni C, Imberty M, Gratier M. Live maternal speech and singing have beneficial effects on hospitalized preterm infants. *Acta Paediatr*. 2013;102(10):1017-1020. doi:10.1111/apa.12356
31. Noori S, Seri I. Evidence-based versus pathophysiology--based approach to diagnosis and treatment of neonatal cardiovascular compromise. *Semin Fetal Neonatal Med*. 2015;20(4):238-245. doi:10.1016/j.siny.2015.03.005
32. Kuguoglu S, Yildiz H, Kurtuncu Tanir M, Demirbag BC. Breastfeeding after a cesarean delivery. In: Salim R, ed. *Cesarean Delivery*. BoD – Books on Demand; 2012:121-160.
33. WHO. Caesarean section rates continue to rise, amid growing inequalities in access. World Health Organization. https://www.who.int/news/item/16-06-2021-caesarean-section-rates-continue-to-rise-amid-growing-inequalities-in-access-who. Published 2021. Accessed June 19, 2021.
34. Alus Tokat M, Serçekuş P, Yenal K, Okumuş H. Early postpartum breast-feeding outcomes and breast-feeding self--efficacy in Turkish mothers undergoing vaginal birth or cesarean birth with different types of anesthesia. *Int J Nurs Knowl*. 2015;26(2):73-79. doi:10.1111/2047-3095.12037
35. Hobbs AJ, Mannion CA, McDonald SW, Brockway M, Tough SC. The impact of caesarean section on breastfeeding initiation, duration and difficulties in the first four months postpartum. *BMC Pregnancy Childbirth*. 2016;16(1):90. doi:10.1186/s12884-016-0876-1
36. Gibbs BG, Forste R, Lybbert E. Breastfeeding, parenting, and infant attachment behaviors. *Matern Child Health J*. 2018;22(4):579-588. doi:10.1007/s10995-018-2427-z
37. Tilokskulchai F, Phatthanasiriwethin S, Vichitsukon K, Serisathien Y. Attachment behaviors in mothers of premature infants: a descriptive study in Thai mothers. *J Perinat Neonatal Nurs*. 2002;16(3):69-83. doi:10.1097/00005237-200212000-00008
38. Lagercrantz H, Changeux JP. The emergence of human consciousness: From fetal to neonatal life. *Pediatr Res*. 2009;65(3):255-260. doi:10.1203/PDR.0b013e3181973b0d

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.7

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 185–193

ORIGINAL PAPER

Dmitriy Nosivets

Intraarticular administration of chondroitin sulfate in experimental osteoarthritis

Oles Honchar Dnipro National University, Dnipro, Ukraine

ABSTRACT

Introduction and aim. Osteoarthritis (OA) is generally a progressive disease that affects synovial joints, resulting in abnormalities to articular cartilage subchondral bone, synovium, and adjacent soft tissues. The purpose of this work was to investigate the specific activity of chondroitin sulfate (CS) in intra-articular and intramuscular administration to laboratory rabbits in experimental OA.

Material and methods. OA was induced in rabbits by a single injection of mono-iodoacetate in knee joint. CS was administered intra-articularly and intramuscularly. The analysis of biochemical markers and macroscopic assessment of rabbit knee joints was performed.

Results. Intramuscular and intra-articular injection of CS reduces the intensity of the degenerative-dystrophic process due to the impact on inflammatory and the activation of anabolic mechanisms. Intra-articular administration of CS leads to a greater increase in the level of factors of bone and cartilage formation and a greater decrease in the levels of factors of the acute phase of inflammation and factors that destroy the cartilage matrix.

Conclusion. Intramuscular administration of CS revealed a lower intensity of destructive changes in the cartilaginous surface of the knee joint, and intramuscular – the absence of cartilage destruction and defects of the cartilaginous surface, which indicates the peculiarity of the topical effect of the CS.

Keywords. chondroitin sulfate, chondroprotection, intra-articular and intramuscular administration, osteoarthritis

Introduction

Osteoarthritis (OA) is traditionally characterized as a heterogeneous group of chronic degenerative-dystrophic, and more recently inflammatory diseases of the joints and spine of various etiologies, but with similar progressive morphological changes of cartilage, subchondral bone, and synovial capsule, which lead to a decrease in the functional activity of patients, the ability to self-care and disability of patients.[1-5]

The social significance of OA is due to the fact that the first signs or manifestations of OA may be at the age of 30, and with age, there is an increase in the incidence of the disease. It is established that the development of OA increases from 2 to 10 times at the age from 30 to 65 years. According to the WHO, 80% of the population aged 50-60 suffer from OA, more than half of them have limitations in daily life activities, and 25% ‑ can not cope with basic daily responsibilities. Everyone under the age of 80 suffers from OA. In addition, patients with OA make up 30% of patients with disabilities due to diseases of the musculoskeletal system.[6,7]

The cause of OA is considered to be a violation of the mechanical load on the joint and the possibility of physiological recovery of hyaline cartilage after this

Corresponding author: Dmitriy Nosivets, e-mail: dsnosivets@ukr.net

Received: 6.03.2022 / Revised: 15.03.2022 / Accepted: 15.03.2022 / Published: 30.06.2022

Nosivets D. *Intraarticular administration of chondroitin sulfate in experimental osteoarthritis*. Eur J Clin Exp Med. 2022;20(2):185–193. doi: 10.15584/ejcem.2022.2.7

load, which leads to the formation of a «vicious circle» associated with the development and maintenance of inflammation in the joint and progressive cartilage destruction.[8-10]

It is known that the main symptom that aggravates the course of OA and causes disability is pain.[1,4,5] To date, the cause of joint pain in OA has not been definitively established.[11,12] It is believed that the answer to the question of the cause of joint pain in OA can be found in the study of molecular biology and biochemistry of OA. From this point of view, OA is a complex process, the reversibility of which depends on the action of the restorative and regenerative processes in the joint by affecting chondrocytes in the direction of anti-inflammatory and chondroprotective action.[13,14]

Treatment of OA is one of the most pressing problems of modern medicine around the world, not only because this pathological condition is very common, characterized by severe course and consequences that lead to disability, but also because of the lack and inconsistency of data on the effectiveness of various measures and drugs. In this aspect, the problem of analgesia and the impact on the structural organization of articular cartilage with the help of drugs of the group Symptomatic Slow-Acting Drugs in Osteoarthritis (SYSADOAs).[15,16]

At present, it is believed that the mechanical properties of articular cartilage depend on the structural organization of the cartilage matrix, namely the interaction of water molecules and macromolecules – collagen, proteoglycans, and non-collagenous proteins.[17,18] The synthesis of matrix macromolecules is determined by the functional activity of chondrocytes, which decreases with age, leading to disruption of the normal ratio of components of articular cartilage and the progress of its degeneration, and disruption of subchondral bone remodeling contributes to the degradation of cartilage tissue. Approaches to conservative treatment with SYSADOAs, PRP-, and SVF-therapy, which are aimed at restoring the cellular composition of cartilage, stimulating the regenerative potential of the joint as a whole, and eliminating the main manifestations of OA, are based on this.[19,20]

Some authors emphasize the importance of the meniscus into the OA knee joint as the function of the meniscus is very important to make the joint surfaces concordant and to better cushion the load during movement and the role of some biomarkers such as lubricin.[21,22]

Aim

The purpose of this work was to investigate the specific activity of chondroitin sulfate (CS) in intra-articular and intramuscular administration to laboratory rabbits in experimental osteoarthritis (OA).

Material and methods

Design

Experimental studies were performed on nonlinear, healthy, adult 16 Chinchilla rabbits (8 males and 8 females) weighing 2.5 kg. The animals were on a standard diet and in standard vivarium conditions (air temperature: 22±2°C, light/dark cycle: 12/12 hours) in accordance with sanitary and hygienic standards and received food and water ad libitum.[23,24]

Ethics approval

The Committee on Bioethics of the Oles Honchar Dnipro National University approved the study protocol and all procedures related to the maintenance of the animals, their humane treatment, and their use in the experiments. These also complied with Good Laboratory Practice requirements and the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes.

Osteoarthritis model

Groups 2, 3, and 4 of rabbits had osteoarthritis induced by mono-iodoacetate (MIA). The MIA osteoarthritis model involved a single MIA injection (3 mg in 50 μL of sterile saline) into the knee joint, as described by Guingamp et al.[25-27]

Experimental groups

After the formation of the experimental model of OA on the 28th day of the experiment, the rabbits were divided into experimental groups as follows:

Group 1: Intact animals (4 rabbits);

Group 2: OA without «treatment» (4 rabbits);

Group 3: OA + intramuscular administration of CS (4 rabbits);

Group 4: OA + intra-articular administration of CS (4 rabbits).

Chondroitin sulfate administration

CS was ARTRIDA® (manufacturer by «Haupt Pharma Livron», France and promotion by Lithuanian pharmaceutical company «Farmlyga»).

CS was used intra-articularly 0.24 ml 1 time in 3 days 5 times on 28, 31, 34, 37, and 40 days of the experiment and intramuscularly – 0.24 ml 1 time per day every other day for 25 days (from 28 days of the experiment to 53 days).

Biochemical parameters

On the 28th day of the experiment (this period corresponds to the maximum period of formation of the experimental model OA), blood was taken from animals of all experimental groups to assess the level of biochemical markers and animals 3 and 4 groups began administration of CS according to the above scheme.

On days 43 and 53 of the experiment (depending on the method of CS administration) blood was taken from animals of groups 3 and 4 to assess the level of biochemical markers.

The test systems of the following markers manufactured by «MyBioSource» (San Diego, CA, USA) and Elabscience Biotechnology Co., Ltd. (Wuhan, China) were used in the study:

1. Markers of cartilage and bone destruction: C-telopeptide of collagen type 2 (CTX-II) and C-telopeptide of collagen of type 1 (CTX-I) reflect the degree of destruction of cartilage and bone tissue, respectively. Relevant markers are found in the serum due to the destruction of cartilage and bone tissue and the breakdown products of collagen types 1 and 2 into the blood.

2. Markers of enzymes that destroy the cartilage matrix: matrix metalloproteinases (MMP) 3, 9, and 13 reflect the «state» of the cartilage matrix.

3. Markers of bone formation: bone alkaline phosphatase (BALPL) reflects the intensity of anabolic processes in bone tissue. Allows assessing the regenerative potential of bone tissue.

4. Biochemical markers of inflammation (acute serum inflammation proteins): C-reactive protein (CRP), interleukins (IL) 1, 6, and 8, tumor necrosis factor-alpha (TNF-α). Reflect the body's overall response to inflammation. Are non-specific markers of inflammation.

Blood samples were taken from the marginal ear vein of rabbits after fasting for 12 hours. Blood samples were left to coagulate for 2 hours at 4°C. Then they were centrifuged at 4000 rpm for 15 min at 4°C to obtain serum. Serum samples were aliquoted in 0.5 ml tubes and stored at -80°C until analysis.

Serum concentrations of cartilage and bone markers, pro-inflammatory/anti-inflammatory cytokines were measured by quantitative solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using rabbit-specific test kits and following the manufacturer's instructions.

The procedure for studying biochemical markers was performed as follows. To each well of the plates was added a standard solution or serum sample (100 μL), the plate was covered and incubated for 2 hours at 37°C. Then the liquid from each well was removed and added a working solution of biotinylated antibody (100 μL), specific to the corresponding marker, the content of which is examined in the serum of rabbits. The plate was covered and incubated for 1 hour at 37°C. The wells were washed three times with wash buffer (350 μL), avidin conjugated with horseradish peroxidase (100 μL) was added to each well, the plate was covered and incubated for 30 minutes at 37°C. The wells were washed five times with wash buffer (350 μL), a tetramethylbenzidine substrate solution (90 μL) was added to each well, and the plate was incubated in the dark for 15-30 minutes at 37°C. Subsequently, a stop solution (50 μL) was added to each well. The optical density of each well was determined using a Rayto RT-2100C microplate reader (Rayto Life and Analytical Sciences Co., Ltd., China) at 450 nm. The concentration of the test marker was calculated by comparing the optical density of each sample with a standard 4-parameter logistics curve constructed using AssayFit Pro 1.31. Each sample was tested twice according to the test system manufacturer's instructions.

Sectioning and macroscopic parameters

All animals were killed accordind to Ethical Approvement by intraperitoneal administration of a thiopental sodium solution (40 mg/kg body weight) and samples of knees were taken.[28] Rabbits of group 4 were killed on day 43, and rabbits of groups 1, 2, and 3 were killed on day 53.

Macroscopic evaluation of Chinchilla rabbit joint tissues was performed using generally accepted standard methods and surgical instruments.

Statistical analysis

Physical parameters are reported as means $\pm$ errors. Depending on the normality of the distribution (as assessed using the Shapiro-Wilk test) and the groups being compared, the Student's t-test, the paired t-test, the Mann-Whitney U-test, or the paired Wilcoxon test were generally used. For knee circumference, a one-way dispersion analysis and Duncan's test were used. The level for significance was taken to be $P<0.05$. Statistical processing was performed using STATISTICA 6.1 software product provided (StatSoft Inc., serial No AGAR909E-415822FA).

Results

Macroscopic parameters

At the research of tissues of a knee joint at animals of group 1, the normal structure of a joint is defined (Fig. 1). Cartilage has a white, bright color. The cartilage surface is shiny, without pathological usurpations and defects. The capsule of the joint is not thickened the usual color. There are no infiltrative and inflammatory phenomena around the cartilaginous formations. Pathological changes in the structure of the knee joint and soft tissues have not been identified.

At the research of tissues of a knee joint at animals of group 2, the expressed pathological changes (Fig. 2) are defined. The cartilage has a yellow, dull color. The cartilage surface is heterogeneous, focal pathological usurpations and defects are determined. The capsule of the joint is thickened, hyperemic, has a pronounced capillary and vascular network. Infiltrative and

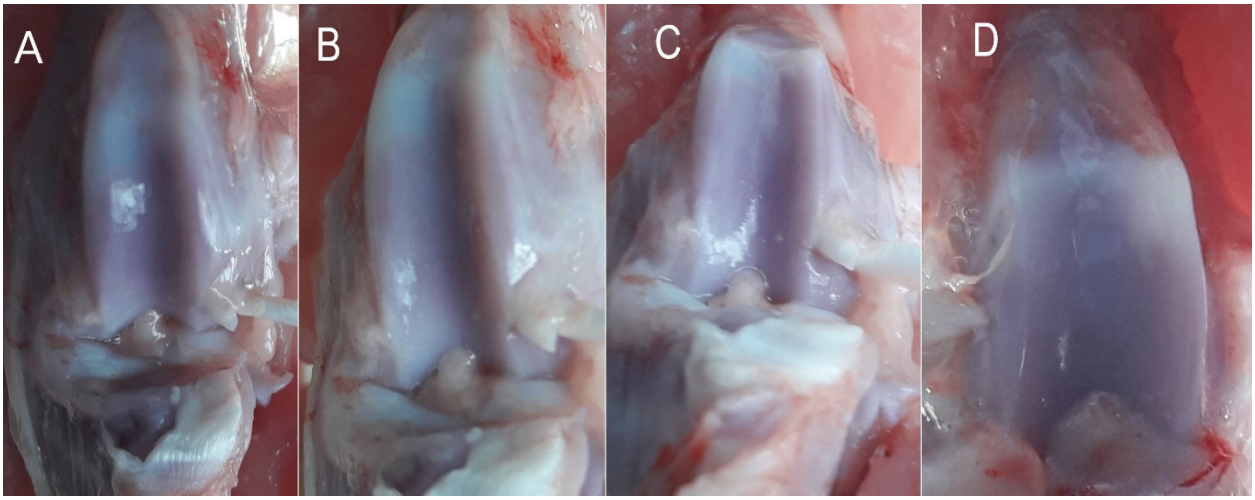

Fig. 1. Macroscopic structure of the tissues of the knee joint of rabbits of group 1 (the distal part of the femur of the rabbit is shown)

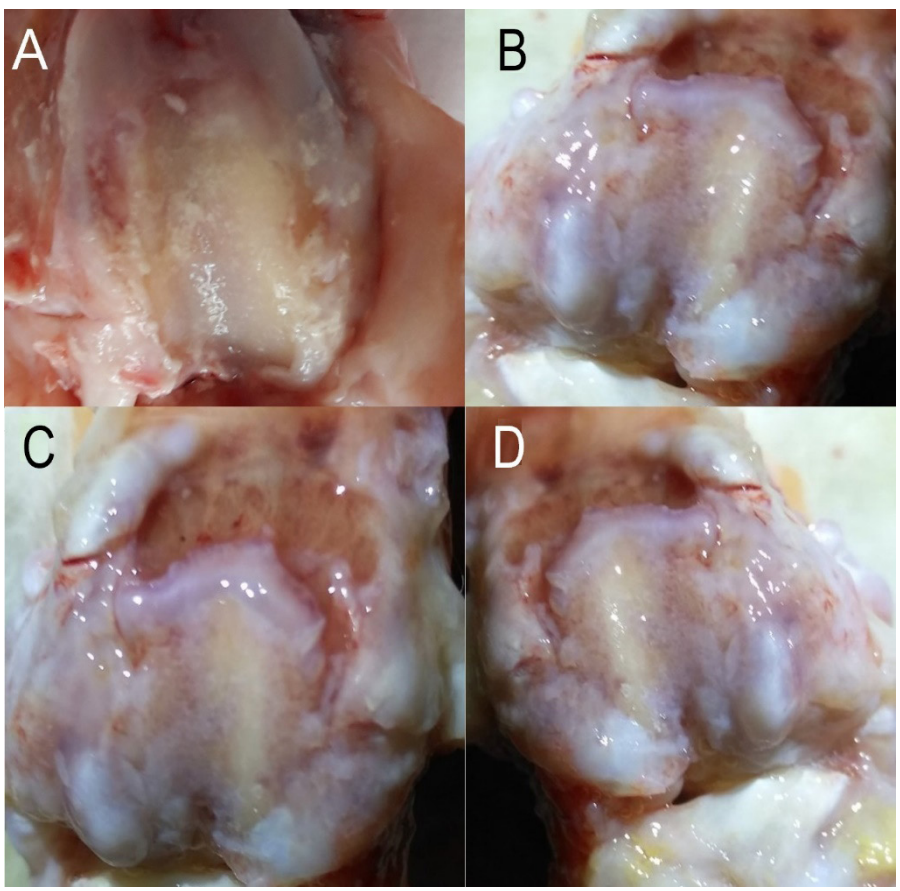

Fig. 2. Macroscopic structure of the tissues of the knee joint of rabbits of group 2 (the distal part of the femur of the rabbit is shown)

inflammatory phenomena around bone and cartilage formations are determined. The identified phenomena confirm the adequacy of the simulation of OA of the knee joint in animals using MIA.

At the research of tissues of a knee joint at animals of groups 3, pathological changes are defined (Fig. 3A-D). The cartilage has a yellow, bright color. In Fig. 3A-B cartilage surface is heterogeneous, individual focal pathological usurpations and defects are identified. In Fig. 3D the cartilage surface is homogeneous, pathological usurpations and defects are not defined. The capsule of the joint is slightly thickened, hyperemic, in Fig. 3A has a pronounced capillary and vascular network. Infiltrative and inflammatory phenomena around the cartilaginous formations are moderate.

At the research of tissues of a knee joint at animals of groups 4, moderate pathological changes (Fig. 4A-D) are defined. The cartilage has a yellow, bright color.

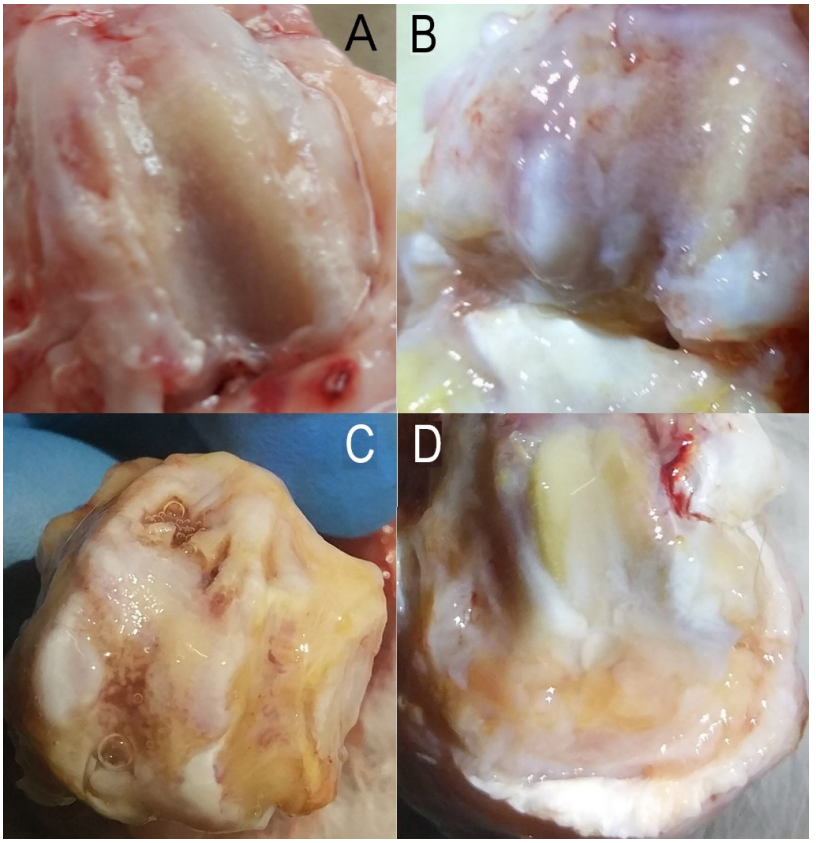

Fig. 3. Macroscopic structure of the tissues of the knee joint of rabbits of group 3 (the distal part of the femur of the rabbit is shown)

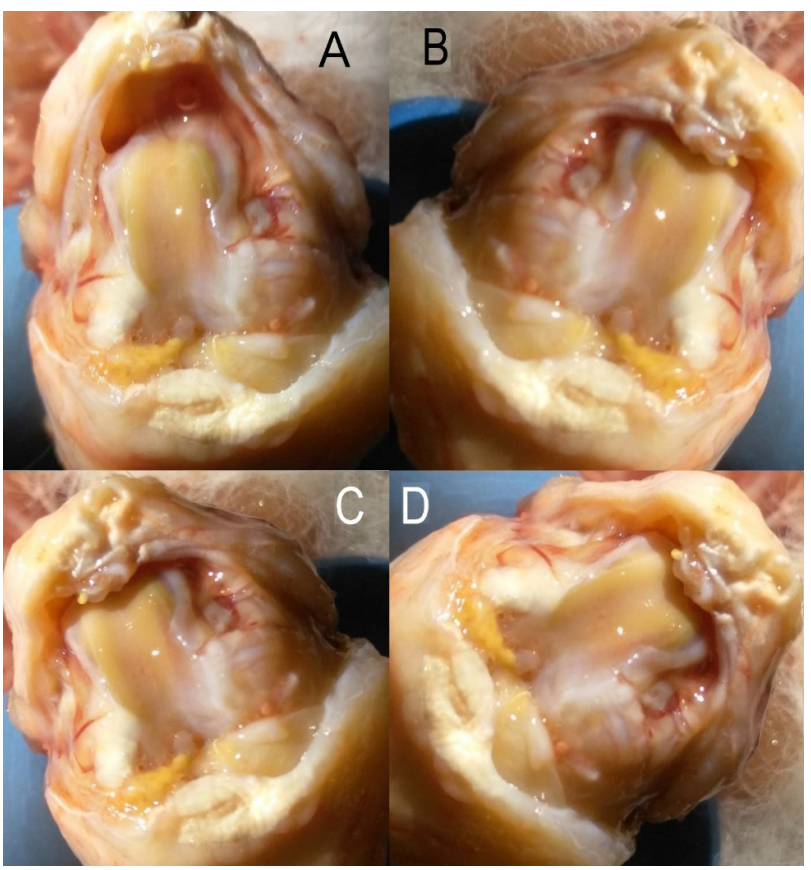

Fig. 4. Macroscopic structure of the tissues of the knee joint of rabbits of group 4 (shown rabbit knee joint intersected along with the joint space)

Table 1. The average values of biochemical parameters in animals of the experimental groups at 28 days (M)

	TNF-α, pg/mL	BALPL, ng/mL	MMP-3, ng/mL	MMP-13, ng/mL	CRP, pg/mL	IL-6, pg/mL	IL-8, pg/mL
Group 1 (n=4)	0	2.66#	**0**	**0**	0	**0**	**0**
Group 2 (n=4)	44.70*	7.87*	3.88*	9.0*	642.50*	68.44*	931.76*
Group 3 (n=4)	41.08*	7.95*	4.16*	10.94*	753.59*	77.86*	968.81*
Group 4 (n=4)	44.35*	7.16*	4.42*	9.69*	581.19*	69.42*	912.64*

*p<0.05 versus Group 1

#p<0.05 versus Group 2

0 – marker is not determined in the blood sample

Table 2. The average values of biochemical parameters in animals of the experimental groups for 43 days (M)

	TNF-α, pg/mL	BALPL, ng/mL	MMP-3, ng/mL	MMP-13, ng/mL	CRP, pg/mL	IL-6, pg/mL	IL-8, pg/mL
Group 1 (n=4)	0	2.63#	**0**	**0**	**0**	**0**	**0**
Group 2 (n=4)	32.45*	7.88*	5.68*	8.90*	590.86*	122.01*	524.35*
Group 3 (n=4)	–	–	–	–	–	–	–
Group 4 (n=4)	15.78*#	14.33*#	2.72*#	4.22*#	110.77*#	35.01#	186.35*#

*p<0.05 versus Group 1

#p<0.05 versus Group 2

0 – marker is not determined in the blood sample

– marker definition was not performed

Table 3. The average values of biochemical parameters in animals of the experimental groups for 53 days (M)

	TNF-α, pg/mL	BALPL, ng/mL	MMP-3, ng/mL	MMP-13, ng/mL	CRP, pg/mL	IL-6, pg/mL	IL-8, pg/mL
Group 1 (n=4)	0	2.07#	**0**	**0**	**0**	**0**	**0**
Group 2 (n=4)	29.62*	6.32*	4.07*	7.78*	754.32*	120.71*	541.11*
Group 3 (n=4)	18.55*	14.11*#	2.93*	4.08*	104.24*#	36.30*#	193.76*#
Group 4 (n=4)	–	–	–	–	–	–	–

*p<0.05 versus Group 1

#p<0.05 versus Group 2

0 – marker is not determined in the blood sample

– marker definition was not performed

The cartilage surface is homogeneous, focal pathological usurpations and defects are not defined. The capsule of the joint is normal, the vascular pattern is not determined. Infiltrative and inflammatory phenomena around the cartilaginous formations are not defined.

Biochemical parameters

The obtained results are presented in Tables 1, 2, and 3. The average values (M) of biochemical parameters on the 28th day of the study in animals of the experimental groups are presented in the Table 1.

The average values (M) of biochemical parameters on the 43rd day of the study in animals of the experimental groups are presented in the Table 2.

The average values (M) of biochemical parameters on the 53rd day of the study in animals of the experimental groups are presented in the Table 3.

Analysis of the results (Table 1-3) suggests that on the 28th day of the experiment in groups 2, 3, and 4 there is an increase in levels of TNF-α, BALPL, MMP-3, MMP-13, CRP, IL-6, and IL- 8 in relation to animals of group 1 (intact), which reflects the activity of the pathological process caused by the action of MIA on cartilage tissue. Levels of markers TNF-α, BALPL, MMP-3, MMP-13, CRP, IL-6, and IL-8 in group 2 animals (without treatment) on 28, 43, and 53 days of the experiment reflect a gradual decrease in the intensity of inflammatory and destructive processes in knee joints of rabbits, which corresponds to the peculiarities of OA in the experimental reproduction of the degenerative-dystrophic process by intra-articular administration of MIA.

On day 43 of the study, high levels of TNF-α, MMP-3, MMP-13, CRP, IL-6, and IL-8 were observed in groups 2 with respect to groups 1 and 4, reflecting the further development of OA. However, in group 4 there is a decrease in TNF-α, MMP-3, MMP-13, CRP, IL-6, and IL-8 relative to group 2, which corresponds to a decrease in the intensity of catabolic and inflammatory processes, and an increase in BALPL indicates a more

pronounced intensity anabolic process in bone and cartilage.

Almost a similar pattern is observed on the 53rd day of the experiment. In group 2, high levels of markers TNF-α, MMP-3, MMP-13, CRP, IL-6, and IL-8 were observed relative to groups 1 and 3, which reflects the further development of OA. At the same time, in group 3 there is a decrease in TNF-α, MMP-3, MMP-13, CRP, IL-6, and IL-8 relative to group 2, which corresponds to a decrease in the intensity of catabolic and inflammatory processes, and an increase in BALPL indicates the more pronounced intensity of anabolic processes in bone and cartilage.

Noteworthy is the change in BALPL levels in group 4 on day 43 of the study and in group 3 on day 53 of the experiment, which reflects the intensity of anabolic processes in the knee joints of rabbits. These values are 14.33 and 14.11 ng/mL, respectively. When comparing the obtained values with the indicators of group 2 (without treatment) there is an increase in the intensity of anabolic processes 1.9 and 2.23 times, respectively (Tables 2 and 3).

Analyzing the dynamics of changes in the levels of markers TNF-α, BALPL, MMP-3, MMP-13, CRP, IL-6, and IL-8 in group 3 (intramuscular administration) and group 4 (intraarticular administration) can be argued that intramuscular and intraarticular administration of the CS leads to a decrease in the intensity of the degenerative-dystrophic process due to the impact on inflammatory phenomena and activation of anabolic mechanisms.

A comparison of the results obtained in the group 3 (intramuscular administration) and the group 4 (intra-articular administration) suggests that intra-articular administration of CS leads to a greater increase in BALPL and a greater decrease in markers TNF-α, MMP-3, IL-6, and IL-8. However, no statistically significant reliability was obtained between these indicators of groups 3 and 4 (Tables 2 and 3).

Discussion

When comparing the results obtained between the experimental groups of animals, a sharp difference in macroscopic changes with group 1 is determined, which indicates the presence of degenerative-dystrophic changes in the knee joints of animals of groups 2, 3, and 4. The most pronounced intensity of pathological changes was determined in animals of group 2, which confirms the adequacy of the performed OA model. When comparing macroscopic changes between groups 2 and 3, a lower intensity of destructive changes in the cartilaginous surface of the knee joint was determined. When comparing macroscopic changes between groups 2 and 4, the absence of pathological usurpation and cartilaginous surface defects was determined.

Thus, based on the obtained results, it can be stated that the intramuscular administration of CS revealed a lower intensity of destructive changes in the cartilaginous surface of the knee joint, and intra-articular – the absence of pathological usurpation and cartilage surface defects, indicating the topical effect of CS. In our opinion, the obtained results may depend on the CS drug used. We have previously confirmed the importance of using a high-quality pharmaceutically pure active main ingredient of CS to ensure optimal efficacy and safety of the final product in patients with osteoarthritis.[29]

In the process of biochemical studies, no indicators of CTX-I, CTX-II, IL-1, and MMP-9 were determined due to the fact that the level of studied markers in blood samples was less than the sensitivity of test systems, which does not contradict the literature and reflects study performed.[30-34] However, low levels of CTX-I and CTX-II indicate the absence of severe destructive changes caused by the introduction of MIA, which corresponds to the initial manifestations of OA and adequate conditions for the appointment of the CS.[35-37]

Limitations

The author did not provide some morphological information and related biomarkers expression through histology, histochemistry, and immunohistochemistry techniques.

Conclusion

To date, there is an open discussion about the appropriateness and effectiveness of CS for OA. There are different opinions on these issues, sometimes of the opposite nature. There is also no consensus in the current literature about the effectiveness of intra-articular injection of CS in OA. In our study, we tried to visualize morphological and biochemical changes during intramuscular and intra-articular administration of CS in experimental OA.

We found that intramuscular administration of CS revealed a lower intensity of destructive changes in the cartilaginous surface of the knee joint, and intra-articular - the absence of pathological usurpation and cartilage surface defects, indicating the topical effect of the CS. Intramuscular and intra-articular administration of CS reduces the intensity of the degenerative-dystrophic process due to the impact on inflammatory phenomena and the activation of anabolic mechanisms. However, intra-articular administration of CS leads to a greater increase in the level of markers of bone and cartilage formation and a greater decrease in the levels of markers of the acute phase of inflammation and markers that destroy the cartilage matrix.

Declarations

Funding

The study was performed within the project «Preclinical study of the specific activity of the drug ARTRIDA® solution for injection, manufactured by Haupt Pharma Livron (France) with intra-articular and intramuscular administration to laboratory rabbits» (customer CJSC «Farmlyga» *Republic of Lithuania*)

Author contributions

Conceptualization, D.N.; Methodology, D.N.; Software, D.N.; Validation, D.N.; Formal Analysis, D.N.; Investigation, D.N.; Resources, D.N.; Data Curation, D.N.; Writing – Original Draft Preparation, D.N.; Writing – Review & Editing, D.N.; Visualization, D.N.; Supervision, D.N.; Project Administration, D.N.; Funding Acquisition, D.N.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

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

Ethics approval

The Committee on Bioethics of the Oles Honchar Dnipro National University approved the study protocol and all procedures related to the maintenance of the animals, their humane treatment, and their use in the experiments. These also complied with Good Laboratory Practice requirements and the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes.

References

1. Barnett R. Osteoarthritis. *Lancet*. 2018;391(10134):1985.
2. Hügle T. Le point sur l'arthrose [Update Osteoarthritis]. *Rev Med Suisse*. 2020;16(685):500-502.
3. Vina ER, Kwoh CK. Epidemiology of osteoarthritis: literature update. *Curr Opin Rheumatol*. 2018;30(2):160-167.
4. Mandl LA. Osteoarthritis year in review 2018: clinical. *Osteoarthritis Cartilage*. 2019;27(3):359-364.
5. Hunter DJ, March L, Chew M. Osteoarthritis in 2020 and beyond: a Lancet Commission. *Lancet*. 2020;396(10264):1711-1712.
6. O'Neill TW, McCabe PS, McBeth J. Update on the epidemiology, risk factors and disease outcomes of osteoarthritis. *Best Pract Res Clin Rheumatol*. 2018;32(2):312-326.
7. Sacitharan PK. Ageing and osteoarthritis. *Subcell Biochem*. 2019;91:123-159.
8. Vincent TL. Mechanoflammation in osteoarthritis pathogenesis. *Semin Arthritis Rheum*. 2019;49(3S):36-38.
9. Mobasheri A, Rayman MP, Gualillo O, Sellam J, van der Kraan P, Fearon U. The role of metabolism in the pathogenesis of osteoarthritis. *Nat Rev Rheumatol*. 2017;13(5):302-311.
10. Astephen Wilson JL, Kobsar D. Osteoarthritis year in review 2020: mechanics. *Osteoarthritis Cartilage*. 2021;29(2):161-169.
11. Malfait AM, Miller RE, Miller RJ. Basic mechanisms of pain in osteoarthritis: experimental observations and new perspectives. *Rheum Dis Clin North Am*. 2021;47(2):165-180.
12. Allen KD, Walsh DA. Modelling pathology: pain relationships in osteoarthritis. *Osteoarthritis Cartilage*. 2021;29(10):1386-1388.
13. Xia B, Di Chen, Zhang J, Hu S, Jin H, Tong P. Osteoarthritis pathogenesis: a review of molecular mechanisms. *Calcif Tissue Int*. 2014;95(6):495-505.
14. Fang T, Zhou X, Jin M, Nie J, Li X. Molecular mechanisms of mechanical load-induced osteoarthritis. *Int Orthop*. 2021;45(5):1125-1136.
15. Mao L, Wu W, Wang M, et al. Targeted treatment for osteoarthritis: drugs and delivery system. *Drug Deliv*. 2021;28(1):1861-1876.
16. Materkowski M. Efficacy Treatment of Osteoarthritis with Combine Chondroitin and Glucosamine. *Ortop Traumatol Rehabil*. 2021;23(3):239-244.
17. Lin J, Wang L, Lin J, Liu Q. The Role of Extracellular Vesicles in the Pathogenesis, Diagnosis, and Treatment of Osteoarthritis. *Molecules*. 2021;26(16):4987.
18. Mahmoudian A, Lohmander LS, Mobasheri A, Englund M, Luyten FP. Early-stage symptomatic osteoarthritis of the knee - time for action. *Nat Rev Rheumatol*. 2021;17(10):621-632.
19. Zhang X, He J, Wang W. Progress in the use of mesenchymal stromal cells for osteoarthritis treatment. *Cytotherapy*. 2021;23(6):459-470.
20. Franklin SP, Stoker AM, Bozynski CC, et al. Comparison of Platelet-Rich Plasma, Stromal Vascular Fraction (SVF), or SVF with an Injectable PLGA Nanofiber Scaffold for the Treatment of Osteochondral Injury in Dogs. *J Knee Surg*. 2018;31(7):686-697.
21. Musumeci G, Carnazza ML, Leonardi R, Loreto C. Expression of β-defensin-4 in "an in vivo and ex vivo model" of human osteoarthritic knee meniscus. *Knee Surg Sports Traumatol Arthrosc*. 2012;20(2):216-222.
22. Musumeci G, Trovato FM, Loreto C, et al. Lubricin expression in human osteoarthritic knee meniscus and synovial fluid: a morphological, immunohistochemical and biochemical study. *Acta Histochem*. 2014;116(5):965-972.
23. Rukovodstvo po provedeniyu doklinicheskih issledovaniy lekarstvennyih sredstv. Chast' 1. Moskva: Grif K. 2012:944.
24. Guidelines for experimental (preclinical) study of new pharmacological substances. Endorsed by corresponding member of RAMS prof. R. U. Khabrieva. 2005:425.
25. Guingamp C, Gegout-Pottie P, Philippe L, Terlain B, Netter P, Gillet P. Mono-iodoacetate-induced experimental osteoarthritis: a dose-response study of loss of mobility,

morphology, and biochemistry. *Arthritis Rheum.* 1997;40: 1670-1679.
26. Nosivets DS. Experimental models of cartilage tissue pathology. *Zaporozhye medical journal.* 2019;4(115):554-560.
27. Guzman RE, Evans MG, Bove S, Morenko B, Kilgore K. Mono-iodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. *Toxicol Pathol.* 2003;31:619-624.
28. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition – https://www.avma.org/sites/default/files/2020-01/2020_Euthanasia_Final_1-15-20.pdf
29. Nosivets D, Montell E, Opryshko V. Histological changes following the administration of two different chondroitin sulfate products in experimental osteoarthritis models in rats. *Eur J Clin Exp Med.* 2021;19(1):23-32.
30. Choi BR, Kang SJ, Kim JL, Lee YJ, Ku SK. Anti-osteoarthritic effects of a mixture of dried pomegranate concentrate powder, eucommiae cortex, and achyranthis radix 5:4:1 (*g*/*g*) in a surgically induced osteoarthritic rabbit model. *Nutrients*. 2020;12(3):852.
31. Oliver RA, Lovric V, Christou C, Walsh WR. Evaluation of comparative soft tissue response to bone void fillers with antibiotics in a rabbit intramuscular model. *J Biomater Appl*. 2019;34(1):117-129.
32. Zhao H, Lu A, He X. Roles of microRNAs in bone destruction of rheumatoid arthritis. *Front Cell Dev Biol*. 2020;8:600867.
33. Heikal MM, Shaaban AA, Elkashef WF, Ibrahim TM. Effect of febuxostat on biochemical parameters of hyperlipidemia induced by a high-fat diet in rabbits. *Can J Physiol Pharmacol*. 2019;97(7):611-622.
34. Mohan N, Mohanan PV, Sabareeswaran A, Nair P. Chitosan-hyaluronic acid hydrogel for cartilage repair. *Int J Biol Macromol*. 2017;104(Pt B):1936-1945.
35. Nosivets DS. Evaluation of the influence of chondroitin sulfate on morphometric parameters of the knee joint, pain threshold and biochemical indices in rats at experimental osteoarthritis. *Ukr J of Medicine Biology and Sport.* 2020;2(24):77-83.
36. Nosivets DS. Bone and cartilage condition in experimental osteoarthritis and hypothyroidism. *Medicinski Glasnik.* 2022;19(1):68-74.
37. Nosivets DS. Changes in the level of interleukin-8 in the blood serum of rats with experimental osteoarthritis and hypothyroidism. *Ukr Biochem J*. 2020;92(6):167-171.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.8

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 194–201

ORIGINAL PAPER

Artur Szymczak [1,2], Anna Ogorzałek [2], Natalia Leksa [1], Anna Sęk-Mastej [1], Stanisław Orkisz [1]

The outcomes in children with Hirschsprung's disease treated with transanal endorectal pull-through method

[1] Institute of Medical Sciences, Medical College, University of Rzeszow, Rzeszow, Poland
[2] Department of Surgery, State Hospital 2 in Rzeszow, Rzeszów, Poland

ABSTRACT

Introduction and aim. The evaluation of functional results, complications and problems of children with Hirschsprung's disease treated with one-stage surgery (TEPT) or two-stage surgery (colostomy, TEPT) in Paediatric Surgery Clinic in Rzeszów.

Material and methods. Medical documentation of 41 children treated due to Hirschsprung's disease in years 2006-2018 in Rzeszów were retrospectively analysed. The results of the questionnaires conducted among the parents of operated children were surveyed.

Results. The average time of the radical surgery was 189 minutes. The mean length of the resected intestine in the classic form was 19 centimeters, in long-segment 35 centimeters. In the post-operative period, 15 patients had a blood transfusion. The mean time of the children's stay calculated from the date of surgery until the discharge equaled 13.4 days. Early post-operative complications: enterocolitis occurred in 6 patients (1 death in a septic shock mechanism), total dehiscence of anastomosis in 1 patient, abscess of perirectal space in 1 patient, anastomotic retraction in 1 patient and in 3 patients inaccurate intra-operative evaluation of the section (intra), buttock dermatitis appeared in all patients.

Late post-operative complications (a control trial of 38 patients): Soiling was confirmed in 9 patients, periodic constipation in one. The abnormal consistency of stool was signaled in 3 children. Two children were repetitively hospitalized due to enterocolitis. The frequency of defecation almost in all patients was reduced after a three, four-month period since the operation from 10-15 per day to the age norm. In one child, where the retraction of the anastomosis was diagnosed, the soiling and heightened frequency of defecation throughout the day persists. All of the parents of the older children view the outcome of the surgery as positive and the life quality of their children does not differ from their peers.

Conclusion. TEPT is a method which can be performed in newborns, infants, babies as well as in case of a long-segment aganglionosis. Barium enema is not reliable in evaluation of the length of the aganglionic section in the long-segment type of Hirschsprung's disease. The treatment of choice in the early post-operative enterocolitis should be colostomy.

Worse functional results were observed in children after two-stage treatment – especially, in cases where the colostomy was created due to the intestinal re-distention.

Keywords. aganglionosis, constipation, encopresis, soiling

Corresponding author: Artur Szymczak, e-mail: artszymczak@poczta.onet.pl

Received: 20.12.2021 / Revised: 20.03.2022 / Accepted: 6.04.2022 / Published: 30.06.2022

Szymczak A, Ogorzałek A, Leksa N, Sęk-Mastej A. Orkisz S. *The outcomes in children with Hirschsprung's disease treated with transanal endorectal pull-through method.* Eur J Clin Exp Med. 2022;20(2): 194–201. doi: 10.15584/ejcem.2022.2.8.

Introduction

Hirschsprung's disease is a congenital disorder caused by the lack of autonomic intramural ganglia of submucosal plexus (Meissner) and myenteric plexus (Auerbach) of the intestinal wall as a result of which the intestine lacking the proper innervation is in a state of constant spasm creating an obstruction in the passage of the food content. This state is accompanied by the constant and excessive pressure of the internal anal sphincter (achalasia). As a result of the excessive effort, the intestine above the obstruction imitatively becomes dilated.

Fig. 1. Abdominal distension – a typical symptom of Hirschsprung's disease

In 1888 Harald Hirschsprung was the first to present the description of the disease symptoms, observed in two unrelated boys who died from chronic constipation. As a cause of the disease, he mistakenly diagnosed congenital megacolon.[1] Hirschsprung's disease has a multi-factor genetic background, caused by the mutation of genes: RET, ECE1, EDNRB, GDNF, SOX10, PHOX2B, KIAA1279. The most common of them is the mutation inactivating RET proto-oncogene, responsible approximately for 50% of family and 15% of occasional cases of the disorder's prevalence. The signal transduction gene-dependant becomes dysfunctional which leads to faulty migration of neural crest cells along the digestive tract wall toward the caudal. Dysfunctions appear between sixth and twelfth week of gestational age, while the time of activity of migration inhibitory factor determines the length of the aganglionic section.[2-5] According to EUROCAT data, the incidence of Hirschsprung's disease is 1,3 in 10 000 live births. More often it concerns the males (4:1). It can appear in the isolated form (70%) or in a syndromic one. It may be associated with Down syndrome or Shah-Waanderburg syndrome.[6,7] According to data published by Anderson et al., who analyzed the population of 9 million children born in California in 1995-2013, it can be clearly concluded that the scope of incidence of Hirschsprung's disease amounted to 2.2 cases in 10 000 live births with the majority of Afro-Americans and Mongoloid race.[8-10]

The classification of the disease type is based on the length of the aganglionic section. In 75% the aganglionosis relates to rectosigmoid segment – the classic type of the disorder, in 15% the aganglionic intestine reaches the left transverse colon flexure, while in 6% the disease concerns the entire large intestine. In rare cases, the aganglionosis might involve the whole intestine up to the duodenum. The clinical symptoms of the disease appear most often upon birth in a form of neonatal intestinal obstruction. The main ailment is constipation. The pathognomonic symptom is the delayed passage of meconium (>24 h after birth). Additionally, abdominal distention appears (Fig. 1) as well as vomiting with bile content. In some cases, the disease may clinically demonstrate itself in a later stage of life, even in adulthood.[11]

The most dangerous complication, which may appear in children with Hirschsprung's disease, is enterocolitis that may have a violent course and can result in a short time in death in a septic shock mechanism. The diagnostics of the disease is based on taking medical history, clinical examination and running diagnostic tests. In the clinical examination, apart from the abovementioned symptoms of obstruction, the symptom of a sudden defecation and gas release after inserting the catheter into rectum appeared. Barium enema, especially in cases of delayed diagnostics in patients with a re-distention of a correctly innervated intestine, may reveal a transitional zone of the intestine (cone syndrome) as well as the length of the aganglionic intestine (Fig. 2)

The supplementary examination in diagnosis and classification of innervation dysfunction of a digestive tract is manomentry of a lower part of a digestive tract. However, the relaxation of the internal sphincter (rectoanal reflex RAR) excludes aganglionosis. The examination determining the diagnosis in Hirschsprung's disease is the result of histopathology of a sample of a posterior rectal wall, which should be taken depending on the child's age from 0,5 to 1,5 centimeters above the dentate line (physiologically aganglionic zone). The lack of ganglia cells and the presence of overgrown nerve trunks producing excessive levels of acetyl cholinesterase confirms the diagnosis. The increased activity of this enzyme is diagnosed by histochemical method. In recent years, the immunohistochemical test of calretinin was introduced as a tool for diagnosis of Hirschsprung's disease. The combination of acetyl cholinesterase histo-

chemical test with the immunohistochemical test of calretinin encourages growth in sensitivity and specificity, as well as enhances precision of diagnostics of aganglionosis.[12-15]

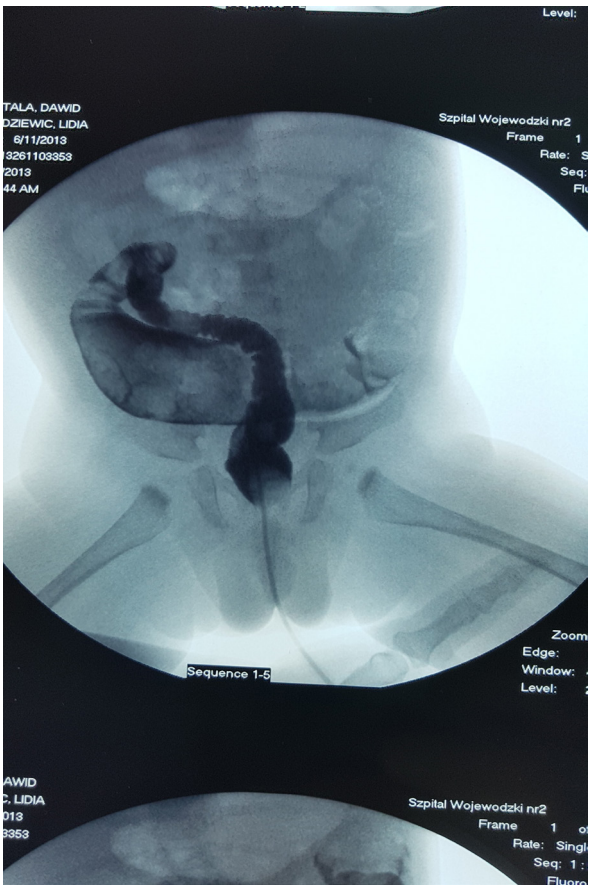

Fig. 2. Barium enema – narrow aganglionic intestine with a syndrome of cone in a transitional zone

The treatment of Hirschsprung's disease is surgical and requires the removal of aganglionic intestine and anastomosis of properly innervated intestine with the rectum just above the external anal sphincter. Depending on the clinical state of the patient, the length of the aganglionic section, the time for diagnosis and as a consequence the diameter of the re-dilated properly innervated intestine section, the treatment might be performed in a one-stage procedure or two and three-stage one.[16]

The standard surgical techniques performed in various modifications with abdominal or abdominal-perineum incision include Rehbein, Duhamel, Swenson and Soave methods.[17-20] In 1998 de La Torre and Ortega modified intra-abdominal Soave technique and presented the outcomes of children's treatment be means of new method, which they called transanal resection of the aganglionic section (TEPT). This technique involves circular incision of the rectum mucosa about 1 centimeter above the dentate line and then dissecting the rectum mucosa from its' muscular cuff up to pelvic diaphragm. In the next stage, the muscle cuff should be circularly incised in order to open the peritoneal cavity and to be able to further dissect the aganglionic intestine supplying it's mesentery up to its' macroscopic dilatation (transitional zone). In a subsequent stage, the sample of the intestinal wall is taken to the intra-operative histopathology biopsy in order to confirm its' normal innervation (Fig. 3). After confirmation of the presence of normal ganglion cells in the intestine, the anastomosis is done between the intestine and the rectal wall about 1 centimeter above the dentate line. De La Torre method in uncomplicated cases comes down to one-stage, successful treatment of children with Hirschsprung's disease and was very quickly accepted by the majority of paediatric surgery centres dealing with this defect.[21]

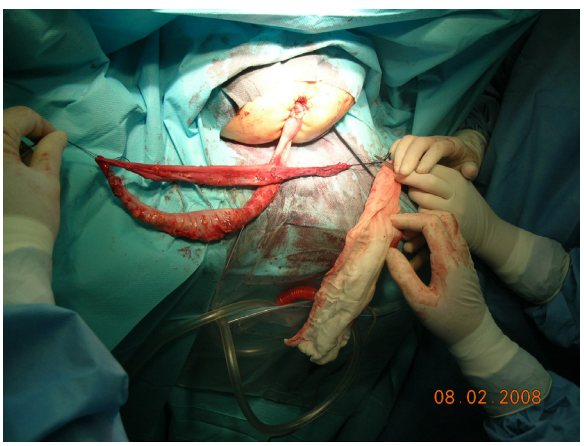

Fig. 3. Transrectal resection of the aganglionic section (TEPT)

Aim

The aim of the study was to compare functional results, complications and problems of children at various ages with Hirschsprung's disease treated surgically with one-stage (TEPT) or two-stage procedure (colostomy, TEPT) along with the validity of performing barium enema in order to assess the length of the aganglionic intestine.

Material and methods

The study was approved by the local bioethics committee (25/02/2019). In 2006-2018 in Paediatric Surgery Clinic in Rzeszów, 41 children with Hirschsprung's disease were treated. This group comprised of 35 boys and 6 girls (Fig. 4).

The classic type of the disease was observed in 36 children, the long-segment type in 4 and 1 had total intestinal aganglionosis (Fig. 5). In 5 cases the Down syndrome was recognized. In 26 children the correct diagnosis was made in the neonatal period, while in 13 in infancy. At the latest, diagnoses were made in children aged 1.5 and 3.5 years. In all patients, the clinical symptoms presented themselves in the neonatal period.

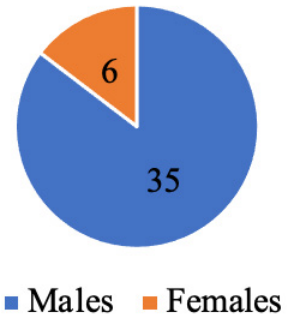

Fig. 4. Children operated on Hirschsprung's disease, division according to sex

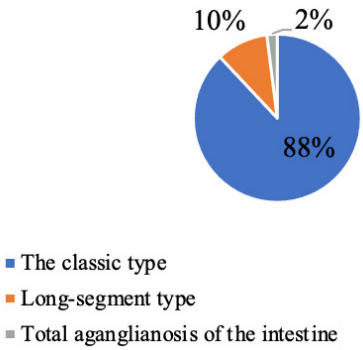

Fig. 5. The length of aganglionic section

The one-stage surgery – TEPT was performed in 25 children (this group included 4 children, in which TEPT was supported with minilaparotomy in order to mobilize the aganglionic section of the intestine. 15 patients received a two-stage treatment – in the first stage the colostomy was created, in the second one TEPT was performed with a simultaneous loop colostomy and anastomosis of a normal intestine with the wall of anal canal just above the external anal sphincter. One child received a three-stage treatment. In the first stage, the colostomy was created, in the second TEPT was performed and after healing of anastomosis and its' dilatation, the child had the continuity of the digestive tract restored (Fig. 6).

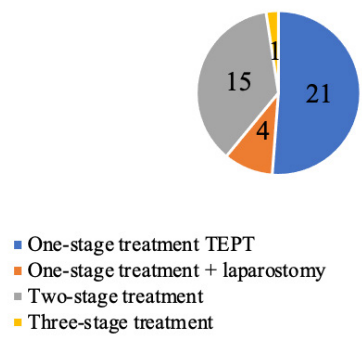

Fig. 6. Children operated on Hirschsprung's disease according to the number of stages of surgical treatment

Colostomy was performed in children due to three reasons. In case of a late diagnosis – in order to decompress and constrict the re-dilated intestine in seven children, associated congenital anomalies (Down syndrome and heart disorders) in two children, and in six children due to the lack of possibility to decompress the intestine in the preoperative period.

Results

Radical surgery was performed in 40.6 week of a child's life on average (the earliest in third week, and at the latest in the age of 4 years and two moths). The average time of the surgery was 188,8 minutes (in the range from 100 minutes to 450 minutes). The median length of the intestine resected in classic type equaled 19 centimeters. In patients with a long-segment disease – 35 centimeters.

None of the patients required a blood transfusion throughout the surgery. In the post-operative period, 15 patients received blood.

All the children were administered wide spectrum antibiotics after the surgery. The average time of children's hospitalization until the date of discharge was 13.4 days (range 7 to 30 days).

In the early post-operative period, in six (14.6%) children symptoms of toxic enterocolitis appeared. In three of them colostomy was created, which was closed on average after 5 months. The other children with toxic enterocolitis received conservative treatment. One child died due to the septic shock (2.4%), in two after the initial improvement of the general condition, later, after the discharge, the symptoms of seasonal, passing obstruction of digestive tract appeared, accompanied by symptoms of enterocolitis – currently, these children have the ileostomy created.

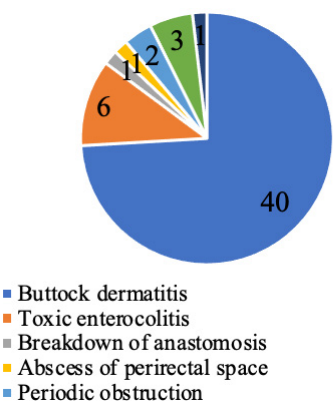

Fig. 7. Early post-operative complications

In one case, on the third day after the surgery, total dehiscence of anastomosis occurred. The child had colostomy created and colorectal re-anastomosis. In one patient, abscess of perirectal space was diagnosed – the

patient had colostomy created, which was closed after 4 moths. In two cases, in the early post-operative period, a passing obstruction of a digestive tract appeared. Both patients received conservative treatment with satisfactory results.

Buttock dermatitis in the anus area appeared in all operated patients (Fig. 7)

In 3 cases, wrong intra-operative evaluation was made, as a result of which in theses patients a radical resection was not made. One patient underwent another TEPT 6 months after the first surgery, in the second case, colostomy was created and afterwards the child underwent a surgery of Duhamel method in another centre. In another patient with the wrong intra-operative diagnosis, the ileostomy was created – the child is in the process of diagnostics in another centre. Two children with the ileostomy created are being diagnosed and treated. In another 38 children, the study was carried out in a form of a survey.

Soiling was stated in 9 patients, periodical constipation in 1. After one-stage TEPT, soiling is present in 4 patients, who were operated in the third week of life (1 patient), in the seventh week (2 patients) and in tenth week (1 patient). In a group of 16 children receiving a two-stage treatment, soiling was reported in 5 of them (among them, 4 with created colostomy due to the late diagnosis and intestine re-dilatation). Incorrect, loose, sticky texture of stool was reported in 3 children. Two children were hospitalized several times due to enterocolitis (Fig. 8)

In two children (one child with a long-segment Hirschsprung's disease and the other one with a post-operative enterocolitis) anastomotic stricture was diagnosed, requiring multiple dilatations in general anesthesia. Improper development was not stated in any case. Frequency of defecation in almost all patients had decreased after three, four-month period since the surgery from 10-15 per day to the age norm. In one child, who was diagnosed with anastomosis retraction, soiling and increased frequency of defecation persist throughout the day.

All of the parents of the older children view the outcome of the surgery as positive and the life quality of their children does not differ from their peers (Tab. 1).

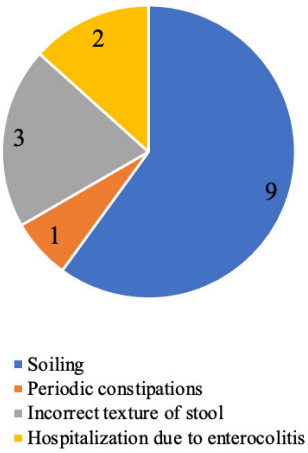

Fig. 8. Late postoperative complications

Discussion

The successful surgical treatment of Hirschsprung's disease aims to resect aganglionic intestine and anastomose of the normally innervated intestine with the rectum wall just above the external anal sphincter. In the second half of 20th century, the methods of Swenson and Bill, Soave, Rehbein and Duhamel gained the recognition and popularity. Treatment of children was performed in stages. In a first stage, the colostomy was created, in the second one, the radical surgery was done. In the third one, the ostomy was closed, restoring the gastric tract continuity. In complication-free course of treatment, the patient underwent laparotomy three times with all possible negative consequences resulting thereof. In recent years, the goal of surgical practice was to perform the radical surgery without ostomy. We presented the outcomes of surgical treatment with TEPT method in our patients with Hirschsprung's disease in years 2006 and 2018. In our material, the majority of patients were boys and the classic type of the disease prevailed – which is consistent with literature. In the first period after the implementation of this method, we operated on children who turned 3 months. Currently, the newborns are operated with TEPT method. The oldest child on the day of surgery was 4 years old. Every patient had barium enema in order to determine the length of the aganglionic section. However, in none of the long-segment cases nor in the total aganglionosis, barium enema was consistent

Table 1. Long-term outcomes of treatment on the basis of surveys conducted among parents

	Improvement of functioning	Soiling	Periodic constipations	Hospitalization due to enterocolitis	Stricture of anastomosis
One-stage treatment n=23 (60%)	23 (100%)	4 (17%)	0	1 (4%)	1 (4.3%)
Two-stage treatment n=14 (37%)	14 (100%)	5 (35%)	1 (7%)	1 (7%)	1 (4.3%)
Three-stage treatment n=1 (3%)	38 (38%)	0	0	0	0
Total n=38 (100%)		9 (23%)		2 (5%)	2 (5%)

with the intra-operative image of agnaglionosis. Moreover, barium enema performed in the neonatal period was not always reliable due to the lack of re-dilatation of normally innervated intestine.[22,23]

In most paediatric surgery centres, the diagnosis of Hirschsprung's disease is made on the basis of the result of histopathological examination obtained by means of suction biopsy, which advantages include little invasiveness of the procedure and lack of complications.[24]

In our centre, the patients are qualified for surgery according to the result of pathomorphological examination of the section taken surgically from the posterior rectal wall. The section of at least 0.8×0.5 cm is taken from above the physiologically aganglionic zone. It is always a full histopathology examination, not intra. It offers a possibility of a reliable pathomorphological evaluation of the whole section and substantially reduces the risk of wrong diagnosis. Furthermore, it shortens the time of the surgery in cases where the decision on the radical surgery is made immediately after obtaining the intra results.[24]

The disadvantage of such management is the waiting time for the results of histopathology test and the scaring in the collection site that during the radical surgery may hinder the dissection of mucosa from the muscle cuff. No complications were observed in any case of the surgical collection of the section.

Until the surgery date the children's digestive tract was decompressed by means of enemas and dilators to anal canal in order to relax the sphincter. Once the effective release of the stool retention was impossible, the patient was qualified for the laparotomy, during which mapping of the intestine was performed and double barrel colostomy was created divided onto the normally innervated intestine. In 21 cases a one-stage TEPT was performed, in another 4 TEPT with minilaparotomy in order to mobilize the intestine. In many centres, in order to mobilize the intestine, the laparoscopic technique is currently used.[24,25]

Out of our 41 patients, 16 received a two-stage treatment. In the first stage, the colostomy was created (delayed diagnosis with re-dilatation of the intestine, children with Down syndrome, in case of ineffective conservative treatment). In the second stage, TEPT was performed taking the normally innervated proximal "barrel" of the fistula down transrectally and anastomosing it with the rectal wall. In these cases the intra-operative histopathology examination was not performed, as a result of which the shortening of surgery time was achieved and avoidance of incorrect diagnosis of intra. A three-stage treatment was performed in 1 patient: colostomy, TEPT, colostomy closure.

In all patients, in the early post-operative period we diagnosed perianal inflammatory lesions of buttock. It is a frequent complication described especially in newborns operated before the third month of life. [24,26,27] In one case, these changes were intensified with skin erosion and fever.

In 3 patients, the intra-operative sections were wrongly evaluated. In one case TEPT was performed again with a satisfactory outcome, in the other, the colostomy was created. Later, this patient underwent Duhamel method surgery in another centre. Another child is in the process of diagnostics. The ileostomy has been created. Other authors emphasize that the correct intra-operative evaluation of the section depends mainly on the experience of professional pathomorphologist, their availability and the quality of a material sample sectioned by the surgeon.[24,28]

The patients with long-segment form of Hirschsprung's disease and a child with a total aganglionosis were operated with TEPT method with laparotomy in order to mobilize the intestine. None of the patients had previously created colostomy. Two patients underwent surgery in the 3rd week of life, another ones in second and third months, and the patient with total aganglionosis in the seventh month of life. All the patients with long-segment type prior to the surgery had barium enema performed, which in none of the cases implied the possibility of long-segment presence. Moreover, the clinical symptoms were not intensified compared with the children with a classic type of the disease.

In 6 patients in the early post-operative period, the symptoms of enterocolitis appeared. 3 of them were treated conservatively. One child died, in two the symptoms of gastrointestinal obstruction occurred later. At present, these children have ileostomy created and require further treatment. In 3 cases of enterocolitis, the colostomy was created (which was closed after 5 months on average). Currently, these children do not make any complaints concerning the digestive tract.

In the group of 41 children operated on for Hirschsprung's disease, 1 died (2.4%) on the 14th day after the operation due to the septic shock secondary to sepsis caused by toxic enterocolitis. In the studies by Elhalaby as well as Anderson, who presented the outcomes obtained in large group of children with aganglionosis, mortality in Hirschsprung's disease in the first year of life equaled approximately 2%.[8,26] Also, in the studies presented by Granstomm and Wester, toxic enterocolitis develops in about 5-42% of children with Hirschsprung's disease and is the most common cause of death.[8,26,29]

Out of 40 patients, 38 completed the treatment. Two have ileostomy created and are under supervision of a referential paediatric surgery centre. Out of 38 patients invited to participate in the control trial, 32 appeared in person. In the remaining 6, medical history was taken over the phone in a form of questionnaire. In the whole group, only one patient has periodic constipation. Also 1 child was hospitalized twice due to the

enterocolitis. Soiling is present in 9 children: in 4 out of 23 treated one-stage and in 5 out of 16 treated two, three-stage, where four of them had a colostomy created due to the late diagnosis of Hirschsprung's disease and re-dilatation of intestine (these were the children who underwent surgery after the 6 month of life and older).

Only in two patients the stricture of anastomosis was diagnosed, which required dilatation in general anaesthesia. On the 7th day after the operation, an everyday dilatation of the anastomosis by means of dilators begins, which lasts after the discharge and is continued by parents for the period of about 6 months. Such manner of conduct prevents leaving permanent scar in the site of anastomosis. Parents of all children observed a gradual decrease in frequency of defecation in the period of 3-4 weeks to few months after the operation. In their subjective opinion, there is no difference in the quality of life between peers or siblings of the treated child. Similar functional results of children treated with TEPT method were presented by Sood et al.[35] The most common late functional problems are encopresis (soiling), enterocolitis and constipation. According to some authors, better results are seen in patients operated on after 3rd month of life with a classic form of the disease. According to others, the patients' age does not greatly influence the results. The most crucial factor is the length of the aganglionic intestine.[16,26,30-33]

According to Hung et al., the risk factors for the development of post-operative enterocolitis is low birth weight, lowered level of IgA, long aganglionic segment and the surgery performed before the 2nd month of life.[34] Nonetheless, all the authors emphasize the gradual improvement of intestinal function along with a reduction in late complications with age.[33-39]

Conclusion

TEPT is the most effective surgical method, which can be applied both in classic type and long-segment one in children with Hirschsprung's disease at various age. Barium enema is not always reliable in evaluation of the length of the aganglionic intestine.

The most serious post-operative complication is toxic enterocolitis, which should be treated by early colostomy creation. Worse functional results were observed in children operated on after 6th month of life, after a two-stage treatment with the colostomy created due to the late diagnosis and re-dilatation of the intestine.

Declarations

Funding

This research received no external funding.

Author contributions

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

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.

Ethics approval

The study was approved by the local bioethics committee.

References

1. Hirschsprung H. Stuhlträgheit Neugeborener in Folge von Dilatation und Hypertrophie des Colons. *Jahrbuch für Kinderheilkunde und physische Erziehung*. 1888;27:1-7.
2. Tang SC, Li, Lai FP, et al. Identification of Genes Associated With Hirschsprung's Disease, Based on Whole--Genome Sequence Analysis, and Potantial Effects on Enteric Nervous System Development. *Gastroenterology.* 2018;155(6):1908-1922.
3. Wei W, Weijue X, Jiangbin L, et al. Whole Exome Sequencing Identifies a Novel Pathogenic RET Variant in Hirschsprung's Disease. *Front Genet.* 2018;9:752.
4. Tilghman J, Ling A, Turner T, et al. Molecular Genetic Anatomy and Risk Profile of Hirschsprung's Disease. *N Engl J Med.* 2019;180:1421-1432.
5. Jurkowicz B, Abu-Bonsrah D, Zhang D, Hutson J, Newgreen D. Transserosal migration of enteric neural stem cells: Developing an avian colon mode. *J Pediatr Surg.*2018;53(12):2435-2439.
6. EUROCAT central Registry:Eurocat Statistical Monitoring Report - 2012-2016.
7. Shahid M, Mahmood A. A Case of Waardenburg-Shah Syndrome Type 4 Presenting with Bilateral Homochromatic Blue Irises from Pakistan. *Cureus.* 2018;10(8):3143.
8. Anderson J, Vanove M, Saadai P, Stark R, Stephenson J, Hirose S. Epidemiology of Hirschsprung's disease in California from 1995 to 2013. *Pediatr Surg Int*. 2018;34(12):1299-1303.
9. Taghavi H, Goddard L, Evans SM, et al. Ethnic variations in the childhood prevalence of Hirschsprung's disease in New Zealand. *ANZ J Surg.* 2018;89(10):1246-1249.
10. Gunadi, Karina SM, Dwihantoro A. Outcomes in patient with Hirschsprung's disease following definitive surgery. *BMC Res Notes.* 2018;11:644.
11. Zelga M, Zelga P, Dziki A, Piaseczna-Piotrowska A. Choroba Hirschsprunga u dzieci i dorosłych – kompendium wiedzy dla chirurga ogólnego. *Nowa Med.* 2017;24(2):59-72.

12. Nabi Z, Shava U, Sekharan A, Nageshwar Reddy D. Diagnosis of Hirschsprung's disease in children: Preliminary evaluation of a novel endoscopic technique for rectal biopsy. *JGH Open*. 2018;2(6):322-326.
13. Zheng Z, Zhang F, Jin Z, et al. Transanal endorectal stepwise gradient muscular cuff cutting pull-through method: Technique refinements and comparison with laparoscopy-assisted procedures. *Exp Ther Med*. 2018;16(3):2144-2151.
14. Jeong H, Jung H, Hwang I, et al. Diagnostic Accuracy of Combined Acetylocholineterase Histochemistry and Calretin Immunohistochemistry of Rectal Biopsy Specimens in Hirschsprung's Disease. *Int J Surg Pathol*. 2018;26(6):507-513l.
15. Rakhshani N, Araste M, Imanzade F, et al. Hirschsprung's Disease Diagnosis: Calretin Marker Role in Determing the Presence or Absence of ganglion Cells. *Iran J Pathol.* 2016;11(4):409-415.
16. Sosnowska P, Błaszczyński M, Moryciński S, Porzucek W, Mańkowski P. Are there any factors influencing the course of multistage treatment in Hirschsprung's disease?. *Przegląd Gastroenetrolgiczny*. 2016;11(2):131-135.
17. Błaszczyński M, Sosnowska P, Analiza technik operacyjnych choroby Hirschsprunga w leczeniu jednoetapowym na przestrzeni lat 2000-2011. *Borgis – Nowa Pediatria.* 2012;3:47-50.
18. Duhamel B. A new operation for the treatment of Hirschsprung's disease. *Arch Dis Child,* 1960;35:38-39.
19. Soave F. A new operation for the treatment of Hirschsprung's disease. *Surgery*. 1964;56:1007-1014.
20. Swenson O, Rheinlander HF, Diamond I. Hirschsprung's disease: a new concept of the etiology. *N Engl J Med*. 1949;241:551-556.
21. de La Torre-Mondragon L, Ortega-Salgado JA. Transanal endorectal pull – through for Hirschsprung's disease. *J Ped Surg.* 1998;33:1283-1286.
22. Jester I, Holland-Cunz S, Loff S, Hosie S, Reinhagen K. Transanal pull -through procedure for Hirschsprung's disease: a 5 year experience. *Eur J Pediatr Surg*. 2009;19(2):68-71.
23. Elhalaby A, Hashish A, Elbarbary M, Soliman H, Wishahy M. Transanal one – stage endorectal pull – through for Hirschsprung's disease: a multicenter study. *J Pediatr Surg*. 2004;29(3):345-351.
24. Sreedher G, Garrison A, Novak R, Keisling M, Ganpathy S. Congenital intestinal hypoganglionosis: A radiologic mimic of Hirschsprung's disease. *Radiol Case Rep*. 2019:14(2):171-174.
25. Marginean CO, Melit LE, Gozar H, Horvath E, Marginean CD. Atypical inset of total colonic Hirschsprung's disease in a small female infant. *Medicine (Baltimore)*. 2018;97(38):12315.
26. de La Torre L, Langer J. Transanal endorectal pull – through for Hirschsprung's disease: technique, nontroversies, pearls, pitfall, and an organized approach to the management of postoperative obstructive symptoms. *Seminars in Pediatric Surgery*. 2010;19:96-106.
27. van de Ven T, Sloots C, Wijnen M, et al. Transanal endorectal pull – through for classic segment Hirschsprung's disease: with or without laparoscopic mobilization of the rectosigmoid? *J Pediatr Surg*. 2013;48(9):1914-1918.
28. Adiguzel U, Agengin K, Kiristioglu I, Dogruyol H. Transanal endorectal pull – through for Hirschsprung's disease: experience with 50 patients. *Ir J Med Sci*. 2017;186(2):433-437.
29. Gupta D, Khanna K, Sharma S. Experience with the Redo Pull – Through for Hirschsprung's Disease. *I Indian Assoc Pediatr* Surg. 2019;24(1):45-51.
30. Granstromm A, Wester T. Mortality in Swedish patients with Hirschsprung's disease. *Pediatric Surg Int*. 2017;33(11):1177-1181.
31. Zhu T, Sun X, Wei M, et al. Optimal time for single--stage pull – through colectomy in infants with short--segment Hirschsprung's disease. *Int J Colorectal Dis*. 2019;34(2):255-259.
32. Chung P, Wong K, Tam P, et al. Are all patients with short segnment Hirschsprung's disease equal? A retrospective multicenter study. *Pediatr Surg Int*. 2018;34(1):47-53.
33. Hadidi A. Transanal endorectal pull – through for Hirschsprung's disease: experience with 68 patients. *J Pediatr Surg*. 2003;38(9):1337-1340.
34. Neuvonen M, Kyrklund K, Rintala R, Pakarinen M. Bowel Function and Quality of Life After Transanal Endorectal Pull – Through for Hirschsprung's Disease: Controlled Outcomes up to Adulthood. *Ann Surg*. 2017;265(3):622-629.
35. Huang W, Li X, Zhang J, Zhang S. Prevalence, Risk Factors, and Prognosis of Postoperative Complications after Surgery for Hirschsprung's Disease. *J Gastrointest Surg.* 2018;22(2):335-342.
36. Sood S, Lim R, Collins L, et al. The long – term quality of life outcomes in adolescents with Hirschsprung's disease. *J Pediatr Surg*. 2018;53(12):2430-2434.
37. Dahal G, Wang J, Guo L. Long-term outcome of children after single-stage transanal endorectal pull – through for Hirschsprung's disease. *World J Pediatr*. 2011;7(1):65-69.
38. Stensrud KJ, Emblem R, Bjornland K. Functional outcome after operation for Hirschsprung's disease-transanal vs transabdominal approach. *J Pediatr Surg.* 2010;45(8):1640-1644.
39. Bragagnini R, Gonzalez Ruiz Y, Siles Hinojosa A, et al. Functional outcomes in postsurgery for Hirschsprung's disease. *Cir Pediatr.* 2017;30(4):191-196.
40. Aslan M, Karaman A, Erdogan D, Cavusoglu Y, Cakmak O. Our experience with transanal pull-through in Hirschsprung's disease. *Eur J Pediatr Surg*. 2007;17(5):335-339.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.9

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 202–211

ORIGINAL PAPER

Mateusz Mołoń [1,2], Małgorzata Janda [2,3]

The impact of COVID-19 pandemic and distance learning on physical and mental health of Polish students

[1] Department of Biology, Institute of Biology and Biotechnology, University of Rzeszów, Rzeszów, Poland
[2] Social Primary School No. 1 in Rzeszów, Rzeszów, Poland
[3] University of Rzeszów Polonia Centre, Rzeszów, Poland

ABSTRACT

Introduction and aim. In Poland, on 25 March 2020, distance learning, which for many people was known only from literature, entered into force. As a result, education could no longer be provided in its current form and was therefore provided online. Therefore, the main aim of the presented research was an attempt to verify the pandemic and distance education impact on students psychophysical conditions and satisfaction.

Material and methods. Some primary school students (grades 6th-8th), secondary school students from the Subcarpathian Voivodeship in Poland and also some university students from foreign countries completed an anonymous survey on the impact of the COVID-19 pandemic on their physical and mental condition as well as on some aspects of distance education. The research was conducted in December 2021 and 804 students (Primary and Secondary School) and 64 university students completed the survey.

Results. Both the primary and the secondary school students claimed they were quite satisfied with distance education. The mostly approved aspect of online education was the fact that the time of learning was fitted to students' needs. Difficulties in distance learning indicated by students were mainly connected with their mental health. Therefore, many students suffered from mental health and concentration problems, unwillingness to be active, apathy. The pandemic was also a stress factor for students and a reason why they were in a bad mood. The pandemic was the reason for an increased interest in natural sciences among 31.3% of primary school students, among 20.8% of secondary school students and among 17.3% of university students from foreign countries. Distance education influenced physical health of almost half of the respondents in both types of schools. The pandemic had an impact on mental health state of almost half of primary school students. 65% of secondary school students said that the pandemic influenced both their mental and physical health. In case of university students the pandemic did not influence their physical health in a significant way (65.4%). The study shows that primary school students used the Internet mainly to play games (almost 50% of the respondents), watch films, listen to music, do homework. Online lessons were only 20% of the answers.

Conclusion. There has been a significant impact of COVID-19 on students in elementary school, high school, and university. This resulted in negative mental and physical effects. Respondents were stressed, depressed, and unmotivated. Approximately half of them engaged in physical activity very rarely or rarely. Even though they were satisfied with online learning, which was caused by the COVID-2019 pandemic, the greatest problem was mental concentration problems, lack of motivation, and social isolation.

Keywords. COVID-19, distance learning, e-learning, students

Corresponding author: Mateusz Mołoń, e-mail: mmolon@ur.edu.pl

Received: 5.04.2022 / Revised: 29.04.2022 / Accepted: 5.05.2022 / Published: 30.06.2022

Mołoń M, Janda M. *The impact of COVID-19 pandemic and distance learning on physical and mental health of Polish students*. Eur J Clin Exp Med. 2022;20(2):202–211. doi: 10.15584/ejcem.2022.2.9

Introduction

A pandemic is defined as "an epidemic that occurs worldwide, or over a very wide area, crossing international boundaries and usually affecting a large number of people".[1] Although there has been much debate about the flu virus, its variants, and epidemic, 2020 changed our lives with the advent of a new epidemic.[2-4] The first cases of COVID-19 disease caused by the SARS-CoV-2 coronavirus were identified on November 17, 2019 in Wuhan City (Hubei Province, Central China). However, the officially infectious disease COVID-19 was declared a pandemic by the World Health Organization (WHO) on 11 March 2020. Initially, the disease occurred only in Wuhan, and then in whole China. In mid-February 2020, the first outbreaks of infections were recorded in South Korea, Iran and Italy. The first case of SARS-CoV-2 infection in Poland was reported on 4 March 2020. Until April 19, 2022, 5,985,818 cases of infection were recorded in Poland, 115,838 deaths were registered. But why is this coronavirus variant so dangerous? This is mainly because the virus spreads easily between humans. So far, the virus has been detected in nasal and pharyngeal secretions, sputum, urine, feces, tears and blood.[5,6] Additionally, the possibility of infection by the virus is made possible by its ability to 'survive' on metal, glass or plastic surfaces for several days at room temperature. However, surface disinfection can effectively inactivate viruses by procedures with 62-71% ethanol, 0.5% hydrogen peroxide or 0.1% sodium hypochlorite within 1 minute.[7] The SARS-CoV-2 virus infects mainly the respiratory system, causing acute atypical pneumonia and acute respiratory distress syndrome in severe cases. In addition, it can also spread through the nervous system, resulting mainly in the loss of smell and taste due to the virus attacking the olfactory and taste receptors.[8] Coronaviruses, as RNA viruses, use (error-prone) polymerase to replicate their RNA genetic material. Consequently, their genome tends to accumulate mutations during each replication cycle, which is natural and serves to better adapt to human organisms and increase virulence.[9] The increasing number of infections and the emergence of new forms of viruses requires the introduction of restrictions, including education. Therefore, distance education has become a huge challenge for teachers and students. The advantage of online education, also called distance education, electronic education, teaching online, e-learning or e-education is avoiding stagnation among teachers and students. Online education makes it possible for students to adapt to changes, to improve their practical skills, to participate in interesting and varied classes. Kubiak defined distance education as a method of educational process in conditions when teachers and students (university students) are far away (sometimes considerably), apart from traditional ways of communication using also present-date, very modern telecommunication technologies to transmit information, and transferring sounds, videos, computer data and printed materials.[10] What is more, contemporary technologies enable direct contact in real time between teachers and students using audio or video conferences, regardless of the distance between them. Online teaching can take different forms. There is a correspondence model, a multimedia model and a synchronous model, but the most effective is an asynchronous model based on using interactive multimedia and materials published on the Internet.[11] All the methods and forms of work, usually attractive to students and accepted by them, gained special meaning in the time of COVID-19 pandemic which required the distance form of education from March 2020 and maintained it to some extent till December 2021. It turned out that the fact of staying for many months at home, the lack of contacts with peers and teachers, participating in distance education system everyday, was experienced and assessed by children and teenagers in different ways. E-learning was still an attractive, innovative form of work. It made educational process possible to continue and in a specific form online education enabled contacts with peers, teachers and educators. However, it was not a direct contact. It was impossible to function in informal, free, stationary education realities. There appeared some problems with concentration, motivation to work, functioning in a new schedule, problems with technical equipment and others.

Aim

The main aim of the presented research was an attempt to verify the pandemic and distance education impact on students psychophysical conditions and satisfaction. Students assessment of the new dominant form of education, their fears concerning the pandemic or accepting the existing situation were verified with the use of surveys, discussed and interpreted in this study

Material and methods

Some primary school students (grades 6th-8th), secondary school students from the Podkarpackie (Subcarpathian) Voivodeship in Poland and also some university students from foreign countries completed an anonymous survey on the impact of the COVID-19 pandemic on their physical and mental condition as well as on some aspects of distance education. Students assessed online lessons, their access to the computer equipment, the influence of their surroundings and their place of staying on the quality of education. On the one hand they stated the most problematic situations and on the other hand they pointed at the factors motivating them to study. Respondents also specified if the COVID-19 pandemic was a danger for them and described its impact on their physical and mental health, they assessed their interests in natural

sciences and some aspects of their physical activities before and during the COVID-19 pandemic.

The anonymous surveys were sent to several chosen primary and secondary schools in Podkarpackie Voivodeship and also to university students from foreign countries studying Polish as a foreign language at universities in Rzeszów, Poland. The research was conducted in December 2021 and 804 students and 64 university students completed the survey (Tab. 1). Most of the respondents in primary schools were girls (155), the minority were boys (114). It was similar in secondary schools with corresponding 395 girls and 140 boys. Among university students from foreign countries 77% were women. The majority of the respondents live in towns/cities.

Table 1. Sex, type of school and place of residence

Primary school (6th-8th grade)		Secondary school	
Female	Male	Female	Male
155	114	395	140
Place of residence			
town/city	village	town/city	village
54	215	288	247
Σ 269		Σ 535	
Σ 804			

Results

As shown in Figure 1, both the primary and the secondary school students claimed they were quite satisfied with distance education (40%). Only 10% expressed their strong dissatisfaction with the above mentioned form of education.

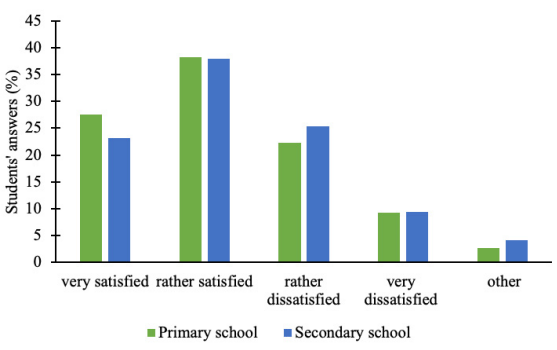

Fig. 1. Level of satisfaction with distance learning among primary and secondary school students

The optimum satisfaction with online lessons seems to be justified taking into account the fact that information technology is fully accepted by the youth of today. Information technology is a friendly working environment for young people where they pursue their hobbies and special interests, gain new information and even stay in contact with other people.

For students "the digital world, new information and communication technology is a natural world. They live, learn and fulfil their own dreams in that world".[12] Taking into account new technologies seems "normal to them, the knowledge conveyed in a traditional way without using available technology is not quite attractive.[12] It is also confirmed by students in their assessment of the positive and negative aspects of online education.

Innovative, virtual forms of classes were approved by over 30% of the respondents (Tab. 2). But the mostly approved aspect of online education was the fact that the time of learning was fitted to students' needs. Secondary school students presented a different evaluation.

Table 2. Positive and negative aspects of distance education

Positive aspects of distance education		
Answers	**Primary school (%)**	**Secondary school (%)**
innovative, virtual classes	31.3	19.5
the time of learning is fitted to students' needs	39.6	61.0
permanent contact with teachers and peers	21.6	6.6
other	7.5	12.9
the lack of direct contact with others	34.3	33.1
difficulties in understanding and remembering the information learned during lessons	28.7	27.7
technical problems	29.5	27.5
other	7.5	11.6

Their priority was fitting the time of learning to their needs (over 60%), meanwhile innovative and virtual forms of classes were approved by 19.5% of students.

Distant learners experienced lower level of organisational stress and higher level of work satisfaction.[13] Many secondary school students claim that online education is appropriate for them "considering peace and quiet, the lack of school stress, the opportunity to devote more time to their own interests".[14] However, at the same time "they mostly suffer from severe lack of direct contact with other people, peers, teachers. It is no surprise to indicate deficiencies in the field of personal contact, taking into account the fact that students value it significantly".[14]

It should be emphasised that the above mentioned regularity can be seen among primary as well as among secondary school students. Moreover, both groups described difficulties in understanding and remembering the information learned during lessons (28.7% primary school students and 27.7% secondary school students) and technical problems (29.5% primary school students and 27.5% secondary school students). Nevertheless, the

biggest problem in distance education proves to be different. The biggest problem in online education for the secondary school students was the problem with concentration (26.8%) and the lack of motivation (21.9%) (Tab. 3). For the primary school students the biggest problems were: the problem with concentration (23.5%) and the lack of contact with friends (20.1%).

Table 3. What was the biggest problem in distance learning for you?

Answers	Type of school	
	Primary school (%)	Secondary school (%)
planning everyday learning	11.9	8.1
dealing with some stress	6	9.7
the lack of the direct contact with a teacher	9.3	5.2
problems with concentration	23.5	26.8
sharing the computer, technical problems	15.7	10.3
the lack of contact with friends	20.1	18.0
the lack of motivation	13.4	21.9

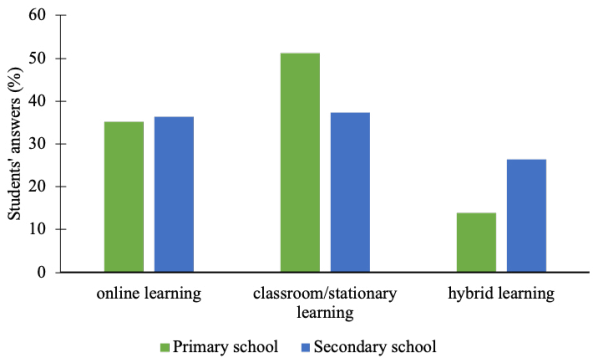

Fig. 2. Choosing the educational method preferred by students

Difficulties in distance learning indicated by students were mainly connected with their mental health. Young people need to have contacts with peers and to create their own identity. The necessity of staying at home in isolation during the COVID-19 pandemic and limited opportunities of development had a negative impact on natural needs of young people. Therefore, many students suffered from mental health and concentration problems, unwillingness to be active, apathy. In that context, students' answers about their preferred form of learning appear to be significant.

The majority of respondents were in favour of traditional education: 51.1% primary school students and 37.3% secondary school students (Fig. 2). The least desired way of learning was hybrid education because it meant a limited teachers' contact with their students.

Teachers were not able to pay attention to one group of students only, they shared their attention between students in the classroom and online students.

The relation between teachers and students is crucial, especially for secondary school students (70.6%) but also for primary school students (54.9%).

Table 4. Does the COVID-19 pandemic influence the teacher-student relations?

Answers	Type of school	
	Primary school (%)	Secondary school (%)
yes	54.9	70.6
no	45.1	29.4

According to Jacek Pyżalski „the two most important areas of relations (…) are peer relations inside class groups and teacher-students relations".[15] They were specific during the isolation caused by lockdown. Nevertheless, students understand that physical distance cannot significantly influence students' contacts with other people. It cannot limit them, or even disable the supporting, motivating relations that help to overcome some difficulties.

Considering the fact that relations with teachers and peers are important for respondents, it is surprising who they chose to ask for help during online education. The great majority of students (60.1% – primary school students, 66.3% – secondary school students) declared that they coped with online learning difficulties on their own (Tab. 5).

Table 5. Who helps you during the distance education?

Answers	Type of school	
	Primary school (%)	Secondary school (%)
parents	20.1	6.2
siblings	9	4.1
I have lessons with a private tutor	6	15.4
I learn on my own	60.1	66.3
I cannot cope with learning	4.9	8.1

Furthermore, the people who are most frequently addressed by children and youth during difficult situations are: their mother (55.2% – primary school students, 37.8% – secondary school students) and friends (19% – primary school students, 36.1% – secondary school students) (Tab. 6). The pandemic became the time when children and youth looked at their relatives and friends from a different perspective.

Parents are able to help "to see a wider perspective beyond the limits and changes resulting from epidemic situation", whereas friends have the ability to participate empathically in peers' life.[16]

Facing the pandemic situation and the necessary participation in online education, factors motivating to work were really essential. It turned out that in both study groups the grades were very important (opinion

expressed by 37.7% primary school students, 35% secondary school students). Primary school students mentioned their parents' satisfaction (25.7%) and the fact that they wanted to gain some knowledge (24.6%). For secondary school students the grades were almost as important as getting some knowledge (36%) (Tab. 7).

Table 6. Who did you ask for help when you had some problems?

Answers	Type of school	
	Primary school (%)	Secondary school (%)
my mum	55.2	37.8
my dad	8.2	3.9
my siblings	8.6	10.5
my grandmother or my grandfather	2.2	1.7
a form teacher/ a teacher	4.1	2.4
an educator/ a psychologist	0.7	1.9
a priest	1.1	4.1
a helpline/an institution providing help	0.7	1.3
a friend	19	36.1
the head teacher	0	0.2

Table 7. What motivated you?

Answers	Type of school	
	Primary school (%)	Secondary school (%)
the grades	37.7	35
I wanted to gain some knowledge	24.6	36
teachers praised me	5.2	2.1
my parents were pleased	25.7	14.4
other	6.7	12.5

The willingness to get some knowledge in both educational groups is worth paying attention to, especially if we take into account all the difficulties that students had to cope with: isolation, psychosomatic problems, concentration problems. Nevertheless, the awareness of developing some knowledge and skills created a mature attitude in children and teenagers and it did not eliminate one of the basic educational aims.

According to WHO health is defined as a state of complete physical, mental and social well-being. Thus it is a key factor determining motivation. Therefore, we asked the respondents about their feelings during the COVID-19 pandemic. Interestingly, as it is shown in Table 8 regardless of the age and school, approximately 40% of students were reluctant to do any activities.

The pandemic was also a stress factor for students and a reason why they were in a bad mood. It was manifested as depression and sadness. Primary school students expressed their opinion that the pandemic did not have a significant impact on their mental health (17.2%). They actually pointed to tiredness resulting from computer work. Some secondary school students claimed that all the responses suggested by the authors were related to their health during the pandemic, but some individuals said that the pandemic was a factor causing social anxiety, loneliness, helplessness. Only 17% of secondary school respondents claimed that the pandemic was the positive time for their feelings because they were able to develop their hobbies, concentrate on secondary school leaving examination preparations, manage time in a better way, relax and motivate to gain general knowledge.

Table 8. How did you feel because of the COVID-19 pandemic?

Answers	Type of school		
	Primary school (%)	Secondary school (%)	University (%)
stressed	25.7	18	27
sad	16.8	23.4	20
reluctant to perform any actions	40.3	36.7	38
other	17.2	21.9	15

University students from foreign countries who came from the eastern Europe (Ukraine, Belarus) as well as from Asian countries (Vietnam, South Korea) and studying Polish as a foreign language necessary to start a university in Poland were also taken into account. In majority they were also reluctant to do any activities (38%) in spite of the fact that the first months spent in Poland were supposed to prepare them for the future education and to make it possible for them to learn about Polish culture, language, existing in society. They also felt stressed (27%) and depressed (20%) which made it difficult for them to learn and also made them isolated. Learning a foreign language was an excessive burden not only because they had to be fully involved and cope with linguistic difficulties but mainly because of the pandemic.

The stress factor often motivates people to learn about its reasons and results. It is often seen in disasters, wars or epidemics. That is why students were asked if they had learnt anything about viruses and natural sciences during the COVID-19 pandemic. As it is presented in Table 9 the knowledge about viruses did not increase in case of only 9.7% of primary school students, 7.7% of secondary school students and 8.1% of university students from foreign countries.

Summing up, the knowledge about viruses was higher among secondary school students, it can be seen in the "yes, definitely" answers (45.5%) and among university students (51.9%). Clearly, students developed their knowledge about viruses on their own or with the help of their teachers. It seems that teach-

ers had a key role in education process because the amount of information that appeared in public (on the Internet, on TV, in newspapers) and was often fake, was proportionately increasing during the pandemic. Therefore, it was substantial to filter the latest scientific knowledge published in reviewed and prestigious magazines.

Table 9. Did you learn a lot about viruses during the COVID-19 pandemic?

Answers	Type of school		
	Primary school (%)	Secondary school (%)	University (%)
definitely yes	38.8	45.5	51.9
yes, a little	51.5	46.8	40
definitely no	9.7	7.7	8.1

However, as it is shown in Table 10 the pandemic itself was the reason for an increased interest in natural sciences among 31.3% of primary school students, among 20.8% of secondary school students and among 17.3% of university students from foreign countries. The result seems to be surprising, especially among secondary school students and university students who chose natural sciences and who had specific interests and attended specific class profiles chosen by themselves (rarely general profiles).

Table 10. Have you become more interested in natural science in relation to the COVID-19 pandemic?

Answers	Type of school		
	Primary school (%)	Secondary school (%)	University (%)
yes	31.3	20.8	17.3
no	68.7	79.2	82.7

As it was shown before, students during the COVID-19 pandemic felt stressed, sad, reluctant to perform any activities. However, the above mentioned general statement was influenced by different factors including: contacts with family, parents and friends, increasing restrictions, lockdown, dealing with problems but also the direct presence of the virus. Therefore, the respondents were asked if the COVID-19 virus was a danger for them. Interestingly, as it is shown in Table 11, the majority of students in both school types claimed that the virus was just a little danger for them. About 25% of primary school students and 21% of secondary school students said that the virus was a significant danger for them.

In both types of schools less than 20% of respondents indicated that the virus was not a danger for them at all. University students from foreign countries answered in a similar way. 67.3% among them said that the virus was just a little danger for them. 19.2% of the respondents claimed that it was a significant danger for them. 13.5% pointed at the virus as no danger at all.

Table 11. Is the COVID-19 virus a danger for you?

Answers	Type of school		
	Primary school (%)	Secondary school (%)	University (%)
yes, very much	24.6	21	19.2
just a little	57.5	60.5	67.3
it is not a danger	17.9	18.5	13.5

A hypothesis was formulated that the main stress factor among students was not the virus itself but the pandemic state, as it is shown in Table 12. That is why respondents were asked about their physical and mental health. As it is presented in Table 12, the pandemic influenced physical health of almost half of the respondents in both types of schools. Interestingly, mental health state depended on the age of the respondents.

Table 12. Does the COVID-19 pandemic influence your physical and mental health?

Influence on physical health			
Answers	**Primary school (%)**	**Secondary school (%)**	**University (%)**
yes	48.9	48.9	34.6
no	51.1	51.1	65.4
Influence on mental health			
yes	48.9	65.2	50
no	51.1	34.8	50

The pandemic had an impact on mental health state of almost half of primary school students. 65% of secondary school students said that the pandemic influenced both their mental and physical health.

In case of university students the pandemic did not influence their physical health in a significant way (65.4%). However, the impact on their mental health was the same when we compare the positive and negative answers (respectively 50%). Therefore, it is clearly shown that the pandemic had a crucial impact on university students' mental as well as physical health.

Online education during the COVID-19 pandemic is on the one hand a way of contact between teachers and students. On the other hand, as experts repeatedly inform, it caused sight problems. Therefore, students were asked how much time they had spent in front of computer screens, tablets or smartphones and what they had used the Internet for. Interestingly, about 20% of primary and secondary school students spent more than 6 hours a day using the Internet (Table 13.)

The majority of students in both groups spent 3 hours a day in front of the screens. As it is presented in

Table 14 the Internet was not used mainly in order to take part in online lessons as it is suggested by many educationists. The study shows that primary school students used the Internet mainly to play games (almost 50% of the respondents), watch films, listen to music, do homework. Online lessons were only 20% of the answers. Secondary school students used the Internet mainly to listen to music, use social media, watch films, contact with other people. Online lessons time was only 7% of the time spent on the Internet. Additionally, secondary school students used the Internet to play games and to do homework significantly less than primary school students.

Table 13. How much time do you spend on the Internet a day?

Answers	Type of school		
	Primary school (%)	Secondary school (%)	University (%)
1 hour	12.3	3	0
2 hours	15.7	5.8	3.8
3 hours	21.3	26	13.5
4 hours	17.2	23.8	23.1
5 hours	8.6	14.6	32.7
6 hours	5.2	10.9	19.2
more	19.8	21.3	7.7

In case of university students from foreign countries the largest group (32.7%) spent 5 hours a day using the Internet. They used social media most often (19.1%), listened to music (17.4%), and only approximately 10% took part in Polish as a foreign language lessons using the Internet. Their online lessons attendance was satisfactory. However, it is alarming that they spent a great number of hours in front of their computer screens, watching films or using social networking sites.

The pandemic and spending time in front of TV and computers screens in a passive way had a bad impact on students' physical health. Polish children body mass has increased in the fastest way in Europe. According to WHO in the last 20 years the number of overweight children has grown three times and Polish teenagers are in the world lead as far as obesity is concerned. Currently in Poland 31% of boys and 20% of girls are overweight and 13% of boys and 5% of girls suffer from obesity. Unfortunately, the pandemic and several lockdowns will certainly increase the numbers. Therefore, respondents were asked how they were keeping fit. As it is showed in Table 15 the type of school did not influence the frequency of physical activities during online education. About 50% of students in both types of schools claimed that they often or very often did physical exercises. Un-

Table 14. What do you use the Internet for? (choose max. 3 answers)

Answers	Type of school		
	Primary school (%)	Secondary school (%)	University (%)
having online lessons	19.4	6.9	10.4
playing games	46.6	23	6.1
doing homework	37.7	27.2	2.6
using social media	28	50.7	19.1
watching films	39.9	43.3	15.7
listening to music	38.4	61	17.4
developing my interests	15.7	19.7	11.3
contacting other people	41.4	44.9	17.4

Table 15. How often were you physically active during online education?

Answers	Type of school		
	Primary school (%)	Secondary school (%)	University (%)
very rarely	12.8	10.1	3.8
rarely	34.6	34.1	59.6
often	37.2	36.3	30.8
very often	15.4	19.5	5.8

Table 16. How often did you do any physical exercises before the COVID-19 pandemic and during online education?

Answers	Type of school					
	Primary school (%)		Secondary school (%)		University (%)	
	Befor pandemic	During pandemic	Befor pandemic	During pandemic	Befor pandemic	During pandemic
once a week	9	15.8	5.8	10.7	11.5	19.2
twice a week	10.2	11.7	19.1	11.4	17.3	19.2
three times a week	12	18.8	25.5	14.8	23.1	11.5
four times a week	18.8	8.3	11.4	9.9	19.2	11.5
five times a week	10.2	6	4.1	9	5.8	1.9
six times a week	3.8	2.6	1.7	3	0	0
everyday	20.7	16.9	7.3	11.8	3.8	3.8
very rarely, several times a month	7.9	7.9	13.3	14.0	11.5	17.3
I didn't do any exercises	7.5	12	11.8	15.4	7.7	15.4

fortunately, the second half of students answered "rarely" or "very rarely".

The results concerning physical exercises frequency before and during the pandemic were shown in Table 16. About 20% of primary school students and about 30% of secondary school students chose the answer "rarely" (less than several times a month) or did not exercise at all. However, it seems positive, that 60% of students were active in some way at least once a week. 60% of university students answered "rarely" and only over 30% "often". Before the pandemic majority of respondents were active twice, three or even four times a week. During the pandemic they were often active once or twice a week. There is also quite a large group of university students who did not do any physical activities at all – the number of them increased during the COVID-19 pandemic.

Discussion

In Poland, on 25 March 2020, distance learning, which for many people was known only from literature, entered into force. As a result, education could no longer be provided in its current form and was therefore provided online. This new approach was a great logistical challenge not only for teachers and students (often including parents), but also verified the IT competences of teachers at all levels of education. Due to the pandemic, all didactic and educational tasks in schools were to be carried out using distance learning techniques. According to the definition, distance learning is a method of conducting the didactic process in conditions where teachers and students are distant from each other and are not in the same place.[10] Teachers at work had to take into account their own and their students' technical capabilities, hardware, and software necessary to manage distance learning. The digital technologies-based distance learning process has become a reality. A significant challenge during the pandemic in the context of education was to ensure appropriate teacher-student relations. It is an important aspect of the functioning of the school under the conditions of direct classical teaching. An individual approach was a difficult task from the student's perspective, especially for students with educational problems. With the lesson time limited to 30 minutes, it seems that this task was almost impossible to accomplish. However, working with students at distance forced teachers to conduct lessons creatively. Nevertheless, the most important thing in remote education in the context of students was maintaining mental comfort in a situation of discouragement, anger, or bitterness. It seems that a hard lockdown and large limitations in movement and contacts with peers were of key importance in the mental sphere of the students. Distance learning had many advantages and disadvantages. The disadvantages include the lack of contact in the teacher-student and student-student relationship, difficulties with concentration of students, problems with checking the student's independence, low physical activity resulting from too long work at the computer, a lot of material to learn, and problems in the organization of work. On the other hand, the advantages include individual adaptation of the workplace to learning and active use of interactive sources of knowledge (provided by educational platforms, including publishing houses). A certain advantage was also the time savings resulting from the lack of commuting.

Here, we are the first to show that distance learning has a negative impact on both the mental health of students and their physical condition. In the context of physical condition and the increase in the number of people with obesity in children in Poland, it seems that the subsequent studies that will be carried out after the pandemic will show even greater health problems for children and adolescents. From the perspective of physical activity, a big problem was the lack of physical education lessons, resulting in an even greater reduction in physical activity. Many teachers, doctors and school management expressed their concerns about the long time spent in front of computer screens, tablets, or smartphones. Interestingly, our research shows unequivocally that during the pandemic, distance learning was only a small fraction of the time students spent their time. As it turns out, primary school students in particular used this time for games. Therefore, it seems that the lack of self-discipline, hygiene at work, and some problems in the child-parent relationship resulted in too much time spent in front of the screens of the devices. As we have shown, a big problem during distance learning was the lowering of mood and general mental state in the vast majority of respondents, regardless of the study group. However, in our opinion, in the context of science education, the pandemic had a positive impact by increasing interest in science education, which in the future may have a positive impact on the choice of an appropriate career development path.

Some recent date from Ecuadorian students show that 63.78% of students want to return to on-site classes regardless of their conditions of Internet connection and their available learning tools. In addition, older students and students from higher semesters were found to think that online classes are better than face-to-face classes and want to continue in online education.[17] Stressful situations can have a significant impact on education success; however, there is not clear link between anxiety, work, and social dysfunction related to learning impairments. In the case of Brazilian students, 42.9% of the respondents experienced symptoms of generalized anxiety disorders and more than half had moderate to severe functional impairment. These data showed that psychological distress and anxiety states might influence

performance during remote learning, which highlights the importance of investigating measures of anxiety and functional impairment as part of the remote-learning curriculum.[18] In turn, recent data also showed that Dutch students were missing a proper structure in lessons and was a decreased in the understanding and enjoyment of the students in all courses. What crucial, teacher data demonstrate that the teachers needed guidance from the schoolboard.[19] The COVID-19 pandemic has resulted in a devastating threat to human society in terms e.g. health, and lifestyle. As was reported, immobilization due to hospitalization or inactivity due to quarantine and social distancing can negatively regulate the ability of many organ systems to resist to infection and increase the probability of disease.[20] In pandemic context and physical activity, the WHO plan is WHO plan. To prevent and reduce inactivity, the WHO designed a global plan named the Global Action Plan on Physical Activity 2018–2030 (GAPPA) in 2017 (in 2016 goals were established). As was rightly argued, a COVID-19 pandemic affected various aspects of our lives, including physical activity.[21,22] Some studies have shown that physical activity decreased during the pandemic. Therefore, the WHO should review the GAPPA and update the goals according to the state of physical activity after the pandemic.[23]

In general, our findings seem to be in line with general trends around the world. And this mainly concerns the deterioration of the psychophysical state of the human population. After the pandemic has resolved, subsequent psychophysical health monitoring studies should be performed to verify the impact of the pandemic on children and adolescents.

Conclusion

There has been a significant impact of COVID 19 on students in elementary school, high school, and college. This resulted in negative mental and physical effects. Respondents were stressed, depressed, and unmotivated. Approximately half of them engaged in physical activity very rarely or rarely. Even though they were satisfied with online learning, which was caused by the Coronavirus pandemic, the greatest problem was mental - concentration problems, lack of motivation, and social isolation.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, M.M.; Methodology, M.M.; Software, M.M..; Validation, M.M. and M.J.; Formal Analysis, M.M. and M.J; Investigation, M.M.; Data Curation, M.M. and M.J.; Writing – Original Draft Preparation, M.M. and M.J.; Writing – Review & Editing, M.M.; Visualization, M.M.; Supervision, M.M.; Project Administration, M.M.; Funding Acquisition, M.M.

Conflicts of interest

The authors declare that they have no competing interests.

Data availability

The data sets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

Not applicable

Reference

1. Last JM, editor. A dictionary of epidemiology, 4th edition. New York: Oxford University Press; 2001
2. Neto M, Gomes TD, Cunha CS, et al. Lessons from the past in the present: news from the Spanish flu pandemic to COVID-19. *Revista Brasileira De Enfermagem.* 2022;75(1): e20201161.
3. Yadav S, Rawal G. Swine flu-have we learnt any lesson from the past? *Pan Afr Med J.* 2015;22:118.
4. Lenglet AD, Hernando V, Rodrigo P, Larrauri A, Donado JDM, de Mateo S. Impact of flu on hospital admissions during 4 flu seasons in Spain, 2000-2004. *BMC Public Health.* 2007;7:197.
5. Chan JFW, Yuan SF, Kok KH, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet.* 2020;395(10223):514-523.
6. Xia JH, Tong JP, Liu MY, Shen Y, Guo DY. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. *J Med Virol.* 2020;92(6):589-594.
7. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. *J Hosp Infect.* 2020;104(3):246-251.
8. Butowt R, Bilinska K. SARS-CoV-2: Olfaction, Brain Infection, and the Urgent Need for Clinical Samples Allowing Earlier Virus Detection. *Acs Chemical Neuroscience.* 2020;11(9):1200-1203.
9. Chen JL. Pathogenicity and transmissibility of 2019-nCoV--A quick overview and comparison with other emerging viruses. *Microbes Infect.* 2020;22(2):69-71.
10. Kubiak MJ. *Szkoła. Internet. Intranet. Wirtualna edukacja,* Warszawa 2000:12.
11. Edukacja w Internecie. http://consilr.info.uaic.ro/uploads_lt4el/resources/pdfpolAn%20education%20on%20the% 20Internet.pdf. Accessed March 15, 2022.

12. Winiarczyk A, Warzocha T. Edukacja zdalna w czasach pandemii COVID-19. *Forum Oświatowe*. 2021:33(1).
13. Konradt U, Hertel G. Quality of management by objectives, task-related stressors, and non-task-relatrd stressors as predictor of stress and job satisfaction among teleworkers. *European Journal of Work and Organizational Psychology*, 2003;12(1):61-79.
14. Kochan I. Nauczanie zdalne w opinii uczniów szkół średnich w czasie trwania pandemii COVID-19. *Studia Edukacyjne*. 2020;59:127.
15. Pyżalski J. Ważne relacje uczniów i nauczycieli w czasie edukacji zdalnej. Ptaszek G, ed., *Edukacja zdalna: co stało się z uczniami, ich rodzicami nauczycielami?* Gdańsk 2020.
16. Dąbkowska M. Psychospołeczne konsekwencje pandemii koronawirusa (COVID-19) u dzieci i młodzieży – przegląd wybranych opracowań. *Niepełnosprawność. Dyskursy pedagogiki specjalnej*. 2021;39.
17. Benalcazar ME, Barona L, Valdivieso AL, Vimos VH, Velastegui D, Santacruz CJ. Educational Impact on Ecuadorian University Students Due to the COVID-19 Context. *Education Sciences*. 2022;12(1):17.
18. Godoy LD, Falcoski R, Incrocci RM, Versuti FM, Padovan-Neto FE. The Psychological Impact of the COVID-19 Pandemic in Remote Learning in Higher Education. *Education Sciences*. 2021;11(9):473.
19. Lauret D, Bayram-Jacobs D. COVID-19 Lockdown Education: The Importance of Structure in a Suddenly Changed Learning Environment. *Education Sciences*. 2021;11(5):221.
20. Woods JA, Hutchinson NT, Powers SK, et al. The COVID-19 pandemic and physical activity. *Sports Med Health Sci*. 2020;2(2):55-64.
21. Giustino V, Parroco AM, Gennaro A, Musumeci G, Palma A, Battaglia G. Physical Activity Levels and Related Energy Expenditure during COVID-19 Quarantine among the Sicilian Active Population: A Cross-Sectional Online Survey Study. *Sustainability*. 2020;12(11):4356.
22. Meyer J, McDowell C, Lansing J, et al. Changes in Physical Activity and Sedentary Behavior in Response to COVID-19 and Their Associations with Mental Health in 3052 US Adults. *Int J Environ Res Public Health*. 2020;17(18):6469.
23. Amini H, Habibi S, Islamoglu AH, Isanejad E, Uz C, Daniyari H. COVID-19 pandemic-induced physical inactivity: the necessity of updating the Global Action Plan on Physical Activity 2018-2030. *Environ Health Prev Med*. 2021;26(1):32.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.11

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 212–216

REVIEW PAPER

Yethindra Vityala [1], Abhijit Krishna [2], Dinesh Pandla [2], Krishna Priya Kanteti [3], Jahnavi Sadhu [4], Harsha Vardhan Boddeti [2], Tejesh Kintali [2], Mohammad Shaour Khalid [2]

Pathophysiology of thromboembolism in patients with COVID-19

[1] Department of Pathology, International Higher School of Medicine, International University of Kyrgyzstan, Bishkek, Kyrgyzstan
[2] International Higher School of Medicine, International University of Kyrgyzstan, Bishkek, Kyrgyzstan
[3] Beihua University, Jilin, China
[4] Apollo College of Physiotherapy, Telangana, India

ABSTRACT

Introduction and aim. A small number of critically ill patients with coronavirus disease (COVID-19) develop thromboembolism (arterial or venous), both micro- and macrovascular complications such as deep vein thrombosis, pulmonary embolism, and pulmonary arterial thrombosis. The objective of the study is to describe the pathophysiology of venous thromboembolism in patients with COVID-19.

Material and methods. In this article a narrative review regarding pathophysiology of thromboembolism in patients with COVID-19.

Analysis of the literature. The development of coagulopathy is a consequence of the intense inflammatory response associated with hypercoagulability, platelet activation, and endothelial dysfunction. The pathophysiology that relates pulmonary thromboembolism (PTE) with COVID-19 is associated with a hypercoagulable state. PTE is suspected in hospitalized patients presenting dyspnea, decreased oxygen requirement, hemodynamic instability, and dissociation between hemodynamic and respiratory changes. In COVID-19-associated coagulopathy, initially, patients present with elevated levels of fibrinogen and D-dimer, with minimal changes in prothrombin time and platelet count. The main risk factor for the development of pulmonary embolism is the increase in D-dimer that is associated with the development of PTE. The administration of iodine-based contrast agent to patients with COVID-19 would affect P-creatinine and renal function, where Ultrasound is viewed as cost-effective and highly portable, can be performed at the bedside.

Conclusion. Acute respiratory distress syndrome severity in patients with COVID-19 can explain PTE as a consequence of an exaggerated immune response.

Keywords. acute respiratory distress syndrome, COVID-19, pathophysiology, pulmonary embolism, thromboembolism

Corresponding author: Yethindra Vityala, e-mail: yethindravityala10@gmail.com

Received: 16.03.2022 / Revised: 31.03.2022 / Accepted: 11.04.2022 / Published: 30.06.2022

Vityala Y, Krishna A, Pandla D, et al. *Pathophysiology of thromboembolism in patients with COVID-19*. Eur J Clin Exp Med. 2022;20(2):212–216. doi: 10.15584/ejcem.2022.2.11.

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in December 2019 in Wuhan, China, and the number of COVID-19 cases continues to increase, causing global medical, social, and economic issues.[1-3]

In severe cases, COVID-19 can be complicated by the acute respiratory distress syndrome (ARDS), sepsis and septic shock, multiorgan failure, including acute kidney injury and cardiac injury.[4] ARDS causes lung injury that occurs in response to various events and is characterized by inflammation, increased lung vascular permeability, and lung tissue with reduced aeration. Reduced lung compliance is linearly related to impaired oxygenation indicates loss of aeration leading to hypoxemia (a hallmark of ARDS pathophysiology).[5] A small number of critically ill patients with COVID-19 develop thromboembolism (arterial or venous), both micro- and macrovascular complications such as deep vein thrombosis, pulmonary embolism (PE), and pulmonary arterial thrombosis. Thromboembolic events (TEE) are a serious complication that produces an increase in morbidity and mortality.[6]

Aim

Along with the measures used to stop the spread of infections, a useful strategy was the massive acquisition and execution of diagnostic tests. The objective of the study is to describe the pathophysiology of venous thromboembolism in patients with COVID-19.

Material and methods

In this article a narrative review regarding pathophysiology of thromboembolism in patients with COVID-19.

Analysis of the literature

Etiopathogenesis of COVID-19-associated coagulopathy

The development of coagulopathy is a consequence of the intense inflammatory response associated with hypercoagulability, platelet activation, and endothelial dysfunction. Coagulopathy is mainly thrombotic, initially in the lung and later systemic, micro- and macrovascular complications associated with endothelial damage, inflammation, neutrophil extracellular traps (TENs), macrophage activation, and cytokine storm that perpetuate the vicious circle of thrombosis and inflammation (Fig. 1). The pathophysiology of thrombotic complications is complex, there are prothrombotic risk factors, including a severe inflammatory response with the production of cytokines, hypoxia, prolonged immobilization, and disseminated intravascular coagulation.[12]

Immune response

SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) receptors to infect lung cells, similarly, tissue damage occurs in endothelial cells, releasing cytokines, interleukin IL-1, IL-2, IL-6, and tumor necrosis factor-alpha that activate the coagulation cascade. During the early stage of COVID-19, ACE2 decreases followed by an increase in plasma angiotensin 2 increases, which in turn leads to vasoconstriction, platelet activation, and cytokine release syndrome.[13] ACE2 expression is predominantly seen in pneumocytes, in the endothelium,

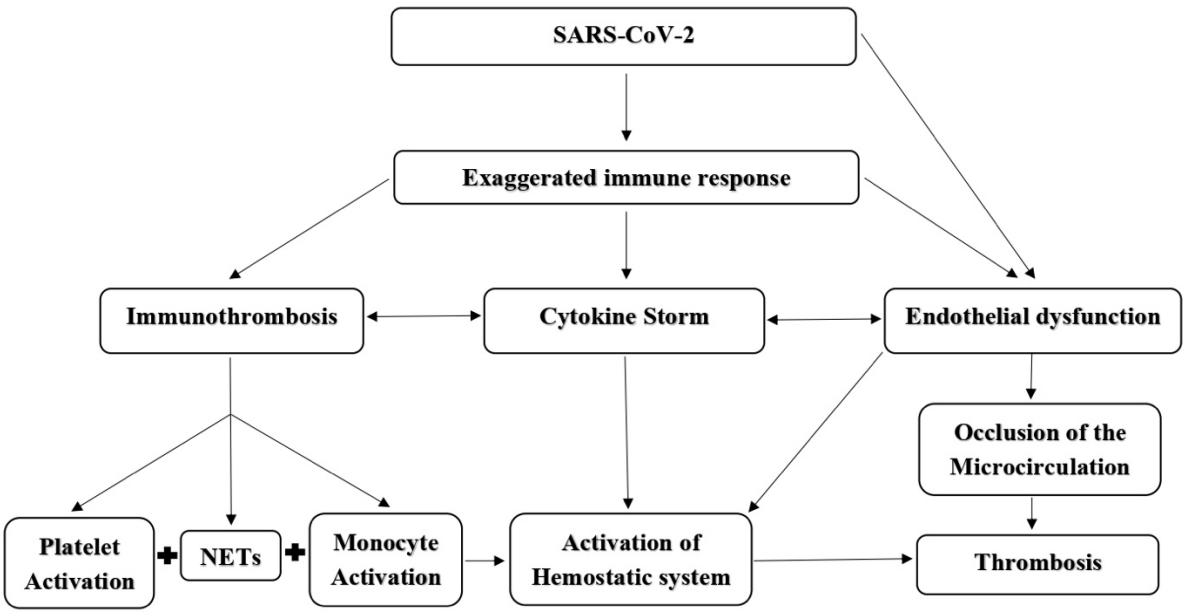

Fig. 1. Schematic representation of the mechanism responsible for the activation of the hemostatic system and thrombosis after SARS-CoV2 infection

and macrophages, apart from lung ACE2 expression are also observed in respiratory epithelial cells, enterocytes, and smooth muscle cells.[14]

SARS-CoV-2 penetrates alveolar epithelial cells through the ACE2 receptor. Viral replication triggers complement activation, with the formation of C3a and C5a, including neutrophils, macrophages, lymphocytes, and monocytes, responsible in turn for the massive release of IL1, IL-6, IL-8, and interferons that favor the tissue factor and thrombomodulin expression and endothelial adhesion molecules, which activates fibrinolysis.[15] The increased expression of tissue factor and thrombomodulin is responsible for the activation of the hemostatic system. An endothelial and platelet activation causes an imbalance between thrombin with fibrin deposition, microangiopathy, and tissue damage.

Thrombosis

Thrombosis is a hemostatic response that leads to the formation of thrombi that obstruct blood flow. An embolism occurs when the clot breaks from the site of origination, travels through the bloodstream, and blocks a blood vessel in another tissue or organ. The alpha-granules of activated platelets release platelet factor 4, attracts and activates neutrophils leading to the formation of NETs and followed by leukocyte-platelet aggregates.[16]

COVID-19 and thromboembolism

COVID-19 predisposes to venous thromboembolism (VTE) with pulmonary thromboembolism (PTE) and reported in autopsies of patients with COVID-19.[16] PTE occurs between 9-12 days after the onset of symptoms and even 23% of patients received thromboprophylaxis developed PTE. So far, there is no evidence linking the development of PE and longer intubation time in critically ill patients with COVID-19.[18] The pathophysiology that relates PTE with COVID-19 is associated with a hypercoagulable state. The systemic inflammatory response syndrome and endothelial dysfunction is associated with activation of coagulation, increased thrombin generation, and decreased anticoagulation. In 11.6% of hospitalized patients with COVID-19 despite thromboprophylaxis, VTE is reported and increased to 15.7% in hospitalized patients with COVID-19 admitted to the intensive care unit.[19] The prevalence of PTE in patients with COVID-19 is 16% higher than in those patients without COVID-19.[20] PTE is suspected in hospitalized patients presenting dyspnea, decreased oxygen requirement, hemodynamic instability, and dissociation between hemodynamic and respiratory changes.

Pathogenesis of COVID-19-associated coagulopathy

In COVID-19-associated coagulopathy, initially, patients present with elevated levels of fibrinogen and D-dimer, with minimal changes in prothrombin time and platelet count. Coagulopathy can be diagnosed by increased D-dimer, elevated levels of fibrinogen (initially fibrinogen increases in the acute phase and later decreases to <100 mg/dL in patients with poor prognosis). Prothrombin time, partial thromboplastin time, and platelet count are within normal limits.[21] The main risk factor for the development of PE is the increase in D-dimer and an increase in D-dimer >6 μg/mL is associated with the development of PTE with an OR 4.8 (CI 3.2-7.2). The elevation of D-dimer is associated with a disease progression from mild or moderate stage to a more severe disease stage, with intensive care unit admission and an increase in mortality due to the fibrinolysis in the airway and the alveoli of the lung. In this extravascular fibrinolysis, inflammatory cells invade lung tissue because of fibrin deterioration and increased D-dimer levels, which justifies the correlation with the severity of the lung disease and not only due to TEE. The concentration of D-dimer, a protein fragment present in the blood resulting from clot degradation that is commonly found in patients with the suspected thrombotic disorder and significantly increased in the patients with ARDS.[22]

Non-hospitalized immobilized patients with COVID-19 had a cumulative effect on TEE risk but hospitalization can also lead to TEE and PE in non-immobilized patients.

Computerized axial tomography scan

Computed Tomography (CT) pulmonary angiogram is the investigation of choice in patients with suspected PE because it allows adequate visualization of the pulmonary arteries till the subsegmental sections (Fig. 2).

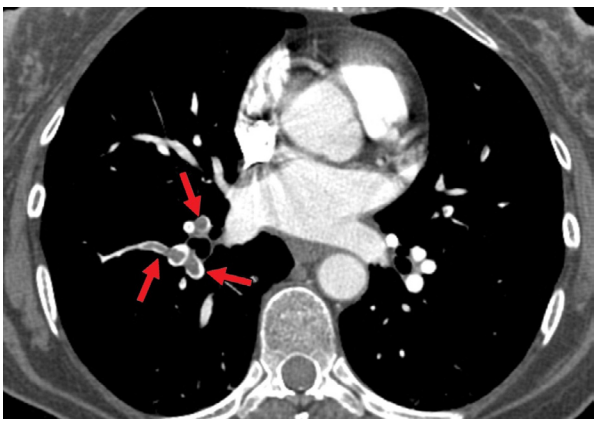

Fig. 2. Computed Tomography pulmonary angiogram showing acute pulmonary embolism (red arrows) in right lower lobe pulmonary arteries

Most number of emboli were observed in segmental section (51%), lobar (31%), central (13%) and subsegmental 5.5%.[23] CT has 83% sensitivity and 96% specificity for the diagnosis of PE and showed a high

negative predictive value of CT angiography. The administration of iodine-based contrast agent to patients with COVID-19 would affect P-creatinine and renal function. Moreover, critically ill patients with COVID-19 are at increased risk of contrast-induced acute kidney injury but acute kidney injury was not associated with the administration of iodinated contrast material.[24] Compared to CT, Ultrasound is viewed as cost-effective and highly portable, can be performed at the bedside, therefore a promising alternative to address these requirements.[25] Currently, extremity venous Doppler ultrasound for deep vein thrombosis is recommended for symptomatic patients and bedside echocardiography helps diagnose PE-associated findings (right ventricular dilatation or dysfunction and intracardiac thrombus), indicating a clot-in-transit.[26]

Conclusion

ARDS severity in patients with COVID-19 can explain PTE as a consequence of an exaggerated immune response.

Declarations

Funding

No financial support was received for the study.

Author contributions

Conceptualization, Y.V., A.K., D.P., K.P.K. and J.S.; Formal Analysis, K.P.K., J.S., H.V.B., T.K. and M.S.K.; Writing – Review & Editing, Y.V., A.K., D.P., K.P.K., J.S., H.V.B., T.K., and M.S.K.

Conflict of interest

The authors declare no conflicts of interest.

Data availability

Data are available from the corresponding author upon reasonable request.

References

1. Bassetti M, Vena A, Giacobbe DR. The novel Chinese coronavirus (2019-nCoV) infections: challenges for fighting the storm. *Eur J Clin Invest*. 2020;50:e13209.
2. CDC. About COVID-19. Centers for Disease Control and Prevention; 2019. Available from: https://www.cdc.gov/coronavirus/2019-ncov/index.html. Accessed: 2020 Dec 08.
3. WHO. Coronavirus. World Health Organization; 2019. Available from: https://www.who.int/health-topics/coronavirus. Accessed: 2021 Dec 08.
4. 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.
5. Gattinoni L, Pesenti A, Avalli L, Rossi F, Bombino M. Pressure-volume curve of total respiratory system in acute respiratory failure. Computed tomographic scan study. *Am Rev Respir Dis*. 1987;136(3):730-736.
6. Carsana L, Sonzogni A, Nasr A, et al. Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study. *Lancet Infect Dis*. 2020;20(10):1135-1140.
7. Jin X, Lian JS, Hu JH, et al. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut*. 2020;69(6):1002-1009.
8. Espy MJ, Uhl JR, Sloan LM, et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. *Clin Microbiol Rev*. 2006;19(1):165-256.
9. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020;25(3):2000045.
10. Corman VM, Müller MA, Costabel U, et al. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. *Euro Surveill*. 2012;17(49):20334.
11. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA*. 2020;323(18):1843-1844.
12. Wu D, Yang XO. TH17 responses in cytokine storm of COVID-19: An emerging target of JAK2 inhibitor Fedratinib. *J Microbiol Immunol Infect*. 2020;53(3):368-370.
13. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and anticoagulation. *Blood*. 2020;135(23):2033-2040.
14. Bertram S, Heurich A, Lavender H, et al. Influenza and SARS-coronavirus activating proteases TMPRSS2 and HAT are expressed at multiple sites in human respiratory and gastrointestinal tracts. *PLoS One*. 2012;7(4):e35876.
15. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: A review. *Clin Immunol*. 2020;215:108427.
16. Middleton EA, He XY, Denorme F, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. *Blood*. 2020;136(10):1169-1179.
17. Llitjos JF, Leclerc M, Chochois C, et al. High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. *J Thromb Haemost*. 2020;18(7):1743-1746.
18. Al-Ani F, Chehade S, Lazo-Langner A. Thrombosis risk associated with COVID-19 infection. A scoping review. *Thromb Res*. 2020;192:152-160.
19. Cui S, Chen S, Li X, Liu S, Wang F. Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. *J Thromb Haemost*. 2020;18(6):1421-1424.
20. Lodigiani C, Iapichino G, Carenzo L, et al. Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. *Thromb Res*. 2020;191:9-14.
21. López-Reyes R, Oscullo G, Jiménez D, Cano I, García-Ortega A. Thrombotic Risk and Covid-19: Review of Current Evidence for a Better Diagnostic and Therapeutic Approach. *Arch Bronconeumol*. 2021;57:55-64.

22. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N Engl J Med.* 2020;383(2):120-128.
23. Grillet F, Behr J, Calame P, Aubry S, Delabrousse E. Acute Pulmonary Embolism Associated with COVID-19 Pneumonia Detected with Pulmonary CT Angiography. *Radiology*. 2020;296(3):186-188.
24. Mathews BK, Koenig S, Kurian L, et al. Clinical Progress Note: Point-of-Care Ultrasound Applications in COVID-19. *J Hosp Med*. 2020;15(6):353-355.
25. Lu W, Zhang S, Chen B et al. A Clinical Study of Noninvasive Assessment of Lung Lesions in Patients with Coronavirus Disease-19 (COVID-19) by Bedside Ultrasound. *Ultraschall Med.* 2020;41(03):300-307.
26. Gogna A, Yogendra P, Lee SHE, et al. Diagnostic ultrasound services during the coronavirus disease (COVID-19) pandemic. *Am J Roentgenol*. 2020;215(5):1130-1135.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.11

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 217–223

REVIEW PAPER

Klaudia Nawrot [1], Ewelina Polak-Szczybyło [1,2,3], Agnieszka Ewa Stępień [1,2,3]

Characteristics of the health-promoting properties of *Cornus mas*

[1] Dietitians' Scientific Club section Food safety at Department of Dietetics, Institute of Health Sciences, College of Medical Sciences, University of Rzeszow, Rzeszów, Poland
[2] Centre for Innovative Research in Medical and Natural Sciences, Laboratory of Innovative Research in Dietetics, College of Medical Sciences, University of Rzeszow, Rzeszów, Poland
[3] Department of Dietetics, Institute of Health Sciences, College of Medical Sciences, University of Rzeszow, Rzeszów, Poland

ABSTRACT

Introduction and aim. The medicinal properties of *Cornus mas L.* have been used in European and Asian folk medicine for many centuries in the prevention and treatment of many diseases. The high biological activity of the plant results primarily from the presence of valuable ingredients, including anthocyanins, flavonoids and iridoid compounds. The aim of the article is to present the role of bioactive ingredients present in *C.mas* that determine its health-promoting properties.

Material and methods. Review and analysis of the scientific literature.

Analysis of the literature. The summarize information about in the field of phytochemical properties and therapeutic effects, among others anticancer, antidiabetic, neuroprotective, cardioprotective and antibacterial**.**

Conclusion. The results of many in vitro and in vivo scientific studies They indicate the possibility of the potential use of Cornelian cherry to obtain valuable nutraceutical and pharmacological substances

Keywords. bioactive compounds, cornelian cherry, *Cornus mas* L., medicinal plant

Introduction

Cornelian cherry (*Cornus mas* L.,) is a fruit plant belonging to the *Cornaceae* family is a tall shrub or a small tree. It occurs naturally mostly in central and south-eastern Europe as well as south-central and western Asia. The latin name *Cornus mas* L. comes from the word „*Cornu*" - horn, and „*maschile*" - hardwood. The beautiful yellow flowers of the edible dogwood develop before the leaves appear and are decisive for its classification as ornamental plants. Ripe, small stone fruits with a dark or cherry-red color, and less often pink or yellow with a sweet and sour taste are used in the kitchen to prepare, among others. marmalades, wines, tinctures.[1,2] Flowers, leaves and fruit have been used in folk medicine for many years due to their numerous medicinal and nutritional values (Fig. 1).[3]

The high biological value results mainly from the content of biologically active substances, mainly antioxidants that determine the antioxidant activity.

The conducted phytochemical studies of Cornelian cherry fruits indicate the presence of numerous valuable bioactive ingredients from the group of polyphenolic compounds (mainly anthocyanins, flavonoids and phenolic acids), triterpenoids and pectin.[4-8] Anthocyanins also affect the color of Cornelian cherry fruits, and the total content of anthocyanins ranges from 35 to 300 mg/100g depending on the color of the fruit. The amount of anthocyanins in the skin fruit can reach even 650-850 mg/100g, while in the flesh it contains only 35-120 mg/100g.[8]

Corresponding author: Ewelina Polak-Szczybyło, e-mail: ewelina.polak@onet.pl

Received: 1.10.2021 / Revised: 8.02.2022 / Accepted: 14.02.2022 / Published: 30.06.2022

Nawrot K, Polak-Szczybyło E, Stępień AE. *Characteristics of the health-promoting properties of Cornus mas*. Eur J Clin Exp Med. 2022;20(2):217–223. doi: 10.15584/ejcem.2022.2.11

Whereas *C. mas* leaves contain 113-117 mg/g dry weight of polyphenolic compounds, and flavonols 33-35 mg/g dry weight but no anthocyanins.[9] The richest source of flavonoids are flowers.[10] The presence of other bioactive ingredients was also found in *C. mas* varieties, such as ascorbic acid and iridoid compounds, mainly loganic acid.[11-15]

Fig. 1. *Cornus mas* L. fruit (photograph by Maria Strzępa)

The content of vitamin C varies greatly depending on the variety and ranges from 50 to 100 mg/100 g, and some varieties may even contain 200 mg/100 g of ascorbic acid.[1,13-17] Carotenoids and fatty acids are also present.[17] The largest amount of fat is in the seeds (4.5%) and the main fatty acid is linoleic acid.[7]

The body's defense mechanism reduces the free radicals formed in the body. Long-term, too high level of free radicals that are not neutralized in the body by the antioxidant mechanism is conducive to the formation of oxidative stress.[18] Oxidative stress increases the risk of developing diseases, including cancer, metabolic disorders, and the antioxidants present in the diet prevent the formation of oxidative stress affecting human health.[19]

The presence of bioactive compounds, mainly antioxidants, in *C. mas* determines its biological activity. *C. mas* showing high antioxidant activity, i.e. the ability to neutralize free radicals thanks to numerous bioactive compounds determined by the DDPH, ABTS and FRAP tests.[9,15, 20-26]

Aim

This article presents the results of *in vitro, in vivo* and human studies determining the pro-health effects of *C. mas*: antitumor, supporting diabetes treatment, cardioprotective and antihyperlipidemic, hepatoprotective, neuroprotective, and antimicrobial. Modern scientific research also focuses on important aspects of phytochemical analysis, evaluation of the pharmacological aspect, toxicology of extracts from fruits, leaves, flowers of *C. mas* for the possibility of their use as a safe medicine or supplement for patients.

Material and methods

Review and analysis of the scientific literature.

Analysis of the literature

Antitumor activity

The results of scientific research indicate that these natural substances, among others phenolic compounds, waveguides present in fruits, leaves and flowers of *C. mas* influence the growth of neoplastic cells *in vitro*.[27-29] By inhibiting the proliferation of neoplastic cells (cytostatic effect) and reducing their survival due to induction of apoptosis (cytotoxic effect), they determine the antitumor and cytotoxic properties of *C. mas*.[30-32]

In their research, Savikin et al. assessed the cytotoxicity, antioxidant properties and chemical composition of methanol extracts from *Cornus mas* leaves and flowers. The cytotoxicity was determined on the basis of the metabolic activity of the cells determined by the MTT test. They showed that methanol extracts at a concentration of 200 μg/mL from *C. mas* flowers or leaves inhibited the growth of human cervix adenocarcinoma cells (HeLa) and human colon carcinoma (LS174) cells lines after 72 h of incubation.[33]

In vitro studies by the Yousefi research team demonstrated cytotoxicity of 80% ethanol extracts from *C.mas* fruit against human ovarian cancer cells (SKOV3), breast adenocarcinoma cells (MCF-7), prostate adenocarcinoma cells (PC-3) and lung non small cell cancer (A549). The analyzed doses in the concentration range of 5, 20, 100, 250, 500, 1000 μg/mL showed a high cytotoxic effect on the cells of all types of tested neoplasms.[34] Scientific results of Forman et al. they also showed inhibition of the viability of the aqueous extract of *C. mas* fruit against the MCF-7 cell line.[35] In contrast, *C. mas* juice with a concentration of 0.007-1% shows cytotoxic activity against human liver carcinoma cells (HepG2), human colon cancer cells (Caco2 and HT-29).[36]

Antidiabetic activity

Preparations based on *C. mas* reduce the level of glucose in the blood and increase the sensitivity of tissues to insulin. The results of the research proved the relationship between the presence of polyphenols, such as anthocyanins and flavonoids in fruits, and the inhibition of α-glucosidase activity and the reduction of postprandial blood glucose levels.[37-39]

The studies assessed the effect of an extract derived from the fruit of *C. mas* on glycemic control in people with type 2 diabetes. It has been shown that their daily consumption of these extracts increases insulin levels and thus improves glycemic control. In addition, it also reduces the level of serum triglycerides, which are often abnormal in people with type 2 diabetes.[40] On the other hand, ursolic acid present in *C.mas* fruit causes glucose uptake by activating the insulin receptor.[41]

Cardioprotective activity

Research results indicate an important role of Cornelian cherry fruit in the treatment of metabolic disorders. The results of scientific studies indicate a close relationship between the antioxidant properties of *C. mas*, resulting from the presence of anthocyanins and iridoids in fruits, and the cardioprotective and antihyperlipidemic activity in the human body.

Atherosclerosis is recognized as a civilization disease and the main cause of death all over the world. Due to their valuable pro-health properties, anthocyanins, which are phytochemicals, effectively prevent the development of dyslipidemia.[38,42] The research results indicate that the antioxidant and anti-inflammatory properties of *C. mas* may have a positive effect on the health of patients with hypercholesterolaemia.[43] This effect may be due to the presence of anthocyanins, which inhibit the expression of lipogenic enzymes in adipose tissue and liver, and reduce the activity of lipoprotein lipase in visceral adipose tissue and increase it in muscles.[44,45] Sozański et al. in their *in vivo* studies in an animal model showed that the *C. mas* L. fruit lysate present in the diet for 60 days reduced the level of triglycerides in the blood serum by 44% and effective prevention of atherosclerotic changes in the thoracic aorta by activating the expression of PPARα. Additionally, the significant influence of *C. mas* L. on the oxidative stress in the liver induced by the diet and the normalization of the elevated level of pro-inflammatory cytokines in the serum was assessed.[3]

Hosseinpour et al. observed that after administering the dried powder obtained from the fruit of *C.mas* to rats for a period of 4 weeks, a decrease in the level of triglycerides and LDL-C, an increase in HDL-C and an increase in the antioxidant capacity of the liver.[46] Further studies on an animal model confirm the above results of the effect of the dried powder from *C. mas* fruit for 4 weeks to reduce cholesterol and triglycerides.[47]

Subsequent studies focused on the analysis of bioactive compounds present in *C.mas* fruits and their influence on the activity of pancreatic lipase. Iridoids (loganic acid, cornuside) and anthocyanins (pelargonidin 3-O-galactoside) were identified in the fruit extract of *C. mas* using the HPLC-DAD-MS (high-performance liquid chromatography coupled with diode-array detection and tandem mass spectrometry techniques) technique. A subfraction of *C.mas* extract at a concentration of 7.5 μg/mL which contained pelargonidine 3-O-galactoside decreased pancreatic lipase activity by 28.3±1.5%, but loganic acid and cornucid did not inhibit it. The results of the research suggest that the ability of *C. mas* fruit to inhibit the activity of pancreatic enzymes and their use in the prevention of hyperlipidemia.[48]

In the scientific literature, there is a link between endocrine hormones and metabolic diseases. Cortisol plays a significant role in the metabolism of cholesterol.[49] In animal studies, plasma cortisol levels decreased after the introduction of *C.mas* fruit supplementation.[50] Following the introduction of *C. mas* fruit supplementation to the daily diet of children and adolescents with dyslipidemia for 6 weeks, an improvement in the lipid profile and vascular inflammation was observed.

Research by the team of Asgary et al. and Rafieian-Kopaei et al. indicate a very valuable anticoagulant effect of *C. mas*. The results of *in vivo* studies on an animal model showed that consumption of *C. mas* dried fruit powder may be beneficial in the group of patients with atherosclerosis due to its lowering effect on plasma fibrinogen levels.[52,53]

Gholamrezayi et al. in their research on the effect of *C. mas* fruit extract on lipid profiles and leptin parameters in postmenopausal women. After 8 weeks of daily supplementation, the weight of the group of women did not change, as did the waist circumference, the ratio of LDL/HDL and TC/HDL and the leptin concentration, but the HDL levels increased compared to the control group.[54]

Hepatoprotective activity

The metabolism of xenobiotics in the body can lead to liver damage, resulting in carcinogenic transformation or inflammation. *In vivo* studies on an animal model have proven the protective effect of *C. mas* fruit extract on the liver by maintaining its biological functions and reducing the degree of damage.[55-58]

Alavian et al. demonstrated the protective effect of *C. mas* fruit extract, rich in antioxidants and anthocyanins, on the liver of male rats against hepatotoxicity induced by CCl_4. After 14 days of oral administration of *C. mas* fruit extract (200 and 500 mg/kg body weight/d) to these rats, they observed a reduction in elevated levels of the following enzymes: aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).[55]

In subsequent *in vivo* studies, the protective effect of *Cornus mas* fruit extract on the liver of healthy rats was indicated. These extracts (50, 200 and 400 mg/kg body weight) administered daily for 3 weeks to rats caused a decrease in the levels of the enzymes: AST, ALT and ALP in the blood serum at doses of 200 and 400 mg/kg. There were also no pathological changes in the liver tissues.[56]

Some drugs used in the treatment of cancer and autoimmune diseases cause side effects by toxic to the liver and lead to its damage. Scientists have attempted to evaluate the medicinal effect of *C. mas* fruit extract on drug-induced liver damage.

In vivo studies were conducted with the administration of the anti-cancer drug Methotrexate to hepatoxic rats. Rats were simultaneously administered the drug and the extract of freeze-dried fruit *C. mas* (300, 700, 1400 mg/kg body weight). The levels of some enzymes in the liver were determined: direct and indirect total bilirubin, AST, ALT, ALP, and lactate dehydrogenase (LDH). It was observed that the dose of 700 and 1400 mg/kg of body weight of *C. mas* extract significantly counteracted the changes caused by this drug, including: the levels of AST, ALT, ALP enzymes; direct bilirubin and LDH demonstrating a hepatoprotective effect.[57]

On the other hand, Abasi et al. undertook an evaluation of the effect of *C. mas* fruit extracts (CMFE) on liver changes caused by the anticancer drug cisplatin. The drug administered to rats caused a decrease in the antioxidant activity of the liver enzymes: SOD, GPx, TAC and CAT, and increased the activity of MDA and decreased the levels of AST, ALT and ALP in the serum. For 16 days, the administration of freeze-dried fruit extract of *C. mas* (300 and 700 mg/kg body weight) to rats increased the level of antioxidant activity of liver enzymes and enzymes in the serum in relation to changes caused by the action of cisplatin. It was also observed that after treatment with *C. mas* extract, the histological structure of the liver tissue was close to that of normal histology. The content of antioxidants and phenolic compounds in *C. mas* fruit counteracts oxidative stress and histological changes in the liver in rats caused by the action of this drug.[58]

Neuroprotective activity

Neurological disorders are caused by excess free radicals in the body. The excess of free radicals promotes the development of oxidative stress negatively affecting the human body, especially brain tissue.[59] *C. mas* as a rich source of antioxidants from the group of polyphenols mainly flavonoids, anthocyanins and vitamin C may reduce the level of free radicals. Flavonoid derivatives as antioxidants play an important role in pathophysiology of many diseases, including neurological disorders (eg. Alzheimer's disease). Resveratrol, belonging to the flavonoid group, protects neurons from the toxic effects of beta-amyloid protein, which plays a role in the development of Alzheimer's disease, and reduces the frequency of neuronal death in the hippocampus.[59] In contrast, catechin and epicatechin flavonoids protect brain cells against damage caused by free radicals. Vitamin C actively participates in the process of myelination of the brain and is a neuromodulator.[59] As an antioxidant, it reduces lipid peroxidation and strengthens the structure of cell membranes. neuroprotective.[3,4,22,60]

Francik et al. evaluated the effect of freeze-dried *C. mas* fruit on the diets of control, fructose and high fat rats. The addition of dried fruit of *C. mas* to the diet led to an increase in the activity of catalase (an antioxidant enzyme) present in the brain tissue and inhibition of the process of protein oxidation, leading to a reduced amount of carbonyl and thiol groups in the brain tissue and plasma.[59] The results of studies assessing the activity of paraoxonase-1 (PON1) in rats after a 5-week diet with 10% of *C.mas* fruit juice showed its increase in plasma, but not in the liver. The increase in the activity of PON1 protects the LDL fraction and prevents its oxidation caused by oxidative stress. Because PON1 is an enzyme that plays a role in protecting against oxidation of, inter alia, two cholesterol fractions; LDL and HDL ensure the hydrolysis of activated phospholipids and lipid peroxides.[61]

The results of the research show that *C. mas*, a rich source of antioxidants, can be used to support treatment and to prevent neurological diseases.

Antimicrobial activity

The results of scientific works also indicate the antimicrobial properties of *C. mas*.[9,62-65] The results indicate that after the incubation of water extracts of *C. mas* fruit with the strains of bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* causing infectious diseases, their growth was inhibited.[64] Milenković-Andjelković et al. analyzed the antibacterial and antifungal activity of fruit, leaves and seeds, bark of *C. mas*. They observed that only methanol and ethanol extracts from seeds, leaves and bark showed antimicrobial activity. The greatest effect was shown by leaf extracts against the bacteria *S. aureus* and the fungus *Candida albicans* compared to methanol extracts from seeds and bark of *C. mas*.[24] Subsequent studies indicate high antimicrobial activity against *Bacillus subtilis, B. cereus, Escherichia coli* and *Serratia marcescens* juice, methanolic and aqueous fruit extracts of *C. mas*.[66]

Krzystak et al. assessed the antimicrobial potential of extracts derived from seeds, leaves, bark and fruit. Among the tested microorganisms, the most susceptible strains were: *S. aureus, E. coli* and the fungus *C. albicans*.[67] Additionally, Antolak et al. indicated that phytochemicals found in plant raw materials may re-

duce the adhesion bacteria of the genus *Asaia spp.* present in food products and juices. Identified by HPLC and LC-MS techniques in the juice of *C. mas* anthocyanins (cyanidin-3-glucoside: 0.28 ± 0.039 μg/mL, petunidin-3-glucoside: 0.380±0.052 μg/mL, cyanidin-3-robinobioside: 0.321±0.041 μg/mL, pelargonidin-3-robinobioside: 0.302±0.022 μg/mL) and phenolic compounds (quercetin, quercetin-3-glucoside, myricetin-3-galactoside) prevent the loss of the organoleptic quality of food and plant preparations [68]

Toxic activity

It is very important to define the safe amount of use of medicinal products derived from *C. mas* for patients as a herbal medicine. The results of clinical trials with human subjects indicate that daily consumption of 100 g of fresh *C. mas* fruit for 6 weeks does not show any toxic effects on the human body.[51] Subsequent studies investigated the safety of taking anthocyanins isolated from *C. mas* as supplements. They showed no adverse effects at the amount of 600 mg/day taken by adult diabetic patients over a period of 6 weeks. [40]

Conclusion

C. mas is a very popular plant in Europe and Asia with high biological activity. Its beneficial pro-health effects are confirmed by the results of many *in vitro* and *in vivo* scientific studies presented in this article. They indicate the possibility of the potential use of Cornelian cherry to obtain valuable nutraceutical and pharmacological substances. However, it is necessary to conduct further scientific research, mainly clinical on a large study group and long-term, together with an assessment of possible levels of toxicity, in order to consider leaves, fruits, flowers as a safe herbal medicine. It is also important to analyze the aspect of interaction of *C. mas* extracts with drugs already used in the treatment of a given disease entity.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, K.N., E.P.S. and A.E.S.; Writing – Original Draft Preparation, K.N., E.P.S., A.E.S.; Writing – Review & Editing, E.P.S. and A.E.S.

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. Klimenko S. The cornelian cherry (*Cornus mas L.*): collection, preservation, and utilization of genetic resources. *J Fruit Ornam Plant Res.* 2004;12:93-98.
2. Brindza P, Brindza J, Tóth D, Klimenko O, Grigorieva O. Slovakian cornelian cherry (*Cornus mas L.*): Potential for cultivation. *Acta Hortic (ISHS).* 2007;760:433-437.
3. Sozański T, Kucharska AZ, Szumny A, et al.The protective effect of the *Cornus mas* fruits (cornelian cherry) on hypertriglyceridemia and atherosclerosis through PPARα activation in hypercholesterolemic rabbits. *Phytomedicine*. 2014;21:1774-1784.
4. Dinda B, Kyriakopoulos AM, Dinda S, et al. *Cornus mas L.* (cornelian cherry), an important European and Asian traditional food and medicine: Ethnomedicine, phytochemistry and pharmacology for its commercial utilization in drug industry. *J Ethnopharmacol*. 2016;193:670-690.
5. Czerwieńska ME, Melzig MF. *Cornus mas* and *Cornus officinalis*—Analogies and Differences of Two Medicinal Plants Traditionally Used. *Front Pharmacol*. 2018; 9:894.
6. Jaćimović V, BoŽović D, Ercisli S, et al. Some fruit characteristics of selected cornelian cherries (*Cornus mas L.*) from Montenegro. *Erwerbs-Obstbau*. 2015; 57:119-124.
7. Bilejić SM, Golšin BR, Todorović NJ, et al. Physicochemical fruit characteristics of cornelian cherry (*Cornus mas L.*) genotypes from Serbia. *Hort Sci*. 2011;46:849-853.
8. Kazimierski M, Regula J, Molska M. Cornelian cherry (*Cornus mas L.*) – characteristics, nutritional and pro-health properties. *Acta Sci Pol Technol Aliment*. 2019;18(1):5-12.
9. Milenković-Andjelković AS, Andjelković MZ, Radovanović AN, et al. Phenol composition, DPPH radical scavenging and antimicrobial activity of Cornelian cherry (*Cornus mas*) fruit and leaf extracts. *Hem Ind.* 2015;69:331-337.
10. Stankovic MS, Zia-Ul-Haq M, Bojovic BM, Topuzovic MD. Total phenolics, flavonoid content and antioxidant power of leaf, flower and fruits from Cornelian cherry (*Cornus mas L.*). *Bulg J Agric Sci*. 2014;20:358-336.
11. West BJ, Deng S, Jensen CJ, et al. Antioxidant, toxicity, and iridoids tests of processed Cornelian cherry fruits. *Int J Food Sci Technol.* 2021;47:1392-1397.
12. Kucharska AZ, Szumny A, Sokół-Łetowska A, et al. Iridoids and anthocyanins in cornelian cherry (*Cornus mas L.*) cultivars. *J Food Comp Anal*. 2015;40:95-102.
13. Biaggi DM, Donno D, Mellano MG, et al. *Cornus mas (L.)* Fruit as a Potential Source of Natural Health-Promoting Compounds: Physico-Chemical Characterisation of Bioactive Components. *Plant Foods Hum Nutr*. 2018;73:89-94.
14. Boris P, Stajner DI, Keversan S, et al. Antioxidant capacity of cornelian cherry (*Cornus mas L.*) - Comparison between permanganate reducing antioxidant capacity and other antioxidant methods. *Food Chem*. 2012;134(2):734-741.
15. Piórecki N. Dereń jadalny (*Cornus mas L.*) – właściwości i możliwości. *Szkółkarstwo*, 2007;3:86-88.

16. Guleryuz M, Bolat I, Pirlak L. Selection of table cornelian cherry (*Cornus mas L.*) types in Coruh Valley. *Turk J Agric Forestry*. 1998;22:357-364.
17. Pantelidis GE, Vasilakakis M, Manganaris GA, et al. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, red currants, gooseberries and cornelian cherries. *Food Chem*. 2007;102:777-783.
18. Horváth G, Turcsi E, Molnár P, et al. Isolation and identification of carotenoids in the fruit of cornelian cherry (*Cornus mas L.*). *Planta Med*. 2007;73:286.
19. Benzie IFF. Evolution of dietary antioxidants. *Comp Biochem Physiol Part A Mol Integr Physiol*. 2003;136(1):113-126.
20. Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. *Eur J Med Chem*. 2021;209:112891.
21. Leja M, Mareczek A, Nanaszko B. Antyoksydacyjne właściwości owoców wybranych gatunków dziko rosnących drzew i krzewów. *Rocz AR Pozn Ogrodn*. 2007;41:327-331.
22. Celep E, Aydin A, Yesilada EA. Comparative study on the *in vitro* antioxidant potentials of three edible fruits: cornelian cherry, Japanese persimmon and cherry laurel. *Food Chem Toxicol*. 2012;50:3329-3335.
23. Popović BM, Štajner D, Slavko K, et al. Antioxidant capacity of cornelian cherry (*Cornus mas L.*)–comparison between permanganate reducing antioxidant capacity and other antioxidant methods. *Food Chem*. 2012;134:734-741.
24. Pyrkosz-Biardzka K, Kucharska A, Sokol-Łetowska A, et al. A comprehensive study on antioxidant properties of crude extracts from fruits of *Berberis vulgaris L., Cornus mas L.* and *Mahonia aquifolium. Nutt Pol J Food Nutr Sci* 2014;64:91-99.
25. Hosu A, Cimpoiu C, David L, et al. Study of the antioxidant property variation of cornelian cherry fruits during storage using HPTLC and spectrophotometric assays. *J Anal Methods Chem.* 2016;2016:2345375.
26. Moldovan B, Filip A, Clichici S, et al. Antioxidant activity of Cornelian cherry (*Cornus mas L.*) fruits extract and the in vivo evaluation of its anti-inflammatory effects. *J Funct Foods*. 2016;26:77-87.
27. Desai SJ, Prickril B, Rasooly A. Mechanisms of Phytonutrient Modulation of Cyclooxygenase-2 (COX-2) and Inflammation Related to Cancer. *Nutr Cancer*. 2018;70(3):350-375.
28. Choi YH, Jin GY, Li GZ, et al. Cornuside suppresses lipopolysaccharide-induced inflammatory mediators by inhibiting nuclear factor-kappa B activation in RAW 264.7 macrophages. *Biol Pharm Bull*. 2011;34(7):959-966.
29. Peyman MK, Maziar SB, Shahin A, et al. Therapeutic uses and pharmacological effects of *Cornus mas* A review. *J Pharm Biomed Sci*. 2014;6:1732-1738.
30. Guy M, John A H. Apoptosis and cancer chemotherapy. *Cell Tissue Resh*. 2000;301:143-152.
31. Ghobrial IM, Witzig TE, Adjei AA. Targeting apoptosis pathways in cancer therapy. *Cancer J Clin*. 2005;55:178-194.
32. Sak K. Cytotoxicity of dietary flavonoids on different human cancer types. *Pharmacogn Rev*. 2014;8(16):122-146.
33. Savikin K, Zdunic G, Jankovic T, et al. *In vitro* cytotoxic and antioxidative activity of Cornus mas and Cotinus coggygria. *Nat Prod Res*. 2009;18:1731-1739.
34. Yousefi B, Abasi M, Abbasi MM, Jahanban-Esfahlan R, Anti-proliferative properties of *Cornus mass* fruit in different human cancer cells. *Asian Pacific J Cancer Prev.* 2015;16(14):5727-5731.
35. Forman V, Haladová M, Grančai D, Ficková M, Antiproliferative activities of water infusions from leaves of five *Cornus L.* species. *Molecules.,* 2015;20(12):22546-22552.
36. Tiptiri-Kourpeti A, Fitsiou E, Spyridopoulou K, Vasileiadis S, Iliopoulos C, Galanis A, Chlichlia K. Evaluation of antioxidant and antiproliferative properties of *Cornus mas L.* fruit juice. *Antioxidants,* 2019;8(9):377.
37. Park CH, Noh JS, Tanaka T, et al. The effects of corni fructus extract and its fractions against alpha-glucosidase inhibitory activities in vitro and sucrose tolerance in normal rats. *Am J Chin Med*. 2011;39:367-380.
38. Asgary S, Rafieian-Kopaei M, Shamsi F, et al. Biochemical and histopathological study of the anti-hyperglycemic and anti-hyperlipidemic effects of cornelian cherry (*Cornus mas L.*) in alloxan-induced diabetic rats. *J Complement Integr Med*. 2014;11:63-69.
39. Shishehbor F, Azemi ME, Zameni D, et al. Inhibitory effects of hydroalcoholic extracts of barberry, sour cherry and Cornelian cherry on α-amylase and α-glucosidase activities. *Int J Pharm Res Allied Sci*. 2016;5:423-428.
40. Soltani R, Gorji A, Asgary S, et al. Evaluation of the Effects of *Cornus mas L.* Fruit Extract on Glycemic Control and Insulin Level in Type 2 Diabetic Adult Patients: A Randomized Double-Blind Placebo-Controlled Clinical Trial. *Evid Based Complement Alternat Med.* 2015;2015:740954.
41. Zhang W, Hong D, Zhou Y, et al. Ursolic acid and its derivative inhibit protein tyrosine phosphatase 1B, enhancing insulin receptor phosphorylation and stimulating glucose uptake. *Biochim Biophys Acta*. 2006;1760(10):1505-1512.
42. Sozański T, Kucharska AZ, Rapak A, et al. Iridoid-loganic acid versus anthocyanins from the *Cornus mas* fruits (cornelian cherry): Common and different effects on diet-induced atherosclerosis, PPARs expression and inflammation. *Atherosclerosis.* 2016;254:151-160.
43. Asgary S, Rafieian-Kopaei M, Shamsi F, et al. The effects of cornelian cherry on atherosclerosis and atherogenic factors in hypercholesterolemic rabbits. *J Med Plants Res.* 2011;5(13):2670-2676.
44. A Tsuda T, Horio F, Uchida K, et al. Dietary cyanidin 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *J Nutr*. 2003;133(7):2125-2130.

45. Lefevre M, Wiles JE, Zhang X, et al. Expression microarray analysis of the effects of grape anthocyanins in mice: a test of a hypothesis-generating paradigm. *Metabolism*. 2008;57(7):52-57.
46. Hosseinpour F, Shomali T, Rafieian-Kopaei M. Hypocholesterolemic activity of cornelian cherry (*Cornus mas L*.) fruits. *J Complement Integr Med*. 2017;14(4). doi:10.1515/jcim-2017-0007
47. Gholipour S, Shomali T, Rafieian-Kopaei M. Anti-hypertriglyceridemic activity of *Cornus mas* in diabetic rats. *J Clin Diag Res*. 2018;12(8):1-5.
48. Świerczewska A, Buchholz T, Melzig MF, Czerwińska ME, *In vitro* α-amylase and pancreatic lipase inhibitory activity of *Cornus mas L*. and *Cornus alba L*. fruit extracts. *J Food and Drug Anals*. 2019;27(1):249-258.
49. Fraser R, Ingram MC, Anderson NH, et al. Cortisol effects on body mass, blood pressure, and cholesterol in the general population. Hypertension. 1999; 33(6):1364-1368.
50. Lofti A, Shahryar A, Rasoolian H. Effects of cornelian cherry (*Cornus mas L*.) fruit on plasma lipids, cortisol, T3 and T4 levels in hamsters. *J Anim Plant Sci*. 2014;24:459-462.
51. Asgary S, Kelishadi R, Rafieian-Kopaei M, et al. Investigation of the lipid-modifying and antiinflammatory effects of *Cornus mas L*. supplementation on dyslipidemic children and adolescents. *Pediatr Cardiol*. 2013;34(7):1729-1735.
52. Asgary S, Rafieian-Kopaei M, Adelnia A, et al. Comparing the effects of lovastatin and *Cornus mas* fruit on fibrinogen level in hypercholesterolemic rabbits. *ARYA Atheroscler*. 2010;6(1):1-5.
53. Rafieian-Kopaei M, Asgary S, Adelnia A, et al. The effects of cornelian cherry on atherosclerosis and atherogenic factors in hypercholesterolemic rabbits. *J Med Plants Res*. 2011;5:2670-2676.
54. Gholamrezayi A, Aryaeian N, Rimaz S, Abolghasemi J, Fallah S, Moradi N, Taghizadeh M. The effect of *Cornus mas* fruit extract consumption on lipid profile, glycemic indices, and leptin in postmenopausal women- a randomized clinical trial. *Phytotherapy Res*. 2019;33(11):2979-2988.
55. Alavian SM, Banihabib N, Es Haghi M, et al. Protective Effect of *Cornus mas* Fruits Extract on Serum Biomarkers in CCl4-Induced Hepatotoxicity in Male Rats. *Hepat Mon*. 2014;14(4):10330.
56. Abbasi M, Abdollahi B, Milani P, et al. Effects of hydro-methanolic extract of *Cornus mas* on histopathological and biochemical parameters of rats' liver and kidney. *Bothalia J*. 2014;44:250-259.
57. Saei H, Hatami H, Azarmi M, et al. Hepatoprotective effect of *Cornus mas* fruits extract on serum biomarkers in methotrexate induced liver injury in male rats. *Pharmacol*. 2016;1:91-98.
58. Abbasi MM, Hassanalilou T, Khordadmehr M, et al. Effects of *Cornus mas* Fruit Hydro-Methanolic Extract on Liver Antioxidants and Histopathologic Changes Induced by Cisplatin in Rats. *Indian J Clin Biochem*. 2020;35(2):218-224.
59. Cooper EL, Ma MJ. Alzheimer disease: clues from traditional and complementary medicine. J. Tradit. *Complement. Med*. 2017;7:380-385.
60. Francik R, Kryczyk J, Krośniak M, et al. The neuroprotective effect of *Cornus mas* on brain tissue of Wistar rats. *Scientific World Journal*. 2014;847368.
61. Francik R, Kryczyk-Kozioł J, Krośniak M, Francik S. Activity of para oxonase 1 and lipid profile in rats fed cornelian cherry or chokeberry in different types of diet. *Act Pol Pharma - Drug Research*, 2017;74(6):1683-1679.
62. Turker A, Yildirim A, Karakas F. Antibacterial and antitumor activities of some wild fruits grown in Turkey. *Biotechnol Biotechnol Eq*. 2012;26:2765-2772.
63. Radovanović BC, Milenković-Andelković AS, Radovanović AB, et al. Antioxidant and antimicrobial activity of polyphenol extracts from wild berry fruits grown in Southeast Serbia. *Trop J Pharmaceut Res*. 2013;12:813-819.
64. Kyriakopoulos AM, Dinda B. *Cornus mas (Linnaeus*) novel devised medicinal preparations: bactericidal effect against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Molecules* 2015;20:11202-11218.
65. Hosseinpour-Jaghdani F, Shomali T, Gholipour-Shahraki A, et al. *Cornus mas*: a review on traditional uses and pharmacological properties. *J Complement Integr Med* 2017;14:20160137.
66. Krisch J, Galgóczy L, Tölgyesì M, Papp T, Vágvölgyi C. Effect of fruit juices and pomace extracts on the growth of gram-positive and gram-negative bacteria. *Acta Biol Szeg*. 2008;52(2):267-270.
67. Krzyściak P, Krosniak M, Gąstoł M, et al. Antimicrobial activity of Cornelian cherry (*Cornus mas L*.). *Postępy Fitoterapii*, 2011;4:227-231.
68. Antolak H, Czyzowska A, Sakač M, et al. Phenolic Compounds Contained in Little-known Wild Fruits as Antiadhesive Agents Against the Beverage-Spoiling Bacteria *Asaia spp. Molecules*. 2017;22(8):1256.

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.12

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 224–231

CASUISTIC PAPER

Katarzyna Fuksa

The importance of electroencephalography in the diagnosis of hepatic encephalopathy due to cirrhosis – a case report

Department of Neurology, MSWiA Hospital in Rzeszow, Rzeszow, Poland

ABSTRACT

Introduction and aim. Hepatic encephalopathy (HE) associated with cirrhosis of the liver is a neuropsychiatric syndrome, with symptoms ranging from barely detectable changes to deep coma. It frequently occurs in the form of episodes and relapses and can be triggered by external factors. HE severity is graded according to the West Haven criteria. The aim of the study is to draw attention to the ever-important and often key role of electroencephalography in the diagnosis of hepatic encephalopathy, even in today's era of increasingly advanced diagnostic methods.

Description of the case. A 57-year-old patient, professionally active at the time, was admitted to the hospital's Neurology Department on an emergency basis due to difficulties with standing and moving, orientation disorders and limb tremor.

Conclusion. While HE pathogenesis is multifactorial, the most important factors include increased brain exposure to ammonia, intestinal dysbiosis, and endotoxemia inducing a systemic inflammatory response. Patient observation, blood laboratory tests, neuropsychological tests and neurophysiological tests (EEG and evoked potentials) play an important role in establishing the diagnosis. Treatment and secondary prevention of hepatic encephalopathy include elimination of triggers and reduction of ammonia production and improvement of its metabolism.

Keywords. ammonia, cirrhosis, electroencephalography, encephalopathy, triphasic waves

Abbreviations: EEG – electroencephalography, HE – hepatic encephalopathy, MRI – magnetic resonance imaging, CT – computed tomography

Introduction

Hepatic encephalopathy (HE) is one of major complications of liver cirrhosis, dramatically impairing the general condition of cirrhotic patients and increasing the risk of death due to hepatic causes. Upon diagnosis of cirrhosis, HE is present in 10-14 of patients, while the risk of the first episode occurring within 5 years is 5-25%.[1,2] During cirrhosis decompensation, ECT is diagnosed in 16-21% of cases. Improvement in liver function usually leads to regression of HE symptoms, although neuropsychiatric changes may sometimes become permanent.[3] The condition was first described in 1893 by Nencki, Pavlov and Zaleski, who found ammonia imbalance caused by portal-systemic anastomosis in dogs to be the reason for neurobehavioral changes (aggression, irritability, ataxia, convulsions and coma).[4]

Ammonia is produced in the intestines through the bacterial metabolism of amines, amino acids, purines and urea. It is produced in smaller amounts in the intestinal epithelium. Under physiological conditions, ammonia that has entered the bloodstream is largely metabolised by the liver; trace amounts of ammonia are metabolised by the skeletal muscles and the brain. Ammonia is converted to urea in the hepatocytes of the peripheral zone of the hepatic lobe, Krebs cycle, and to glutamine in the hepatocytes of the central zone of the

Corresponding author: Katarzyna Fuksa, e-mail: katarzfuk@gmail.com

Received: 10.02.2022 / Revised: 25.02.2022 / Accepted: 27.02.2022 / Published: 30.06.2022

Fuksa K. *The importance of electroencephalography in the diagnosis of hepatic encephalopathy due to cirrhosis – a case report.* Eur J Clin Exp Med. 2022;20(2):224–231. doi: 10.15584/ejcem.2022.2.12

lobe.[5] In decompensated cirrhosis, the liver's capacity for metabolising ammonia decreases by approximately 80%, while its metabolism by the skeletal muscles and the brain increases accordingly.[6,7]

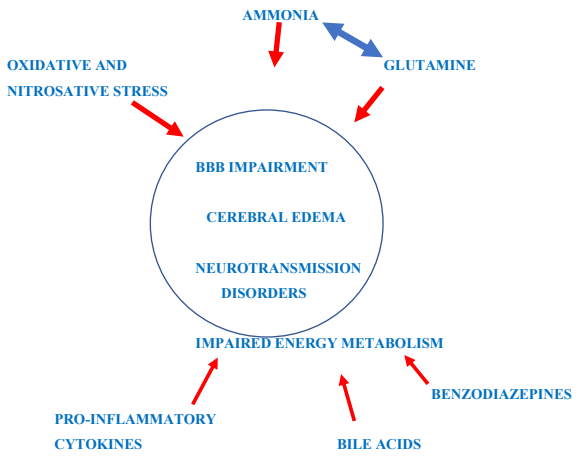

Fig. 1. Disturbances in the circulation of ammonia

As a gaseous substance, ammonia crosses the blood-brain barrier inhibiting cellular membrane potentials and impairing the function of neurotransmitters (Fig. 1). However, ammonia concentration does not correlate with the clinical expression of HE, which suggests involvement of other brain toxic bacterial products, such as mercaptans, γ-aminobutyric acid (GABA), phenols, short- and medium-chain fatty acids and oxindoles.[8,9] The latter, by inhibiting the activity of sodium channels in the brain, may lead to impaired consciousness and coma.

Hepatic encephalopathy covers a whole range of potentially reversible neurological and psychiatric disorders that affect intellectual, cognitive and personality functioning.[10] Patients suffer from, *inter alia*, memory impairment, orientation and concentration disorders, structural apraxia, behavioural changes (irritability, euphoria) and disturbance of the circadian rhythm of sleep.[11] Neurological examination reveals slowness of movement, dysarthria, coarse tremor and/or pyramidal signs. Impaired consciousness may progress from clouding to deep coma without response to stimuli.[12]

HE has been divided into 5 grades of clinical advancement according to the West Haven classification (Table 1).[13] This division is largely subjective as the transition between subsequent stages is continuous rather than staged.

The simplified West Haven classification divides hepatic encephalopathy into latent and overt forms. The latent form includes minimal hepatic encephalopathy (MHE), but also Grade 1 HE, with discreet consciousness disorders, mood changes, impaired focus, impaired attention and problems with performing simple mathematical operations.[14] Characteristic features of MHE are the absence of abnormal neurological examination and discreet cognitive and attention disorders that can only be detected through a neuropsychological examination. This form occurs in over 50% of patients with liver cirrhosis and its diagnosis requires a series of neuropsychiatric tests, preferably two due to the likelihood of impairment of various cognitive functions.[15]

The first symptoms of overt hepatic encephalopathy are confusion and lower motor activity. Coma may occur frequently within several days. Many patients show characteristic trembling and fluttering of the hands (tremor, asterixis) and have unpleasant mouth odour. Trembling tongue or eyelids are equivalents of asterixis. Asterixis is not specific to HE (it may occur even in patients with impaired kidney function, congestive heart failure, after overdosing of certain drugs or in carbon monoxide poisoning) and is a consequence of loss of nervous system control over the motor activity of skeletal muscles. The handgrip strength in patients with

Table 1. Grading of hepatic encephalopathy according to the West Haven criteria

Grade	State of consciousness	Intellect and behaviour	Neurological findings
0	Normal	No or minimum impairment of memory, intellectual function and motor coordination	Normal examination (no asterixis)
1	Sleep-wake disorders – hypersomnia or insomnia	Shortened attention span Lower intellectual function – impaired addition or subtraction; forgetfulness Irritability; alternating euphoria and depression	Mild asterixis Change of handwriting
2	Disorientation Lethargy and apathy	Slurred speed Significantly impaired intellectual function; personality changes; inappropriate behaviour	Obvious asterixis and shaky hands Dysarthria Weakened tendon reflexes
3	Confusion Somnolent but arousable	Inability to perform intellectual tasks Gross disorientation as to time and place Retrograde amnesia; periodic bouts of rage; delusions; incomprehensive speech	Exaggerated tendon reflexes Babinski reflex Nystagmus; extrapyramidal symptoms
4	Coma with or without response to painful stimuli	Impossible to assess (coma)	Decerebration Dilated pupils not reacting to light

asterixis varies between strong and weak. In addition, patients with HE display extrapyramidal symptoms resembling Parkinson's disease (bradykinesia, slowness of speech, hypomimia, tremor, dyskinesia).[16]

Hepatic myelopathy as a symptom of late-stage cirrhosis may mimic HE. Such patients suffer from motor function disorders that prevail over the neuropsychiatric disorders; they may take the form of spastic paraplegia with exorbitant tendon reflexes. In the event of convulsions, severe hyponatraemia should be excluded in the first instance.[17]

The International Society of Hepatic Encephalopathy and Nitrogen Metabolism recommends diagnosis of overt HE only after appearance of disorientation or coarse hand tremor.[18]

Taking into account the duration of symptoms, clinically evident HE was classified into episodic (symptoms recurring after more than 6 months), recurrent (when the subsequent relapse occurs within 6 months) and chronic. Chronic HE occurs on an ongoing basis but its severity fluctuates. In its induced form, hepatic encephalopathy accounts for about 90% of all cases. The most common HE-inducing factors are bacterial infections, diuretics, bleeding, constipation, electrolyte disturbances or therapeutic paracentesis.[16,19]

Diagnosis of HE is primarily based on the patient's medical history and physical examination to check for typical symptoms, such as metabolic tremor. Laboratory testing comprises basic biochemical tests, including assessment of liver enzymes, blood ammonia concentration, peritoneal puncture fluid test, coagulogram, albumin, as well as neuropsychological and neurophysiological tests (EEG and evoked potentials). EEG may reveal nonspecific changes, such as generalised slowing of basic activity, or abnormalities more typical of HE, such as bilaterally synchronous delta waves and triphasic waves, mainly above the frontal lobes. Sharp waves are superimposed over slow waves in the frontal lobe area or appear in a generalised way. The EEG test results may help diagnosis; however, no correlation has yet been found between severity of the pathology in the EEG test and the clinical advancement of HE.[20,21] Neuroimaging is mainly helpful in differential diagnosis. The brain CT scan result is often correct. Sometimes cerebral edema is found in the acute stage of the disease, and atrophy in the chronic stage. On the other hand, MRI is more sensitive in detecting cerebral edema; it also allows to exclude other cerebral pathologies. Patients with liver cirrhosis frequently display bilateral increase in the signal intensity within the globus pallidus, possibly caused by manganese deposits; the signal intensity does not correlate with the clinical advancement of the disease. MRI spectroscopy can show an increase in concentration of the osmotically active agents of the brain, such as myoinositol, glutamate/glutamine and choline.[22]

Due to the lack of specific diagnostic tests, the diagnosis of HE requires exclusion of neurological and metabolic diseases unrelated to cirrhosis.[23,24] HE treatment relies on reducing intestinal ammonia production (lactulose, rifaximin, probiotics) and improving its metabolism (L-ornithine-L-aspartate, branched-chain amino acids).[25]

Aim

The aim of the study is to draw attention to the ever-important and often key role of electroencephalography in the diagnosis of hepatic encephalopathy, even in today's era of increasingly advanced diagnostic methods.

Description of the case

A 57-year-old patient, professionally active at the time, was admitted to the hospital's Neurology Department on an emergency basis due to difficulties with standing and moving, orientation disorders and limb tremor. The symptoms had appeared two days before. In 2015, she was diagnosed with alcoholic cirrhosis of the liver, grade 2 esophageal varices, thrombocytopenia and arterial hypertension. She had a history of alcohol abuse but according to the patient and her family she had been abstinent for 6 months. Upon admittance she was conscious, with psychomotor slowing, oriented about herself and her location but disoriented as to time; she was able to follow simple instructions. Neurological examination showed no meningeal symptoms; other symptoms included speech dysarthria; no paresis; ataxia of the trunk and limbs; coarse positional tremor of the upper limbs; decreased muscle tone in four limbs; uncertain gait, wide-based, requiring assistance. The CT scan of the head revealed a chronic ischemic vascular lesion in the left frontal lobe, with no freshly extravasated blood or other focal lesions. The USG test of the abdominal cavity showed no liver enlargement; the liver was of uneven outline and small nodular appearance, and had normal echogenicity. The pancreas showed no enlargement and had reduced echogenicity suggesting chronic inflammatory changes; the spleen was enlarged and had a homogeneous structure and normal echogenicity. The venous vessels in the splenic hilum were dilated. No free fluid in the peritoneal cavity was noted. Basic laboratory blood tests revealed an increased bilirubin level of 2.2 mg/dL [normal range 0.2-1.2 mg/dL], a slightly elevated liver enzymes concentration with GGT of 57 U/L [normal range 9-36 U/L] and AST of 36 U/L [normal range 5-34 U/L], and a decreased albumin level of 3.2 g/dL [normal range 3.5-5.2 g/dL].

During the second day of stay improvement of the patient's condition was noted, with reduction of limb tremors, improvement of gait and correct orientation. The patient did not report any significant complaints.

Diagnostic tests were extended to include head MRI and EEG test. MRI showed numerous foci suggesting

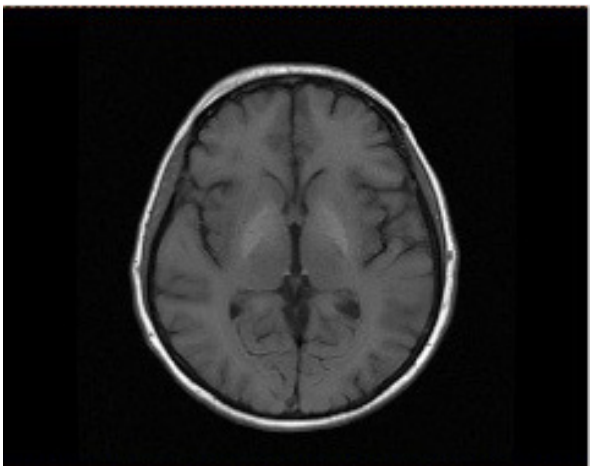

Fig. 2. MRI T1

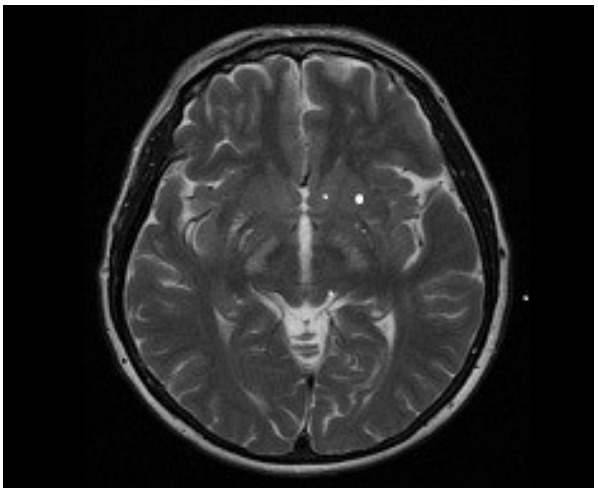

Fig. 3. MR T2

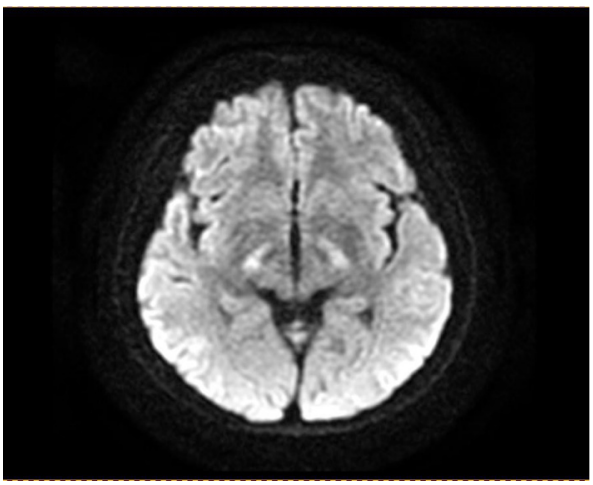

Fig. 4. MR DWI

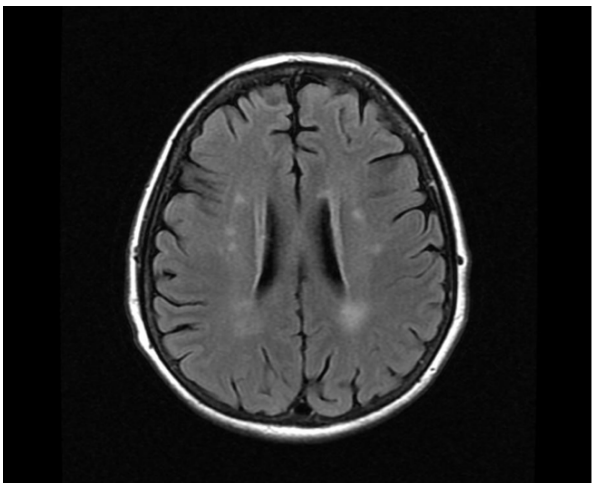

Fig. 5. MR FLAIR

chronic hypoxic-ischemic changes; in the globus pallidus, ventrolateral thalamus and substantia nigra and the brain branches, symmetrical banded foci were noted on both sides with higher signal intensity at T1- and T2-weighted imaging, and the FLAIR and DWI sequences (with no water diffusion restrictions) – the image may correspond to the acquired hepatocerebral degeneration in the course of liver cirrhosis (Fig. 1-4).

The EEG recording showed synchronous and symmetrical biphasic and triphasic waves and complexes of sharp and slow waves against the backdrop of generalised slowing of basic activity. For stop signals, HV and FS the notation pattern remained unchanged (Fig. 6 and 7).

The patient was subjected to psychological assessment (4th day of stay); at the time of the assessment, she was fully oriented, in good logical verbal contact and in a mood adequate to her disease situation. In order to assess the patient's cognitive performance in a more detailed way, further examination by mental health specialists was recommended. Additionally, the patient was consulted by a speech therapist (assessment on the 3rd day of stay), where she appeared conscious, in logical verbal contact and correctly oriented. The patient's verbal perception and expression were undisturbed, and her voice was low, dull and discreetly dysphonic.

The patient's ammonia concentration was determined and hyperammonaemia was found (128.65 *µmol*/l; normal range 18-72 *µmol*/l). In connection with cognitive impairment upon admission and changes in EEG, the patient was diagnosed with overt hepatic encephalopathy. The treatment included use of Lactulose and Xifaxan yielding further improvement.

In October 2021, the patient again experienced disturbed consciousness after the night, with weakness and imbalance preventing movement. At that time, she was hospitalised at the Neurology Department, where biochemical tests also revealed hyperammonaemia (ammonia 210 µg/dL; normal range 19-54 µg/dL); MRI was comparable to the September 2021 results. The EEG recording showed polymorphic slow delta waves as well as symmetrical and synchronous biphasic and triphasic

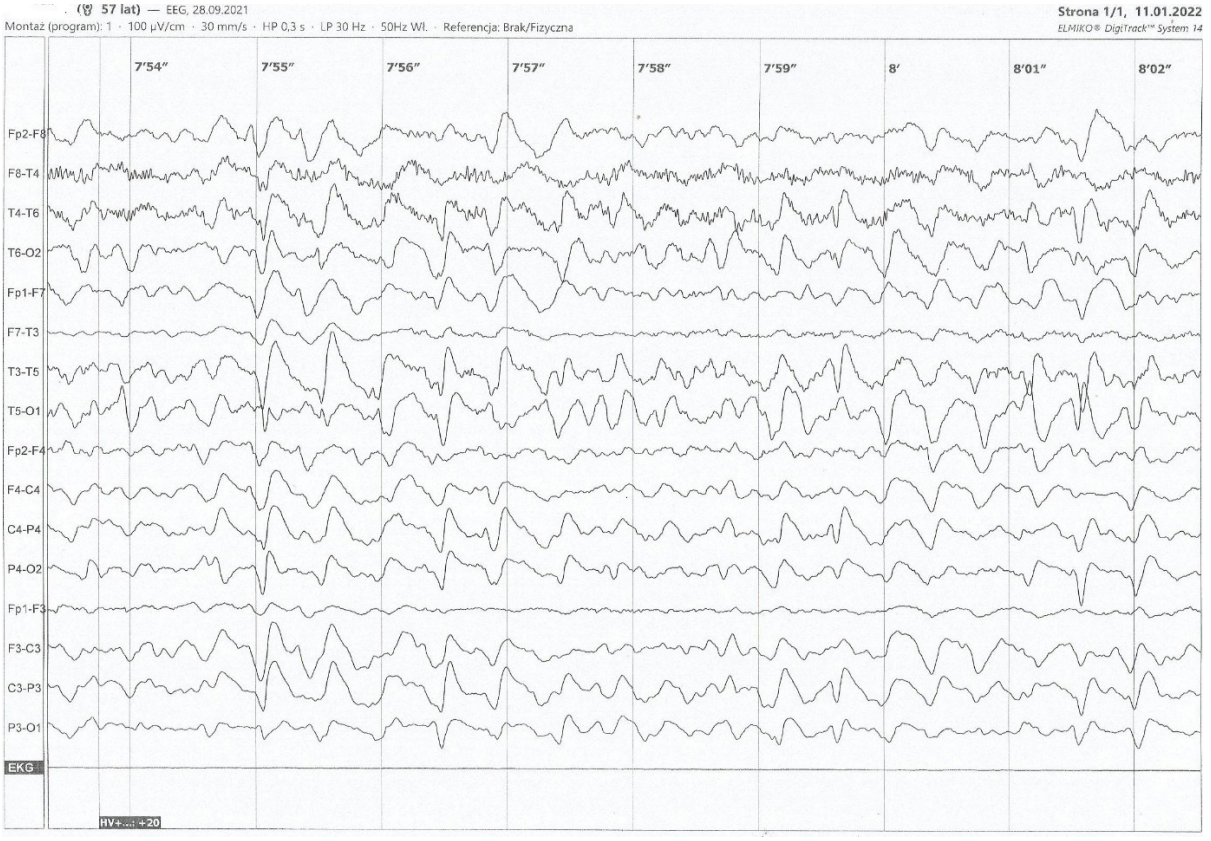

Fig. 6. Synchronous medium and high voltage biphasic and triphasic waves and complexes of sharp and slow waves visible against the background of generalised slowing of basic activity

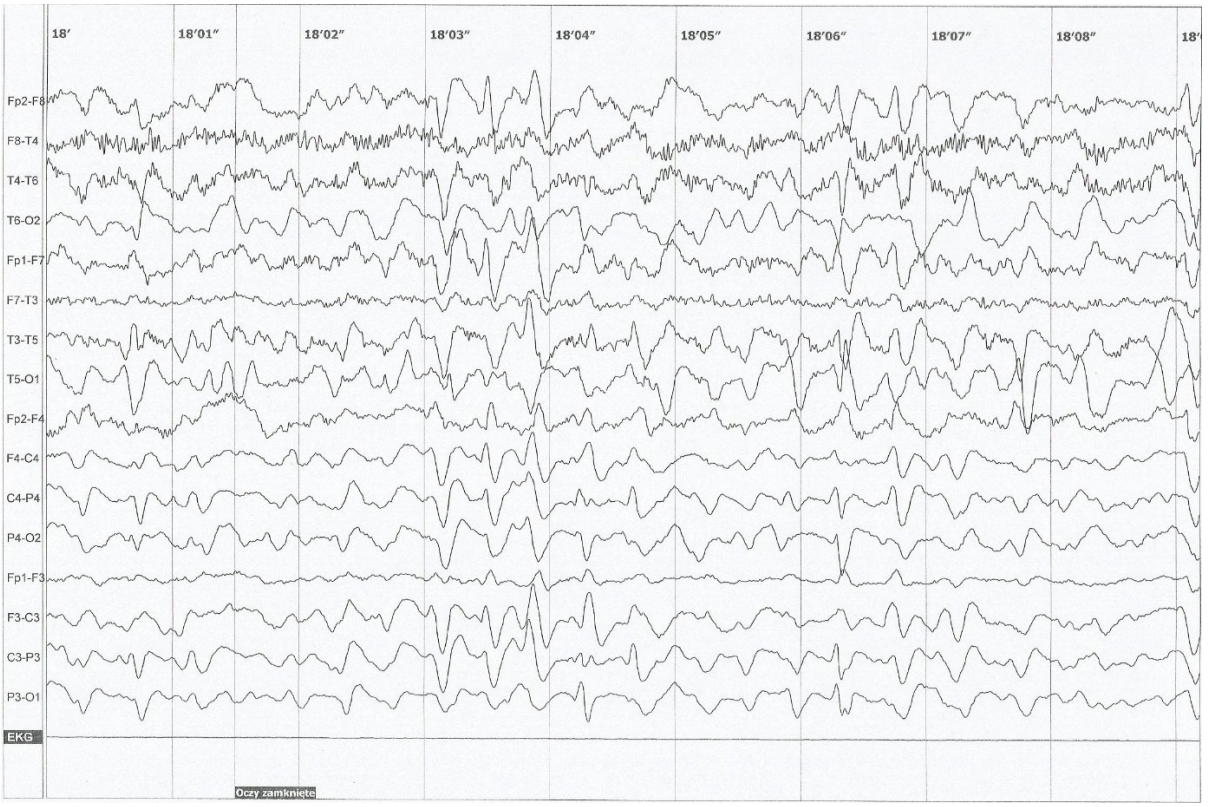

Fig. 7. EEG recording (cont.)

Fig. 8. Follow-up EEG test after 3 months. Slowed-down spatially differentiated EEG recording

waves against the backdrop of disoriented and slower basic activity. The patient was discharged with improvement.

The EEG follow-up test performed after 3 months on an outpatient basis at the Neurological Clinic of the local hospital showed improvement of the recording: the basic activity continued to be slowed down; however, delta waves as well as biphasic and triphasic waves were not visible (Fig. 8).

The patient was in logical verbal contact; deviations included slightly wide-based gait.

Discussion

Changes in the EEG recording in cirrhotic patients were described in the 1950s.[21] The main exponent of HE is generalised slowing of function. Furthermore, posterior dominant rhythm is disturbed, slowed down or absent. Depending on the severity of HE and the degree of consciousness impairment, the slowing may be mild or very severe; it is usually symmetrical unless the pathology is originated from focal lesions unrelated to the disease. In such cases, features of focal and generalised slowing may be visible simultaneously.[12,13]

The degree of slowing of basic activity usually correlates with serum ammonia concentration and liver damage severity. EEG changes occur in both full-blown and minimal hepatic encephalopathy and are particularly useful for guiding diagnosis of poorly expressed symptoms.[26] At an early stage of the disease, lower-frequency alpha waves with diffused theta waves are visible in the frontal and temporal lobes, or in all leads.[27] As the clinical condition deteriorates, theta waves and subsequently delta waves begin to prevail in the recording.[21] For patients in deep coma, a gradual decrease in wave frequency and amplitude is observed up to disappearance of EEG activity.[28,29]

An important element of EEG changes in HE is the presence of periodically occurring triphasic waves. The concept of triphasic was first introduced by Bickford and Butt in 1955.[30,31] These are medium- to high-voltage sharp waves or delta waves with a sharp pattern and triphasic morphology: a positive sharp wave preceded and followed by a negative potential of lower amplitude. The wave frequency is 1-3 Hz and the waves usually appear in a series against the backdrop of slow basic activity. Sharp waves are superimposed over slow waves in the frontal lobe area or appear in a generalised way.[32]

In cases of classic metabolic diseases, triphasic waves show an anterior-posterior delay, which means that the waves appear in the posterior leads with a delay of about 100 ms compared to the frontal leads. The background of the frontal-posterior lag is unknown. Triphasic waves were initially considered pathognomonic of hepatic encephalopathy.[30,32] It is now known that they occur in about 25% of patients with HE but they can also accompany other metabolic encephalopathies, especially ure-

mic and hypoxic encephalopathies as a result of cardiac arrest, after intoxication with drugs (e.g. baclofen, lithium, levodopa), hypothyroidism or hyperthyroidism, electrolyte disorders, encephalitis, Alzheimer's disease and CJD.

Conclusion

In the presented case, the final diagnosis of hepatic encephalopathy required electroencephalographic examination, as the initial diagnosis was ambiguous due to rapid improvement of the patient's condition. By presenting this particular case of hepatic encephalopathy, I would like to emphasise that as a diagnostic method that has been applied for many years, electroencephalographic examination continues to be an important and often key tool in determining the final diagnosis even in today's era of increasingly advanced diagnostic techniques.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, K.F.; Methodology, K.F.; Investigation, K.F.; Resources, K.F.; Writing – Original Draft Preparation, K.F.; Writing – Review & Editing, K.F.; Supervision, K.F.; Project Administration, K.F.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data sets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

Informed consent was obtained from the patient.

References

1. Elwir S, Rahimi RS. Hepatic Encephalopathy: An Update on the Pathophysiology and Therapeutic Options. *J Clin Transl Hepatol*. 2017;5(2):142-151. doi:10.14218/JCTH.2016.00069
2. Ridola L, Riggio O, Gioia S, Faccioli J, Nardelli S. Clinical management of type C hepatic encephalopathy. *United European Gastroenterology Journal*. 2020;8(5):536-543. doi:10.1177/2050640620909675
3. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol*. 2020;5(3):245-266. doi:10.1016/S2468-1253(19)30349-8
4. Shawcross DL, Olde Damink SWM, Butterworth RF, Jalan R. Ammonia and hepatic encephalopathy: the more things change, the more they remain the same. *Metab Brain Dis*. 2005;20(3):169-179. doi:10.1007/s11011-005-7205-0
5. Mohiuddin SS, Khattar D. Biochemistry, Ammonia. In: *StatPearls*. StatPearls Publishing; 2022. Accessed February 20, 2022. http://www.ncbi.nlm.nih.gov/books/NBK541039/
6. Yoshida Y, Higashi T, Nouso K, et al. Effects of zinc deficiency/zinc supplementation on ammonia metabolism in patients with decompensated liver cirrhosis. *Acta Med Okayama*. 2001;55(6):349-355. doi:10.18926/AMO/32003
7. Fiati Kenston SS, Song X, Li Z, Zhao J. Mechanistic insight, diagnosis, and treatment of ammonia-induced hepatic encephalopathy. *Journal of Gastroenterology and Hepatology*. 2019;34(1):31-39. doi:10.1111/jgh.14408
8. Aldridge DR, Tranah EJ, Shawcross DL. Pathogenesis of Hepatic Encephalopathy: Role of Ammonia and Systemic Inflammation. *J Clin Exp Hepatol*. 2015;5(Suppl 1):S7-S20. doi:10.1016/j.jceh.2014.06.004
9. Jayakumar AR, Norenberg MD. Hyperammonemia in Hepatic Encephalopathy. *J Clin Exp Hepatol*. 2018;8(3):272-280. doi:10.1016/j.jceh.2018.06.007
10. Ferenci P. Hepatic encephalopathy. *Gastroenterol Rep (Oxf)*. 2017;5(2):138-147. doi:10.1093/gastro/gox013
11. Bruyneel M, Sersté T. Sleep disturbances in patients with liver cirrhosis: prevalence, impact, and management challenges. *Nat Sci Sleep*. 2018;10:369-375. doi:10.2147/NSS.S186665
12. Cordoba J. Hepatic Encephalopathy: From the Pathogenesis to the New Treatments. *ISRN Hepatol*. 2014;2014:236268. doi:10.1155/2014/236268
13. Weissenborn K. Hepatic Encephalopathy: Definition, Clinical Grading and Diagnostic Principles. *Drugs*. 2019;79(Suppl 1):5-9. doi:10.1007/s40265-018-1018-z
14. Stinton LM, Jayakumar S. Minimal hepatic encephalopathy. *Can J Gastroenterol*. 2013;27(10):572-574.
15. Zhan T, Stremmel W. The Diagnosis and Treatment of Minimal Hepatic Encephalopathy. *Dtsch Arztebl Int*. 2012;109(10):180-187. doi:10.3238/arztebl.2012.0180
16. Mandiga P, Foris LA, Bollu PC. Hepatic Encephalopathy. In: *StatPearls*. StatPearls Publishing; 2022. Accessed February 20, 2022. http://www.ncbi.nlm.nih.gov/books/NBK430869/
17. Kori P, Sahu R, Jaiswal A, Shukla R. Hepatic myelopathy: an unusual neurological complication of chronic liver disease presenting as quadriparesis. *BMJ Case Rep*. 2013;2013:bcr2013009078. doi:10.1136/bcr-2013-009078
18. Hepatic Encephalopathy in Chronic Liver Disease: 2014 Practice Guideline by the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases. *Journal of Hepatology*. 2014;61(3):642-659. doi:10.1016/j.jhep.2014.05.042
19. Step by Step: Managing the Complications of Cirrhosis | HMER. Accessed February 20, 2022. https://www.dovepress.com/step-by-step-managing-the-complications-of-cirrhosis-peer-reviewed-fulltext-article-HMER

20. Amodio P, Montagnese S. Clinical Neurophysiology of Hepatic Encephalopathy. *J Clin Exp Hepatol*. 2015;5(Suppl 1):60-68. doi:10.1016/j.jceh.2014.06.007
21. Koul R, Maiwall R, Ramalingam A, et al. Role of EEG in Predicting Outcome of Hepatic Encephalopathy Patients. *Neurodiagn J*. 2020;60(4):272-288. doi:10.1080/21646821.2020.1824959
22. Chavarria L, Cordoba J. Magnetic Resonance Imaging and Spectroscopy in Hepatic Encephalopathy. *J Clin Exp Hepatol*. 2015;5(Suppl 1):S69-S74. doi:10.1016/j.jceh.2013.10.001
23. Bleibel W, Al-Osaimi AMS. Hepatic Encephalopathy. *Saudi J Gastroenterol*. 2012;18(5):301-309. doi:10.4103/1319-3767.101123
24. Karanfilian BV, Cheung M, Dellatore P, Park T, Rustgi VK. Laboratory Abnormalities of Hepatic Encephalopathy. *Clin Liver Dis*. 2020;24(2):197-208. doi:10.1016/j.cld.2020.01.011
25. Swaminathan M, Ellul MA, Cross TJ. Hepatic encephalopathy: current challenges and future prospects. *Hepat Med*. 2018;10:1-11. doi:10.2147/HMER.S118964
26. Marchetti P, D'Avanzo C, Orsato R, et al. Electroencephalography in Patients With Cirrhosis. *Gastroenterology*. 2011;141(5):1680-1689.e2. doi:10.1053/j.gastro.2011.06.085
27. Togo M, Kinoshita M. Hepatic encephalopathy revisited: Beyond the triphasic waves. *Clin Neurophysiol*. 2019;130(3):408-409. doi:10.1016/j.clinph.2018.12.003
28. Hawkes ND, Thomas G a. O, Jurewicz A, et al. Non-hepatic hyperammonaemia: an important, potentially reversible cause of encephalopathy. *Postgraduate Medical Journal*. 2001;77(913):717-722. doi:10.1136/pmj.77.913.717
29. Rayi A, Mandalaneni K. Encephalopathic EEG Patterns. In: *StatPearls*. StatPearls Publishing; 2022. Accessed February 20, 2022. http://www.ncbi.nlm.nih.gov/books/NBK564371/
30. Emmady PD, Murr N. EEG Triphasic Waves. In: *StatPearls*. StatPearls Publishing; 2022. Accessed February 20, 2022. http://www.ncbi.nlm.nih.gov/books/NBK557679/
31. Davies MG, Rowan MJ, Feely J. EEG and event related potentials in hepatic encephalopathy. *Metab Brain Dis*. 1991;6(4):175-186. doi:10.1007/BF00996917
32. van der Rijt CC, Schalm SW. Quantitative EEG analysis and evoked potentials to measure (latent) hepatic encephalopathy. *J Hepatol*. 1992;14(2-3):141-142. doi:10.1016/0168-8278(92)90148-i

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.13

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 232–235

LETTER TO THE EDITOR
RETRACTED

Berrin Erok [1], İlknur Mansuroğlu [2], Taha Oğuz Keklikoğlu [1], Hakan Önder [1]

Retraction note to: IgG4 related orbital/ophtalmic disease in COVID-19 after improving from critical pneumonia

[1] Department of Radiology, University of Health Sciences Prof Dr Cemil Tascıoglu City Hospital, İstanbul, Turkey
[2] Department of Pathology, University of Health Sciences Prof Dr Cemil Tascıoglu City Hospital, İstanbul, Turkey

Retraction notice:

The Editors-in-Chief have retracted the article because the authors have been unable to provide documents confirming that patient's consent was obtained for the study. After publication the corresponding author requested a retraction.

Corresponding author: Berrin Erok, e-mail: drberrinerok@hotmail.com

Received: 9.02.2022 / Revised: 10.03.2022 / Accepted: 10.03.2022 / Published: 30.06.2022 / Retracted: 30.06.2022

Erok B, Mansuroğlu İ, Keklikoğlu TO, Önder H. *Retraction: IgG4 related orbital/ophtalmic disease in COVID-19 after improving from critical pneumonia*. Eur J Clin Exp Med. 2022;20(2):232–235. doi: 10.15584/ejcem.2022.1.13

Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.14

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 236–237

LETTER TO THE EDITOR

Pathum Sookaromdee [1], Viroj Wiwanitkit [2]

Food delivery rider and COVID-19 as a preventive measure – increased safety or risk?

[1] Private Academic Consultant, Bangkok, Thailand
[2] Honorary Professor, Dr DY Patil University, Pune, India

Dear Editor,

During COVID-19 pandemic, a decreased social contact is recommended as a basic principle for prevention of disease. Nevertheless, there are many activities that requires going out from home. Seeking food is a basic necessary activity. To support staying at home concept, food delivery service is widely used in many countries during COVID-19 pandemic. It is believed that having food delivery rider to buy and send food directly to home can decreased risk from social contact. However, a little mentioned issue is safety and risk of using food deliver rider system. Prado et al. concluded that "*The self-employed food delivery riders have a high incidence rate of SARS-CoV-2 infection in relation to the national average*."[1] During COVID-19 outbreak, limitation of transportation is a common public health recommendation. In many countries, the control of population movement by curfew is used. A common prohibition usually focused on going out from home. Many settings promote using food delivery rider and claim for an effectiveness in reducing chance of disease spreading by direct buying and selling food between customer and food shop owner. From a logistic study, ordering of food delivery service was associated with perception on disease prevention among local people during outbreak.[2] However, it is usually neglected for the possible risk due to food delivery rider.

it can confirm that some riders have silent COVID-19 and can be a silent disease spreader. A rider has to wander around and meet several people, hence, the policies for allowing on food delivery during outbreak might not be a correct solution for pandemic containment. A common reason for supporting food delivery is social distancing.[3] If we consider a basic concept for disease spreading, the spreading is mainly related to contact time and distance. If a rider is a silent COVID-19 carrier, the contact time with food shop owner might be much and the wandering distance of rider can imply a very wide radius of possible disease spreading area and a very long period that the rider can spread disease to external environment comparing to a simple infectious consumer who go to buy at food shop directly. Also, there will be many contact risk persons to a common infectious rider.

Table 1. Estimated risk for using and not using food delivery rider service

	Using food delivery service	Not using food delivery service
Overall number of situation that a person contact with another person who is a silent carrier	30.4	1.8
Overall distance of carrier wandering	45.6D	3.6D

Here, a simple simulation model can help clarify the scenario. For primary assumption, it is assumed that food shop is a clean disease free area and an average inter-place distance among food shop, a customer home

Corresponding author: Pathum Sookaromdee, e-mail: pathumsook@gmail.com

Received: 14.03.2022 / Accepted: 23.03.2022 / Published: 30.06.2022

Sookaromdee P, Wiwanitkit V. *Food delivery rider and COVID-19 as a preventive measure – increased safety or risk?* Eur J Clin Exp Med. 2022;20(2):236–237. doi: 10.15584/ejcem.2022.2.14

and food rider station within the same community is equal to "D" and the incidence rate of silent COVID-19 among rider and general population, based on previous publications are equal to 15.2% and 1.8%, respectively.[1,4] Based on an amount of 100 food orders per day, the estimated risk for using and not using food delivery rider service is presented in Table 1.

It can show that if the prevalence of asymptomatic COVID-19 among food delivery rider is still high, using a delivery service can increased risk. A higher overall distance that a COVID-19 wandering around and number of situation that a person contact with another person who is a silent carrier is expected. If food delivery policies will be implemented, there must be a measure to confirm for disease-free status of the food delivery rider. Regular disease screening and vaccination for food riders should be mandatory requirements.

Declarations

Funding
The authors declare no conflict of interest.

Author contributions
Conceptualization, P.S. and V.W.; Methodology, P.S.; Software, P.S.; Validation, P.S. and V.W.; Formal Analysis, P.S.; Investigation, P.S. and V.W.; Resources, P.S.; Data Curation, P.S..; Writing – Original Draft Preparation, P.S.; Writing – Review & Editing, P.S.; Project Administration, P.S., V.W..; Funding Acquisition, P.S. and V.W.

Conflicts of interest
The authors declare for no conflict of interest.

Data availability
Data sharing not applicable.

References

1. Prado EO, Trujillo ARH, Rivera-Olivero IA, et al. High prevalence of SARS-CoV-2 infection among food delivery riders. A case study from Quito, Ecuador. *Sci Total Environ*. 2021;770:145225.
2. Mehrolia S, Alagarsamy S, Solaikutty VM. Customers response to online food delivery services during COVID-19 outbreak using binary logistic regression. *Int J Consum Stud.* 2020;45(3):396-408.
3. Nguyen THD, Vu DC. Food Delivery Service During Social Distancing: Proactively Preventing or Potentially Spreading Coronavirus Disease-2019? *Disaster Med Public Health Prep*. 2020;14(3):e9-e10.
4. Caturano V, Manti B, Carbone F, et al. Estimating asymptomatic SARS-CoV-2 infections in a geographic area of low disease incidence. *BMC Infect Dis.* 2021;21:350.

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 (2): 238–245

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.

Please note that tables with captions and figures with captions should be should be inserted immediately after the first paragraph in which they are cited.

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/xxxxxxxxxxxxx

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.