European Journal of Clinical and Experimental Medicine

e-ISSN 2544-1361
ISSN 2544-2406

Formerly: Medical Review

Quarterly

Vol. 17, No. 4
Publication date: December 2019

Rzeszów, Poland 2019

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

ICV 2017 = 83.64
MNiSW: 5.0

Indexing:
Ministry of Science and Higher Education (Poland)
Index Copernicus
The Central EuropeanJournal of SocialSciences and Humanities (CEJSH)
POL-Index
Central Medical Library (Poland)
SPORT Computer Base
ARIANTA – Science and branch Polish electronic journals
J-Gate

e-ISSN 2544-1361
ISSN 2544-2406

EDITORIAL CORRESPONDENCE
European Journal of Clinical and Experimental Medicine Editorial Office
35-959 Rzeszów, ul. Kopisto 2A,
tel. 17 872 11 53, 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

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ISSN 2544-1361 (online); ISSN 2544-2406

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 288

Contents

© Wydawnictwo UR 2019
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.1

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 289–294

ORIGINAL PAPER

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Serosurvey and Cellular Immune Status of HTLV-1/2 and HIV Co-infections in Bauchi State, Nigeria

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ABSTRACT

Introduction. Human T-cell lymphotropic virus types 1 and 2 (HTLV-1 & 2) are frequent co-pathogens among immunosuppressed individuals, particularly HIV/AIDS infected persons. Dual infected persons usually present with false normal or high CD4+ T cells count as a result of the ability of HTLV to induce clonal proliferation of CD4+ T lymphocytes. There is paucity of information on this clinical entity in Nigeria.

Aim. This study aimed to determine the seroprevalence of HTLV-1/2 and associated cellular immune response among antiretroviral naïve and experienced HIV infected persons at Bauchi State, Nigeria.

Material and methods. One hundred and eighty two (182) HIV seropositive patients' blood samples were analyzed for anti HTLV-1/2 IgM and IgG antibodies using ELISA while CD4+ T cells were counted using Flow cytometry technique. Socio-demographic data of the subjects and clinical history were obtained via questionnaire and medical records, respectively.

Results. The seroprevalence of anti-HTLV-1/2 was 14%. This comprised 76 (41.8%) males and 106 (58.2%) females. Six (3%) were seropositive for both ant-HTLV -1&2 IgM and IgG. Of the total positive for anti-HTLV-1/2, 20 (25%) ART-naïve and 6(5.9%) ART-experience subjects. Whole blood CD4+ T cell count was significantly high in HTLV-1/-2 IgG/IgM seropositive subjects compared to their HTLV-1/-2 negative counterpart.

Conclusion. All subjects (100%) who were HTLV-1/-2/HIV co-infected had normal to higher CD4+ T cell counts. It is suggested to be very careful in using only CD4+ counts to monitor HIV progression or as indicators for ART.

Keywords. HTLV**,** cellular Immunity, HIV co-infections, antiretroviral therapy

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 8.09.2019 | Accepted: 13.09.2019
Publication date: December 2019

Babayo A, Azeez AO, Sabo YM, Abdullahi IN, Dangana A. *Serosurvey and Cellular Immune Status of HTLV-1/2 and HIV Co-infections in Bauchi State, Nigeria*. Eur J Clin Exp Med. 2019;17(4):289–294. doi: 10.15584/ejcem.2019.4.1

Introduction

Human T-cell lymphotropic virus types 1 and 2 (HTLV-1 and HTLV-2) are members of the deltaretrovirus genus in the Retroviridae family.[1,2] HTLVs likely originated from cross-species transmission of simian T cell lymphotropic virus (STLVs). Combined, this group of viruses are referred to as Primate T-lymphotropic viruses (PTLVs). While the close phylogenetic relationships of HTLV-1 and STLV-1 indicate simian origin for HTLV-1, HTLV-2 and (STLV-2) are only distantly related, so the exact simian origin of HTLV-2 is unknown. Recently, two novel HTLVs were identified in hunters in Cameroon and were called HTLV-3 and HTLV-4.[3]

HTLV-1 is related to adult T cell leukemia (ATL) and a neurologic syndrome called HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP).[3-10] HTLV-2 has not been categorically linked to any disease, but has been associated with several cases of myelopathy/tropical spastic paraparesis (HAM/TSP) like neurological disease.[11-14]

Human T-cell lymphotropic virus, type 2 (HTLV-2) generally causes no signs or symptoms. However, scientists suspect that some infected people may later develop neurological symptoms, such as sensory neuropathies, gait abnormalities, bladder dysfunction, mild cognitive impairment, motor abnormalities and erectile dysfunction.[15] Although evidence to these is limited. HTLVs has a world-wide distribution, with higher prevalence in some areas including Central and south America, Central Africa, south west of Japan, and Iran.[16]

Since HTLV-1, HTLV-2, and HIV share common modes of transmission, it is not surprising that co-infection are frequently reported, especially among people with high risk behaviors, such as injectable drug users, those who practice unprotected sexual intercourse; transfusion of contaminated blood and transplantation of infected organs and tissues.[17,18] Although, human retroviruses have worldwide distribution, HTLV/HIV co-infected have relatively higher prevalence in large metropolitan area and endemic regions.[18]

Epidemiologically, areas are characterized as endemic when the prevalence of HTLVs falls within 0.5%–20% of the total population and characterized as non-endemic when the prevalence is ≤ 0.1%, since it has been identified in all five continents with an estimate of 15 to 20 million infected people.[18-20] The sero-prevalence rates tend to increase with age, and they are higher in females than males.[20] Areas of high prevalence for HTLVs include Japan, Sub-Saharan Africa, Caribbean basin, South America, Melanesia, and the Middle East.[20] HTLV-1 has been widely studied in different subjects, especially, blood donors, injection drug users, thalassemia patients, and HIV-infected individuals.[21-23] Despite the clinical significance of HTLV/HIV co-infection, there is paucity of information on this clinical entity in Nigeria.

Aim

This study aimed to determine the seroprevalence of HTLV-1/2 and associated cellular immune response among antiretroviral naïve and experienced HIV infected persons at Bauchi State, Nigeria.

Material and methods

Study area and population

This hospital based, cross-sectional study consisted of 182 (76 males and 106 females) HIV infected subjects (ART-Naïve and Experienced) of different age groups (mean±SD: 31.2±12.9 years) attending the General Hospital Ningi, Bauchi State, North-Eastern Nigeria. The subjects were divided into two study groups. Viz; Eighty (44.0%) ART-naïve and 102 (56.0%) ART-experienced.

Ethical consideration

The study was conducted in accordance with the Declaration of the Helsinki and had its ethical aspects evaluated and approved (Reference Number MOH/GEN/S/1409/I) by the Human Ethical Research Committee of the Ministry of Health, Bauchi State, Nigeria. All subjects have signed a written informed consent.

Clinical History of the subjects

With the permission of the physicians, subjects' clinical information was obtained from their hospital record files. Only confirmed HIV sero-positive subjects (ART-Naïve/Experienced), voluntarily willing to participate were purposively enrolled in the study.

Sample collection and preparation

Five milliliter (5ml) of whole blood samples were collected aseptically. Two milliliters of ethylenediaminetetraacetic acid-preserved blood samples were used for CD4+ cell counts. The remaining were spun at 12000/g for 10 mins to harvest their serum samples for HTLV-1/2 serological tests. Samples were collected between from 7th May to 10th October 2018. Blood samples were analyzed consecutively within 1 hour of collection.

Laboratory analytical procedures

Flow Cytometry Assay for Lymphocyte Population

Partec ™ CD4 reagents were used in a closed system based on the manufacturer's instructions. The CD4+ cell counts in the whole blood were analyzed using a Partec™ CyFlow Analyzer (Sysmex, Norderstedt, Germany) Model SL3. The normal range for CD4+ T cell count was 500- 1500 cells/mm^3. This device used the principle of light scattering property (based on dissimilarity in cell size or granularity) and the fluorescence of cells following staining with monoclonal antibodies to markers on the cell surface bound to fluorescent dyes. Flow cytometry data was analyzed using FlowJo v.7.6.5 software. Cell

populations of interest were then gated after identification. The generated percentages were multiplied by the total number of lymphocytes in the hemogram to derive absolute values for circulating lymphocytes. Absolute CD4+ cell counts were subsequently analyzed using a single-platform technique.

Enzyme-linked Immunosorbent Assay for Anti-HTLV IgM and IgG antibodies

Indirect anti-HTLV-1/2 IgM (product code: SL-2422Hu) and IgG (product code: SL2421Hu) ELISA were carried out according description and instructions by kits' manufacturer (Sunlong Biotech®, China).

Statistical analysis

All generated data were analyzed using SPSS software version 26.0 (2016, IBM California, USA). The prevalence of HTLV-1 and -2 was expressed in simple proportions and percentages for the study groups. Chi-square contingency table was used to determine the associations between seroprevalence of HTLV-1/-2 infections and risk factors of infection. Then student T test was used to determine difference in mean CD4+ T cell counts of the 2 study groups. A confidence interval of 95%, p-values <0.05 were considered statistically significant.

Results

Twelve (7%) were anti-HTLV-1/2 IgG seropositive, while 26 (14%) were anti-HTLV-1/2 IgM seropositive. However, 6 (3%) had both anti-HTLV-1/2 IgG and IgM antibodies (Figure 1).

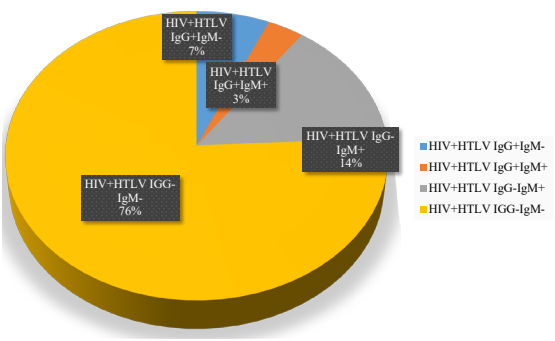

Fig. 1. Seroprevalence of anti- HTLV IgM and IgG in HIV infected subjects

In addition, 38 (21%) HIV infected persons tested seropositive to anti-HTLV-1/ 2 antibodies and 144 (79%) were negative for anti-HTLV 1/2 antibodies (Figure 2).

The HIV infected subjects who were anti- HTLV IgG positive and IgM positive had relatively higher CD4+ T cell count (681.7±191cells/mm3) and least in those had HTLV IgG positive and IgM negative results (554±274 cells/mm3). There was no significant difference between the CD4+ T-cells counts of subjects with varying serological responses to HTLV (p>0.05) (Table 1).

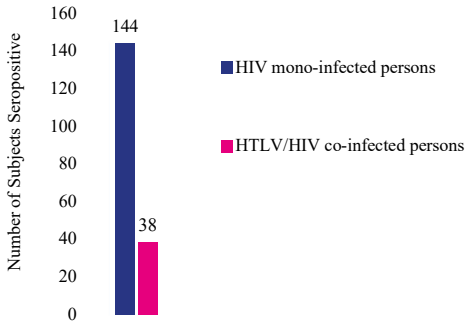

Fig. 2. Seroprevalence of anti-HTLV in HIV infected subjects

Of the 80 ART naïve HIV infected subjects tested, 20 (25%) were anti-HTLV-1/2 IgM seropositive and 14 (17.5%) were anti-HTLV-1/2 IgG seropositive. Conversely, of the 102 ART experienced HIV infected subjects tested, 6 (5.9%) had anti-HTLV-1/2 IgM, while 16 (15.7%) had anti-HTLV-1/2 IgG (Table 2).

Table 1. Comparison of CD4+ T-Cell Count of HIV-Infected Participants with and without HTLV Antibodies Enrolled for the Study

		CD4+ T-cell counts (cells/mm3)	
HTLV antibodies	**Frequency**	**Range**	**Mean± SD**
HTLV IgG Positive	12	289 – 941	554 ± 274
HTLV IgG/IgM positive	6	537 – 927	681.7 ± 191
HTLV IgM Positive	26	220 – 1125	630 ± 290.9
HTLV IgG/IgM Negative	138	194 – 1794	610 ± 445.2
F-statistic value		-	0.1526
p-value		-	0.9279

Table 2. Distribution of HTLV Antibodies according to ART Status of Subjects

ART status	**Total**	**No positive for HTLV-1/2 antibody**	
	Tested	**IgG Positive**	**IgM Positive**
ART-Naïve	80	14(17.5)	20(25)
ART-Experienced	102	16 (15.7)	6(5.9)
P-value		**0.0023***	0.1174
Total	**182**	**30 (33.2)**	**26(14.3)**

Discussion

Human T-cell lymphotropic virus type 1 (HTLV-1) and type 2 (HTLV-2) are retroviruses with global distribu-

tion.[24-26] In this study, an overall prevalence of 14% anti-HTLV IgM seroprevalence was recorded among HIV seropositive patients. This shows that, HTLV-1 /-2 infections are significantly high among HIV patients in the study area and should be subject of concern due to associated immunosuppression this could exert in affected persons.

This findings corroborate with the study of Seaton who reported a similar 14% of HTLV-1 infection in Papua New Guinea.[27] This may be due to the fact that the inhabitants of those areas share similar low economic statuses and high risk social life that have exposed them to these infections However, this findings did not corroborate with that of Nasir et al [28] reported a 4.9% among HIV patients attending University of Abuja Teaching Hospital, Gwagwalada, Abuja, Nigeria. In another study conducted by Yuguda et al., a lower prevalence of 3.2% among blood donors in Jos, Nigeria was reported, while Mohammed et al. documented a prevalence of 6.3% among blood donors in Gombe, Nigeria.[29,30] This disparity could be due to the variations in the subjects, and living conditions as well socio-demographic status of the study population. Conversely, a study by Brites et al. reported a relatively high prevalence of 31.8%.[31] This difference may be due to the persistent exposure of the high transmission rate of HTLV infections in their study area.[32]

This study evaluated CD4 counts of HIV subjects in relation to HTLV-1 and -2 infections. It was found that individuals with lower CD4 counts within the 0-200cells/μl range, had no detectable anti-HTLV-1/-2 antibodies, while those with CD4 counts greater than 200 cells/μl had 7% seroprevalence of anti-HTLV-1/2 IgG and 14% anti-HTLV-1/2 IgM antibodies However, Individuals with higher CD4 >500cells/μl had 3% prevalence of both anti-HTLV-1/-2 IgG and IgM, respectively.

CD4+ cell count is the most used as clinical criterion for ART eligibility in most HIV-infected individuals of low and middle income countries. However, HTLV/HIV coinfection makes persons have pseudu-normal or high CD4+ T cell counts. This may represent a serious problem, especially for clinicians that rely of CD4+ T cell counts for initiation and selection of ART combinations. This is common in settings where there is no availability laboratory reagents/equipment for HIV viral load tests. These findings are in consonance with those of Schechter et al., Fantry et al., Nadler et al., Casseb et al., Scapellato et al., Van Veldhuisen et al. and Gudo et al.[33-39] These studies reported that HTLV/HIV co-infected patients often progress to AIDS irrespective of high and stable CD4+ cell counts. These contrast the depletion of CD4+ cell counts observed in HIV mono-infected patients with AIDS. In cases of HTLV/HIV coinfection, it has been suggested that HIV viral load alongside clinical presentation, rather than CD4+ cell counts, should be used to monitor HIV disease progression.[40]

The predictive value of CD4+ cell count as a marker of HIV-related immunosuppression and disease stage for persons co-infected with HIV and HTLV is not similar with individuals infected with only HIV. HTLV promotes the clonal expansion of CD4-infected T-lymphocytes causing an elevation of less competent CD4+ T-cells in co-infected persons.[41] When compared to HIV-infected patients with CD4+ cell counts greater than 200 cells/mm^3, HIV/HTLV co-infected individuals with similar CD4+ cell counts are at higher risk of developing opportunistic infections.[41,42] ART status indicates a higher HTLV burden in ART-naïve (25%) subjects compare to those whom were ART-experienced (5.9%). This shows that HTLV-1 and -2 tastings/ screening in HIV patients would probably gain more importance in choice of ART combinations.

Conclusions

All subjects (100%) who were HTLV-1/-2/HIV co-infected had normal to higher CD4+ T cell counts. It is suggested to be very careful in using only CD4+ counts to monitor HIV progression or as indicators for ART.

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© Wydawnictwo UR 2019
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.2

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 295–300

ORIGINAL PAPER

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Morphology of extensor digitorum longus of Wistar rats after remobilization by vibratory platform

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ABSTRACT

Introduction. In exercise, vibrations are performed in order to produce rapid and short changes in muscle length. These changes are detected by sensory receptors, in response try to dampen the vibratory waves through a modulation of muscle stiffness. However, its effects on the morphology of muscle tissue are still not fully established, especially after long periods of immobilization.

Aim. To compare the effects of the vibratory platform on the remobilization of the extensor digitorum longus (EDL) muscle of Wistar rats with free remobilization.

Material and methods. 20 rats were divided into: CG (Control), IG (immobilized), IFG (immobilization and free remobilization), IPG (immobilization and remobilization with vibratory platform). The immobilization was performed on the pelvic limb for 15 days. The remobilization with vibratory platform was done for 10 minutes daily, for 2 weeks. The EDL was processed for histological analysis of cross-sections.

Results. The area, larger diameter, smaller diameter and fiber density of the EDL muscle of GI presented significant alteration when opposed to CG, IFG and IPG. The density of nuclei of the EDL muscle of IG presented a significant increase when opposed to the others, and IPG also presented a significant increase when compared to CG.

Conclusion. The morphology and morphometry of the EDL muscle tissue were affected, and both free and vibration platform remobilization re-established the morphological aspects of the muscle fiber, without significant differences between the methods.

Keywords. immobilization, skeletal muscle, vibration

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 20.08.2019 | Accepted: 1.10.2019
Publication date: December 2019

Camilo IR, Wutzke MLS, Costa RM, Bertolini GRF, Ribeiro LFC. *Morphology of extensor digitorum longus of Wistar rats after remobilization by vibratory platform.* Eur J Clin Exp Med. 2019;17(4):295–300. doi: 10.15584/ejcem.2019.4.2

Introduction

The skeletal striated muscle is composed of long, narrow, multinucleated skeletal muscle fibers. Its nuclei are peripherally located in the fiber, below the sarcoplasmic membrane, and have a high capacity of structural and functional adaptation, called neuromuscular plasticity.[1-4] Thus, mass and composition are directly related to function and can be regulated according to workload, activity and pathological conditions.[5-8] In addition, the lack of stimuli can lead to muscle changes, such as immobilization, a therapeutic resource frequently used in the treatment of lesions of the musculoskeletal system.[9]

The greatest effect observed in muscle tissue subjected to long periods of immobilization is muscle atrophy. This process seems to be highly ordered and regulated, characterized by a decrease in the cross-sectional area of the fiber and protein content. These changes result in reduced force production capacity, decreased electrical activation and increased fatigue.[6,10-13] Still, Rocha et al. reported that prolonged immobilization, whether in a shortened or elongated position, seems to reduce the sarcomeres in series number, besides increasing the deposition of intramuscular and perimysium connective tissue, which can reduce the relative strength and muscle dynamics for active movement, leading to functional deficits in the body segment.[14]

In the attempt of a rapid remobilization, several therapeutic meditations have been used, among them physical exercises. However, there are still gaps as to the best type, intensity and period of performance. Thus, it is necessary to search for new modalities and in this sense, exercises that impose hypergravity, due to high acceleration, achieve complex body responses.[15] Whole-body vibration (WBV) has shown good results in the increase of density and acceleration of bone metabolism, besides the increase in strength, balance and muscle power. Still, it can be applied to individuals of various ages and physical conditions, as is the case of those who have limitations to perform exercises that give load.[16-19] In exercise, vibrations are performed in order to produce rapid and short changes in muscle length. These changes are detected by sensory receptors, in response try to dampen the vibratory waves through a modulation of muscle stiffness.[15,20] However, its effects on the morphology of muscle tissue are still not fully established, especially after long periods of immobilization.[20,21]

Aim

Thus, the present study aimed to analyze the morphological effects of the vibratory platform in the remobilization of the extensor digitorum longus (EDL) muscle of Wistar rats compared to free remobilization.

Material and methods

This is an experimental, quantitative study developed at the Laboratory for the Study of Lesions and Physiotherapeutic Resources and at the Laboratory of Structural and Functional Biology of the Universidade Estadual do Oeste do Paraná (UNIOESTE), Cascavel Campus. The study was previously approved by the Ethics Committee on Research Involving Animals of the Universidade Estadual do Oeste do Paraná.

The experimental model used 20 rats, kept in a 12-hour light-dark photoperiod, temperature between 22-24ºC and, hygiene control, with ad libitum water and feed. After acclimatization of one week, the animals were randomly divided into 4 experimental groups, with 5 rats in each group: CG (Control), IG (immobilized), IFG (immobilization and free remobilization), IPG (immobilization and remobilization with vibratory platform).

Immobilization Protocol

To perform immobilization, the animals were anesthetized (xylazine 15 mg/kg and ketamine 80 mg/kg, intraperitoneally) and immobilized with appropriate material to plaster a body segment, the same: ligature of saturated tissue with dehydrated calcium sulfate, in the form of a white powder, characterizing a plastered bandage. The immobilized experimental groups had the orthosis molded from the abdominal region, just below the last ribs, followed to the right pelvic limb, being placed throughout the extension of the limb so that it remained in extension of the knee joint as well as complete plantar flexion of the ankle, that is, in position of stretching of the extensor long muscle of the fingers. The animals were kept in this position for a period of 15 consecutive days.[1]

Remobilization protocol

Animals of the IFG were freely remobilized in the cage for 15 days receiving water and feed at will. For the IPG, remobilization was carried out on the commercial platform, with a frequency of 60 Hz and vibrations with an amplitude of 2 millimeters. The rats were contained in the platform by an apparatus, built in MDF, in white color, with a total area of 25.4 cm^2 subdivided into eight stalls with an area of 2.4 cm^2 and 26.5 cm in height, where each application was done a caster, so that the same rat did not always remain in the same stall. It was performed for 10 minutes, three days interspersed per week, with rest of two days at the end of the week, for 2 weeks.

Histological processing

As soon as the immobilization was removed, the animals of the IG and the other groups, after two weeks of remobilization, were weighed, anesthetized, euthanized in the guillotine and the EDL were collected for histological analysis.

The muscles were dissected and fixed in 7% formaldehyde, stored in 70% alcohol and followed the routine histological procedure for paraffin emblocation. Cross sections of 7 μm thickness were obtained in microtome and the slides were stained with hematoxylin and eosin for general morphological analysis of muscle tissue, and the results were expressed in morphological plates. The slides were analyzed using a light microscope, in which the morphometric characteristics of the muscle tissue were evaluated (area, largest diameter, smallest diameter (100 fibers analyzed per muscle), number of fibers, nuclei and core ratio per fiber of the EDL muscle (10 observation fields per muscle, each field had an area of 3743 μm^2)).

Statistical analysis

The data analysis was performed with the SPSS 20.0 software, by means of Generalized Linear Model (GLM), and a gamma model was adopted for the variables area, largest and smallest diameters, number of nuclei and fiber/core ratio; for the number of fibers the model was linear. The Sidak post-hoc was used, and in all cases the accepted level of significance was 5%. Cohen's effect size was evaluated, considering variations of <0.2 trivial; 0.2-0.5 small; 0.5-0.8 moderate; > 0.8 large.

Results

Morphological Analysis

CG EDL muscles presented muscle fibers in polygonal format, peripheral nuclei and normal fascicular pattern (Fig. 1A). In the animals submitted to the immobilization period (IG), it was verified a disarray in the tissue organization, with apparent signs of atrophy, some fibers with rounded appearance and increase in fiber density per area analyzed after 15 days of immobilization in elongated muscle position (Fig. 1B).

Free remobilization (IFG) for 15 days restored the morphological characteristics, presenting polygonal fibers and peripheral nuclei (Fig. 1C). In the remobilized group with vibratory platform (IPG), fibers with nuclei were observed in the central region and some fibers with rounded appearance, but with a density closer to CG (Fig. 1D).

Morphometric Analysis

The area, larger diameter, smaller diameter and fiber density of the EDL muscle of IG presented significant difference when opposed to CG, IFG and IPG. The EDL muscle nuclei density of IG presented a significant increase when compared to the others, and IPG also pre-

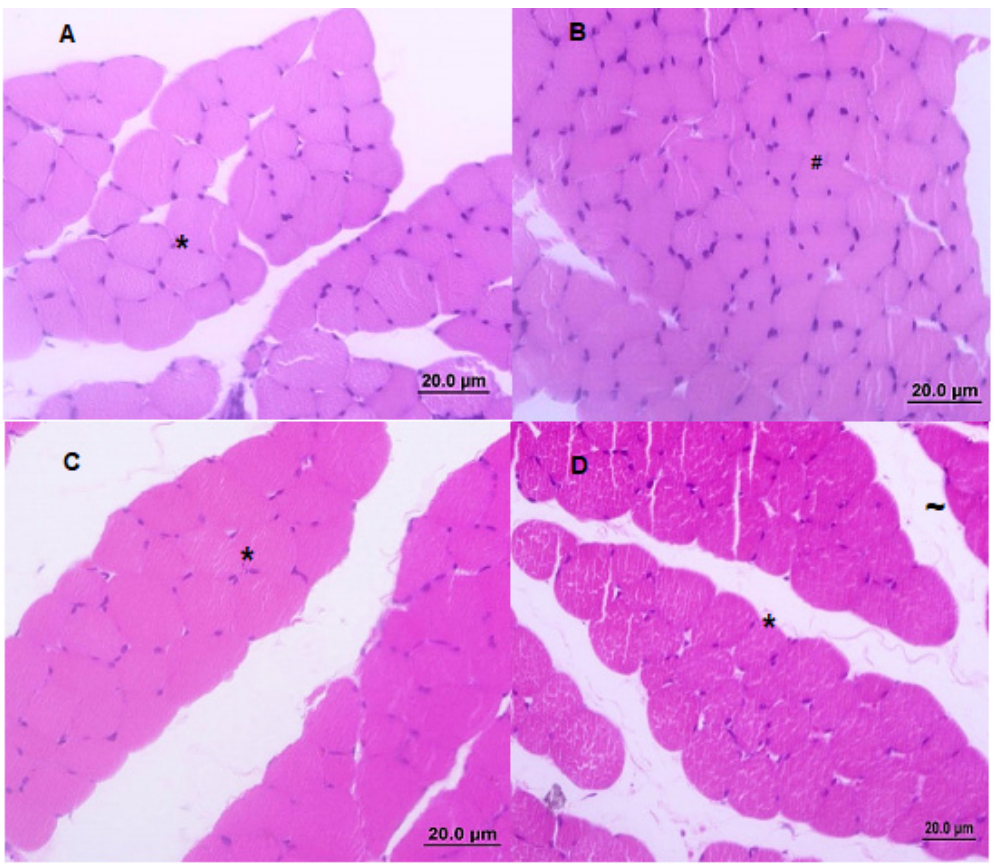

Fig. 1. Photomicrographs of the extensor long muscle of the fingers of Wistar rats, cross-section; hematoxylin and eosin staining. (A), control group (CG), presenting muscle fibers with polygonal shape, nuclei in peripheral position (*). (B), immobilized group (GI), presenting atrophic fibers (#). (C), free remobilization group (GIL) and (D), vibratory platform remobilization group (GIP), fibers with size similar to the control group (*), and central nucleus (~) in GIP (D).

Table 1. Analysis of Morphometric Characteristics. The data are expressed as mean ± standard deviation, below are indicated the Effect Sizes when comparing with the CG. Similar letters indicate no significant differences (p<0.05). CG (control), IG (immobilization), IFG (free remobilization), IPG (remobilization by vibratory platform)

	CG	IG	IFG	IPG
Fiber area	58.23 ± 5.71[a]	41.55 ± 2.28[b] 3.84	54.47 ± 12.58[a] 0.38	57.18 ± 5.64[a] -0.19
Larger diameter	20.54 ± 2.36[a]	14.67 ± 0.78[b] 3.34	20.87 ± 4.72[a] 0.09	20.07 ± 1.96[a] -0.22
Smaller diameter	13.43 ± 1.28[a]	9.45 ± 0.68[b] -3.88	14.11 ± 2.86[a] 0.31	16.33 ± 3.64[a] 1.06
Fiber number	49.13 ± 11.43[a]	101.52 ± 16.72[b] 3.66	53.18 ± 16.22[a] 0.29	59.70 ± 10.65[a] 0.96
Nuclei number	89.37 ± 15.19[a]	175.3 ± 30.77[bc] 3.54	107.76 ± 24.13[ad] 0.91	128.44 ± 32.29[cd] 1.55
Nuclei/fiber ratio	1.75 ± 0.09[a]	1.72 ± 0.11[a] -0.30	2.19 ± 0.20[b] 2.84	2.1 ± 0.20[b] 2.26

sented a significant increase when compared to CG. The fiber nucleus ratio showed a significant increase when comparing IFG and IPG with CG and IG (Table 1).

Discussion

The area, diameter, and diameter of the EDL muscle of IG showed a decrease and fiber number showed a significant increase when compared to CG, IFG and IPG, and the largest effect sizes were found in the comparisons between CG and IG. Such characteristics pointed to a picture of muscular atrophy with immobilization for two weeks. Muscles with a predominantly extensor function present a high degree of adaptation to atrophic stimuli, which was evidenced in the present study.[22]

The nuclei number in IG showed a significant increase when compared to the other groups, however, it was consistent with the increase in the amount of fibers observed per area analyzed, since the ratio of nuclei for fibers had no statistical difference when compared to the control group, again the effect size was greater between the control and immobilization groups. Ferreira et al. stated that muscle atrophy is accompanied by a reduction in the average number of myonuclei per fiber and that apoptosis seems to be underlying this regulated elimination of myonuclei.[23] In the present study, there was no decrease in the nuclei number compared to CG, but it was lower when compared to IFG and IPG.

It is known that hypertrophy is due to the increase in the individual size of muscle fibers, due to increased protein production and the addition of myofilaments, myofibrils and sarcomeres. This process may result from the activation and fusion of satellite cells, resulting in the addition of new myonuclei.[24] In view of the above, it is observed that the findings of the present study are consistent with the literature, because in IFG and IPG an increase in the ratio of nuclei per fiber was observed when compared to IG, which explains the return to baseline values in relation to the cross-sectional area and fiber diameters in IFG and IPG, characterizing muscle hypertrophy and indicating greater muscle metabolism, also pointed by the larger size of effect of these compared to the control.

The present study showed that the fibers of the EDL of the CG presented as polygonal with peripheral nuclei. In IG, degenerative results of immobilization were observed, such as apparent hypotrophy of the muscle fibers. These findings can be explained by Taillandier et al. who state that muscle immobilization can compromise the metabolic homeostasis of muscle fibers, besides causing muscle hypotrophy and changes in the connective tissue of the soleus muscle of rats.[25]

It has been observed that slow muscle fibers (type I), predominantly oxidative, seem to be more vulnerable to disuse atrophy when compared to rapid muscle fibers (type II), due to differences in their metabolismo.[1,23,26] The EDL is predominantly composed of fast fibers type II, however, just as the slow fibers are more vulnerable to immobilization, the muscles with a predominantly extensor function present a greater degree of adaptation to atrophic stimuli than the flexors, which was evidenced in the present study by the apparent atrophy and increase in fiber density per region analyzed in IG.[22]

Similar findings were observed by Polizello et al., who after 14 days of immobilization in position of elongation of the EDL muscle observed atrophy of different types of muscle fibers, in which they justified such findings due to the fact that immobilization in elongation is considered a stimulus to longitudinal tension that does not determine overload in the skeletal muscle, but can induce changes in factors related to myogenic regulation.[27]

In IFG a reestablishment of the morphological pattern found in the animals of the control group (CG) was observed after 14 days of re-mobilization of the animal freely in the cage. Corroborating this study, Polizello et al. showed that the free movement of the animals after

10 days was able to restore the values of diameter and proportion to the values observed in the CG for almost all types of fibers of the EDL muscle of rats immobilized for 14 days.[27] This may have occurred because the discharge of weight in the limbs, exerted by the animal in the cage, provides the muscle with a mechanical tensile load, which, in turn, determines trophic tissue adaptations.[28]

The EDL muscle of the animals treated with vibratory platform presented an apparent return of fiber density to the CG pattern, and also presented some nuclei in the central region, which may be indicative of muscle adaptations. Nuclear centralization suggests that there was sarcolemmal damage and subsequent architectural modification of the costamer proteins, which are of fundamental importance to stabilize the sarcolemma and position the nucleus especially for the EDL muscle.[29,30] These deleterious effects may have occurred owing to the frequency of vibration used in this study. Studies that demonstrate increases in muscle strength using vibration training have employed frequencies between 25 Hz and 45 Hz, requiring studies with different frequencies.[31] In the present study, the frequency of 60 Hz vibrations were used, in which it approaches the limit of the values proven harmful to the musculoskeletal system.

On the other hand, according to Wiggs, these cells with central nucleus can be translators of muscle regeneration, since physical exercise causes a transient inflammatory response, which will have a cytoprotective influence, essential to cell regeneration, translated by the increase in satellite cells.[32,33] Adaptation in the context of skeletal muscle reflects a change in its structure and function in response to stimuli such as physical exercise, immobilization and trauma. The ability of the muscle to respond to these stimuli is based on its regenerative capacity, due to the presence of undifferentiated myogenic cells, known as satellite cells, which are activated and proliferate and/or differentiate themselves through stimulation.[34]

Exercises on vibration platform have the characteristic of increasing the action of gravitational force on skeletal tissues, inducing neuromuscular and neuroendocrine adaptations, showing the possible activation of satellite cells, since the tonic vibration reflex is referred to as the neuromuscular mechanism activated in response to the effect of vibration, resulting in a significant increase in recruitment of motor units.[16,18,21,31,35]

Since the immobilization and consequent muscular atrophy cannot be avoided many times, it is essential for the repair of health and quality of life the recovery of muscle function. In the present study, both free remobilization and vibratory platform were able to restore the normal trophic characteristics of the EDL muscle. However, treatment with the vibratory platform did not promote improvement in relation to free remobilization, but also did not cause deleterious effects, so it could be used in physical rehabilitation programs, for gains in other structures without muscle damage.

Conclusion

It is concluded that immobilization in an elongated position for 15 days affects the morphology and morphometry of the muscle tissue of the EDL muscle, and both free remobilization and vibration platform reestablished the morphological aspects of the muscle fiber, without significant differences between the methods.

Acknowledgments

To the Araucária Foundation for funding with a scientifc initiation scholarship to IRC – 2018.

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© Wydawnictwo UR 2019
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.3

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 301–307

ORIGINAL PAPER

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A new local therapy of periodontitis in the course of stomach pathology and tobacco smoke intoxication

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ABSTRACT

Introduction. Inflammatory periodontal diseases, arising against a background of stomach pathology from tobacco addiction remain an acute problem of modern dentistry.

Aim. The experimental assessment of a new local treatment efficiency during therapy of simulated periodontitis with hyperacid gastritis and the tobacco smoke intoxication.

Material and methods. The work was conducted in 2 stages. At the first stage, all experimental animals were divided into 4 groups: I — intact, II — with simulated periodontitis, III — with simulated periodontitis and hyperacid gastritis, IV — with simulated periodontitis with hyperacid gastritis and tobacco smoking. The local therapy efficiency was evaluated with the use of a new preparation for oral care and a comparison product conducted at the 2nd stage in rats with simulated periodontitis with hyperacid gastritis and tobacco smoking.

Results. Experimental periodontitis with hyperacid gastritis and tobacco smoking provokes considerable changes in the periodontal tissues typical for the inflammatory process: lipid peroxidation activity rises and antioxidant system activity reduces. A local therapy in rats resulted in correction of detected metabolic disorders, improving removal of the damaging factors harmful influence and restoring the periodontal tissues condition.

Conclusion. The medical efficiency of a new gel normalizes the influence of lipid peroxidation processes, inflammation and the oral cavity protective system activation during periodontitis which arises up against a background of the concomitant pathology of stomach – hyperacid gastritis.

Keywords. periodontitis, gastritis, smoking

Introduction

Periodontal disease remain one of the most actual problems of dentistry in connection with the wide prevalence, chronization and its multifactorial nature.[1] The modern ideas about the etiopathogenesis of chronic generalized periodontitis define it as the result of the interaction between microbial factors and the host.[2,3] Modern epidemiological data indicate a high prevalence of chronic

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 18.09.2019 | Accepted: 9.10.2019
Publication date: December 2019

Romanova I, Zolotukhina O, Shnaider S, Kravchenko L, Noneva N. *A new local therapy of periodontitis in the course of stomach pathology and tobacco smoke intoxication.* Eur J Clin Exp Med. 2019;17(4):301–307. doi: 10.15584/ejcem.2019.4.3

generalized periodontitis associated with the influence of both endogenous and exogenous factors. Among exogenous factors, tobacco smoking plays a leading role in the chronic generalized periodontitis etiopathogenesis.[4] There is a close functional connection between the concomitant pathology of the gastrointestinal tract and inflammatory periodontal diseases, which aggravates the concomitant pathology course. Often the periodontal diseases arise as a concomitant with gastrointestinal tract (GIT) pathology because of the endogenous and the exogenous factors influence, the extent of which depends on the form, severity, duration of the basic disease and conditioned by the morphofunctional integrity of the digestive channel.[5] The changes in the oral cavity with the presence of chronic gastritis depend on the state of secretion and acid-forming function of stomach. A rise of gastric juice acidity is often accompanied by salivation, pallor, edema, and inflammation of the oral mucosa (OM).[6] In addition, modern data testifies to the negative influence of *Helicobacter pylori* on the periodontal diseases course.[7] Clinical laboratory experiments in smokers with generalized periodontitis revealed the negative influence of tobacco smoking on the oral cavity tissues, which are the site of primary contact with the toxic and carcinogenic matters of tobacco and tobacco smoke.[8]

Pathogenesis of periodontal disease arising against a background of GIT pathology require a more profound investigation.[9] The modern therapy of this combined pathology taking into account the adverse habits is not effective.

Therefore, we consider searching for and studying the efficiency of new ways of local therapy of periodontal disease as a concomitant pathology of hyperacid gastritis and tobacco smoking for practical dentistry. Hygiene based on honey products with bactericidal and bacteriostatic properties without manifestations of microbial resistance have been recently used in the complex treatment of inflammatory periodontal diseases.[10-17] However, the lack of information about the honey products, in particular, of propolis and wax in the treatment of associated periodontal diseases and the gastrointestinal tract against the background of tobacco smoking was the impetus to searching, developing and studying the efficiency of a new local therapy.

Aim

The experimental assessment of a new local treatment efficiency during therapy of simulated periodontitis with hyperacid gastritis and the tobacco smoke intoxication.

Material and methods

Experiments were conducted on 60 Vistar line rats-males, 1–1.5 months of age, weighting 180–220 g under conditions of the Odessa National Medical University animal facility on a standard diet for laboratory rats. In accordance with the set tasks, the experiments were conducted in 2 stages. In the first stage, the rats were divided into 4 groups. The first group (I) consisted of healthy rats (control, n=10). The second group (II) of rats were subjected to simulated periodontitis (n=10). The third group (III) consisted of the rats who after simulation of hyperacid gastritis were subjected to simulated periodontitis (n=10). The fourth group (IV) consisted rats subjected to a dosed tobacco smoke intoxication and simulated hyperacid gastritis and simulated periodontitis (n=30). After conducting the first series of experiments to investigate the concomitant stomach pathology and the tobacco smoke influence on the metabolic disorders in rat OM tissues with simulated periodontitis, the efficiency of local treatment using a new preparation on the basis of honey products and other biologically active matters with anti-inflammatory, antimicrobial, antioxidant effects – a gel made of honey products (Apigel) and a comparison product – a Propolis extract gel was studied.[18-19] The fourth group (IV) of rats were divided into 3 subgroups (Table 2): the 1st — rats with the simulated periodontitis with hyperacid gastritis and tobacco smoke influence (untreated subgroup, n=10); the 2nd — (the basic one) consisted of rats with simulated periodontitis with hyperacid gastritis and the tobacco smoke intoxication the treated by a new preparation (n=10); the 3rd — (a comparison group) consisted of the rats with simulated periodontitis like in the 1st and 2nd subgroups, subjected to local treatment with a Propolis extract gel (n=10).

Gastroduodenal area damage in rats was induced by addition of ammonium acetate 2 g/L to drinking-water during 10 days, after the third day they were administered 0.4 ml *Helicobacter pylori* suspension 5.108 CFU/mL twice per day during 7 days by a special dispenser.[20-25] Hyperacid gastritis was simulated by a one-time introduction of 5% solution of acetic acid at the rate of 4 mL/kg of weight through the probe 5 days before beginning clinical investigation. For the control, we conducted intragastric pH-metry under intra-abdominal anesthesia of thiopental sodium in a dose of 20 mg/kg of rat weight by introduction to the gastric cavity at supramedian laparotomy of glass electrode (EL-40) with the help of a pH-meter ("pH-340", Plant of Measuring Instruments, Gomel, Belarus). The level of basal acidity during the simulated hyperacid gastritis was 1.80–2.00. In addition, the gastric mucous membrane structure has been studied by histology to confirm the presence of hyperacid gastritis in the experimental animals.

The rats of the III and IV groups after simulation of hyperacid gastritis and II group at the first day of experiment in the first series of tests under the thiopental anesthesia (20 mg/kg) were simulated with periodontitis by the "ligature" model by applying a ligature around the

neck of the central lower incisor with fixation near the gingiva edge by the dental cement. The essence of the model consists in formation of the retention point for the dental plaque, which initiates inflammation and periodontal tissue destruction.[26] The induction of periodontitis in rats by this method was carried out by Y. Chumakova (2014), E. Zhulev (2015), A. Vishnevska (2018), D. Smaiilkulov (2019) and others.[27-39] The conditions of tobacco smoking were created for the IV group rats.

For tobacco smoking simulation, a plastic impermeable chamber with volume of 28 L with three different compartments was used, in which under pressure by motor, tobacco smoke was delivered from 15 cigarettes ("Prima red" PJSC "Imperial Tobacco Production Ukraine" Kiev, Ukraine, with 1.0 mg nicotine and 10 mg tar level) through opening inward during 30 minutes, daily, during 15 days.[40-43] Simultaneously, 7 animals were in the chamber. During fumigation, behavioral reactions of rats were observed: at the beginning of the tobacco smoke delivery to the chamber rat were disturbed, looking for a place for normal breathing, in 10 minutes they calmed down and fell asleep. After the end of inhalation by the tobacco smoke and fresh air supply, the rats activated, began breathing often, and came around in 15 minutes.

The animals were removed from the experiment within a few stages. Euthanasia of rats of the I–III groups and IV (the 1st subgroup) of the first series was carried out immediately after the last procedure of the tobacco smoke inhalation (on the 15th day) under the thiopental anesthesia (20 mg/kg) by the total bloodletting from the heart. All the animals, treated after simulated periodontitis with hyperacid gastritis and smoking (the 2nd and 3rd subgroups of IV group), were decapitated on the 8th and 14th day after the treatment onset.

The gingiva biopsy sampling for the biochemical experiments was conducted. In the gingival homogenate supernatant the level of lipid peroxidation – malonedialdehyde (MDA) end product was conducted by thiobarbituric method, the state of antioxidant defense (AOD) was estimated by catalase activity, the level of inflammation — by elastase activity, the nonspecific defense index — by lysozyme activity.[44-47] The antioxidant-prooxidant index (API) was calculated according to catalase activity and MDA level correlation.

During the conduct of clinical investigation the general principles of animal experiments were used approved by the National Congress on Bioethics (Kiev, Ukraine, 2001) and coordinated with provisions of European Convention conerning vertebrate protection, used for the experimental and other scientific purposes (Strasburg, France, 1985).

The statistical information of obtained data was conducted by the "Statistica 6.0" program with the Student t-test. The changes were considered reliable at $p \leq 0.05$.

Results

The experimental animals, before simulated pathological states, had healthy gingival mucosa, without visible pathological changes, gingival bleeding was not revealed during probing. After ligature-induced periodontitis already on the third day all the rats had clinical symptoms of the periodontal tissues inflammation, namely, plaque was detected at the cervical part of the teeth. Hyperemia, edema, gingival bleeding occurred in the incisors area. Gingival inflammation was determined in 5 days in the molars area too, so the inflammatory process generalization in the periodontal tissues took place. There was bacterial plaque accumulation and the microscopic ulcers appeared on the gingival groove epithelium, which contributes to the penetration of periopathogenic bacteria into the connective tissue. The loss of periodontal ligament and bone resorption occurred within 7 days. The animals with simulated hyperacid gastritis became weak, ate little, had signs of the oral inflammation — hyperemia and edema. After the simulated periodontitis on the 2nd day the rats had a distinct picture of gingival inflammation manifested as edema and marginal edge hyperemia, gingivitis. After 5 days, the gums looked cyanotic, swollen toward the alveolar bone basis. The periodontal pockets with a depth of 1.5 mm appeared at the area of teeth. At the 8–10th day of simulated periodontitis and hyperacid gastritis occurred alveolar process swelling at the upper and lower parts, severe hyperemia of the dental neck, gingival pockets depth up to 3 mm, marginal part is easily detached by flat plastic instrument, gums bleeding. At the 14th day, the distribution of edema on the alveolar process with increasing size remained unchanged. The edema of the anterior part of the alveolar process was white-red, and the lateral parts were blue. The enamel in the cervical region had a dark color. Gingival pocket was 6 mm, easily bleeding during probing. The similar manifestations of the pathological process of the periodontal tissues in rats with simulated gastroduodenitis were underlined by N.I. Sidlairuk, O.V. Avdeev (2016).[48]

Histology of the gastric mucosa of rats with hyperacid gastritis revealed significant structural changes throughout the stomach thickness. The superficial epithelium had multiple small necrotic patches and desquamation. The pronounced desquamation of the superficial epithelium with edema and hyperemia with the formation of surface erosions was observed. The surface erosions localized mainly at the top of folds and were multiple. There were dystrophic and inflammatory changes at the edges and at a distance from defects. These changes were combined with hemomicrocirculatory disorders — hyperemia, stasis, margination in the lumen of dilated vessels. The erosion's bottom was covered by mucus with epithelial cells and leukocytes. Circulatory disturbances were detected — hypertrophy, edema. The similar morpholog-

Table 1. The biochemical parameters changes in the gingival tissues of rats with simulated periodontitis and hyperacid gastritis (M ± m)

Groups of animals	MDA level, mmol/kg	Elastase, mckat/kg	Catalase, mckat/kg	Lysozyme, U/kg	API
I - healthy (control), n=10	8.42±0.34	34.0±2.00	7.18±0.33	276±24	8.52±0.32
II - simulated periodontitis, n=10	14.70±0.62	40.0±3.00	6.74±0.41	188±14	4.58±0.52
P*	<0.05	>0.05	>0.05	<0.05	<0.05
III - hyperacid gastritis and simulated periodontitis, n=10	17.80±1.20	43.0±3.00	5.86±0.48	176±22	3.29±0.84
P*	<0.05	<0.05	<0.05	<0.05	<0.05
P_1 #	<0.05	>0.05	>0.05	>0.05	>0.05
IV - hyperacid gastritis and simulated periodontitis+ tobacco smoke intoxication, n=30	19.30±1.10	46.0±4.00	4.80±0.38	154±26	2.48±0.57
P*	<0.05	<0.05	<0.05	<0.05	<0.05
P_1 #	<0.05	>0.05	<0.05	>0.05	<0.05
P_2 ǂ	>0.05	>0.05	>0.05	>0.05	>0.05

Notes: *: probability in relation to the control group; #: probability in relation to parameters before the treatment; ǂ: probability of distinctions between the basic group and comparison group.

ical changes in the gastric mucosa with experimental gastroduodenitis were observed in the literature sources of Karczewska E., Konturek J., Konturek P. (2002), Misula N., Avdeyev O. (2014).[49-50]

Metabolic alterations of the periodontal tissues were marked in rats of II and III groups as compared with healthy animals. The analysis indicated that the rats with periodontitis with hyperacid gastritis and the tobacco smoke intoxication had the most pronounced changes of the inflammation biochemical markers in the gingival tissues (group IV).

The simulated periodontitis resulted in growth of MDA level in the gingival tissues, which proved the lipid peroxidation intensification with the lowered antioxidant defense activity (the catalase activity decreased) in periodontal the tissues. Still more considerable similar changes took place in the gingival samples of animals with simulated periodontitis and hyperacid gastritis. The most pronounced metabolic disorders of the oral tissues were detected in the IV group (with simulated periodontitis with hyperacid gastritis and tobacco smoking), when combination of two damaging factors took place, especially in the lipid peroxidation-AOD system. The catalase activity decreased as a result of its utilization during the active participation in the lipid peroxidation products deactivation processes. The most low indices of the catalase activity in the gingiva (4.80 ± 0.38 mkat/kg) and the most high level of MDA (19.30 ± 1.10 mmol/kg) are marked in the IV group, which 2.3 times exceeds the given index in healthy animals ($p<0.05$) and 1.3 times — in the rats of II group with periodontitis (Table 1).

In the gingival tissues of the hyperacid gastritis rats with periodontitis, which were under tobacco smoke influence, the elastase activity became 1.35 times as much as in healthy animals ($p<0.05$), without the tobacco smoke intoxication this parameter growing was cut by 25% ($p<0.05$). The concomitant hyperacid gastritis substantially affected the level of the oral metabolic disorders in animals with the induced inflammation of the periodontal tissues, intensifying the oxidative stress phenomena, inhibiting AOD system function, which caused the biological membranes damage, structural-functional changes of OM with the inflammation elements. The elastase activity in the gingival tissues increased in rats with periodontitis and hyperacid gastritis 1.26 times as compared with healthy animals, exceeding this parameter in rats without the concomitant pathology. Simultaneously there was decline of the oral tissues local resistance in the rats of III and IV groups, which was proved by the fact that the lysozyme activity in the gingival homogenates was by 36.3% and 44.3% less as compared with healthy animals. It is known that lysozyme renders a local anti-inflammatory and immune modulating action: inhibits the neutrophils chemotaxis and the toxic oxygen radical production.[51-53] The decline of lysozyme activity can be the cause of local inflammatory process.

Apigel application in the local therapy of simulated periodontitis with hyperacid gastritis and the tobacco smoke intoxication promoted lowering of damage factors influence on the oral cavity of animals and improved the tissue condition. The oral examination revealed a considerably less damage to oral mucosa, namely, the gingival edema and reddening. After a local application of Apigel, the periodontal tissues condition improved already in 5 days after the beginning of the therapy, but with a comparison product application — only in 10 days. The results of conducted biochemi-

Table 2. Correction of metabolic disorder of the rats' periodontal tissues with local treatment of simulated periodontitis and hyperacid gastritis – IV group rats (M ± m)

Subgroups of animals	MDA level, mcmol/kg	Elastase, mckat/kg	Catalase, mckat/kg	Lysozyme, U/kg	API
The 1st – untreated subgroup, n=10	19.7±1.30	46.0±4.0	4.96±0.39	154±22	2.52±0.60
P*	<0.05	<0.05	<0.05	<0.05	<0.05
The 2nd - Apigel subgroup, before treatment, n=10	18.8±0,9	46.0±4.0	4.58±0.4	148±26	2.43±0.52
P*	<0.05	<0.05	<0.05	<0.05	<0.05
The 2nd - Apigel subgroup, after treatment, n=10	9.87±0.48	37.0±2.0	6.88±0.58	218±28	7.07±0.53
P*	<0.05	>0.05	>0.05	>0.05	>0.05
$P_1^{\#}$	<0.05	<0.05	<0.05	>0.05	<0.05
The 3rd - The Propolis subgroup, before treatment, n=10	19.4±1.1	45.0±4.0	4.86±0.36	160±30	2.5±0.6
P*	<0.05	<0.05	<0.05	<0.05	<0.05
The 3rd - The Propolis subgroup, after treatment, n=10	13.31±0.74	40.0±3.0	6.23±0.42	197±22	4.68±0.58
P*	<0.05	>0.05	<0.05	<0.05	<0.05
$P_1^{\#}$	<0.05	>0.05	<0.05	>0.05	<0.05
P_2^{+}	<0.05	>0.05	>0.05	>0.05	<0.05

Notes: *: probability in relation to the control group; #: probability in relation to parameters before the treatment; +: probability of distinctions between the basic group and comparison group.

cal experiments showed that a new preparation considerably lowered the inflammation markers in the gingival tissues. Their level in the substrate, studied in animals treated with Apigel applications on the simulated periodontitis areas, registered the lower values as compared to a comparison group. On the 8th day after the treatment with Apigel the most animals (86%) had indicators normalization of the antioxidant-prooxidant system, the inflammation markers in the gingival tissues.

The applications with a comparison product gave a positive effect only in 38% rats on the 8th day after the beginning of application, but the rest of animals (62%) had metabolic disorders, which were removed mainly by the end of experiment. The complex investigation revealed that Apigel renders a more pronounced curative effect than a comparison product, which was proved by the improvement of biochemical values of the periodontal tissues of rats (Table 2).

Discussion

Today there are many methods and regimens for the treatment of periodontal diseases, which includes various medications and treatment modes, but it is not clear which of the available ones is a sufficiently effective treatment. Periodontitis is a polyethiological disease, the basis of which is the complex of pathological disorders that occur in the oral cavity under the influence of exogenous and endogenous factors. Presently Propolis is widely used in inflammatory dental diseases, as it stimulates reparative processes, activates protective mechanisms and has antimicrobial properties. So, the study of herbal medicines usage undoubtedly has practical and scientific interest, since it promotes the introduction of new methods of treatment and prevention in this category of patients.

Summing up results of experiments, it is possible to establish that Apigel usage as applications in rats with periodontitis and hyperacid gastritis after the tobacco smoke intoxication considerably decreased the inflammation processes in the periodontal tissues, having an effect on normalization of the lipid peroxidation processes, inflammation and activation of the protective systems of the oral cavity.

The obtained data are in agreement with the data of researchers Smaylkulov D., Belov G., Subanova A. (2018), Karczewska E., Konturek J., Konturek P. (2002), Misula N., and Avdeyev O. (2014).[30,49-50]

The results obtained in the experiment indicate to a necessity of studying influence of a developed preparation on the parameters of the nonspecific resistance in the oral cavity at periodontitis with GIT pathology and making indication for its usage in the complex therapy of dental diseases. So, we consider the search and study of efficient new ways of the local therapy of periodontal diseases as a concomitant pathology of hyperacid gastritis under conditions of tobacco smoking for practical dentistry.

The results of experimental studies show that the development of inflammation in the stomach leads to failure of the protective mechanisms of the oral cavity to the action of damaging factors that contribute to the onset or complication of the inflammatory processes in

the periodontium, which must be taken into account in practical dentistry during treatment of patients with concomitant diseases of GIT. In the complex treatment of patients with hyperacid gastritis with inflammatory diseases of the periodontal tissue, we recommend the local therapy with the use of a new Apigel to correct changes in the oral cavity.

Conclusion

During experimental periodontitis with hyperacid gastritis and the tobacco smoke intoxication the changes in the periodontal tissues typical for the inflammatory process develop: the lipid peroxidation activity increases and the antioxidant system activity decreases, inflammation markers increase and the nonspecific defense decreases.

The local therapy of simulated periodontitis with hyperacid gastritis and tobacco smoke intoxication in rats with the use of a new Apigel resulted in correction of the definite metabolic disorders in the gingival homogenates. Apigel removed a harmful influence of damage affects and restored the periodontal tissues better than a comparison product.

Curative effect of Apigel is conditioned by a normalizing influence on the lipid peroxidation processes, inflammation and activating the protective systems of the oral cavity.

The results of experiments give reason to recommend a local application of Apigel for inflammatory processes prevention in the oral tissues with the concomitant hyperacid gastritis and creation of optimal terms for the removal of the structural-functional disorders caused by the endogenous and exogenous factors of risk.

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© Wydawnictwo UR 2019
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.4

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 308–314

ORIGINAL PAPER

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An experimental analysis of different point specific musculoskeletal pain among selected adolescent-club cricketers in Dhaka City

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ABSTRACT

Introduction. Musculoskeletal disorders (MSDs) are considered to be among the most stressful events of human body considering their onset, symptoms and the ultimate consequences.

Aim. This study was conducted to provide a concise overview of cricket-related musculoskeletal pain of the upper limb and lower limb region in male adolescent cricketers.

Material and methods. Data was collected from three clubs in Dhaka city, and the participant's age group was 10-19 years. Data was collected through oral conversations with participants and physical testing. This process was continued over six months, which repeated monthly between same subjects.

Results. 97 cricketers experienced musculoskeletal pain, where maximum reported upper limb musculoskeletal pain was 33.3% shoulder, 21.6% elbow, 27.5% wrist, and 17.6% hand pain. In contrast, 46 candidates were found in the lower limb musculoskeletal pain category containing 19.6%, 30.4%, 30.4% and 19.6% hip joint, knee joint, ankle joint and foot joint musculoskeletal pain, respectively. BMI had no significant effect on the typical upper and lower limb musculoskeletal pain. Batsmen playing for 4 sessions or more per week are the main victims of upper limb musculoskeletal pain. In contrast, bowlers and all-rounders were the main victims of lower limb musculoskeletal pain under similar workloads.

Conclusion. This study reflects an up-to-date overview of regional upper limb and lower limb musculoskeletal pain where the risk of lower limb injury is most common among all types of players.

Keywords. adolescent cricketer, musculoskeletal pain, upper limb pain, lower limb pain, practice session

The list of abbreviations:

MSP – muskuloskeletal pain, **UL-MSP** – upper limb musculoskeletal pain, **LL-MSP** – lower limb musculoskeletal pain, **BMI** – body mass index, **MSDs** – musculoskeletal disorders, **WHO** – World Health Organization, **S/W** – session per week, **H/S** – hour per session

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Received: 30.07.2019 | Accepted: 14.10.2019
Publication date: December 2019

Rashaduzzaman M, Kamrujjaman M, Islam MA, Ahmed S, Al Azad S. *An experimental analysis of different point specific musculoskeletal pain among selected adolescent-club cricketers in Dhaka City.* Eur J Clin Exp Med. 2019;17(4):308–314. doi: 10.15584/ejcem.2019.4.4

Introduction

Musculoskeletal disorders (MSDs) are considered to be among the most stressful events of human body considering their onset, symptoms and the ultimate consequences. MSDs are injuries or pain in the human musculoskeletal system, including joints, ligaments, muscles, nerves, tendons and structures that support limbs, neck and back.[1] MSDs are considered highly stressful for the human being enduring it by WHO.[2] It is reported that evidence of having injuries by playing cricket differs from each other depending on the situation. Obviously, the increased rate of injury of the young crickets is directly proportional to the increased number of playing hours.[3] In contrast, statistically, the majority of the adolescent cricketers (~80%) suffering from different extents of MSDs including knee, lower back and shoulder were the three commonly most susceptible anatomical sites of musculoskeletal pain and injuries while, the batsman are the main ultimate victims (~30%).[4] In all the cases, the coaches and the physiotherapists can play an important role in monitoring incidences of MSDs through positive physical and psychological counselling and early rehabilitation programming.[5] Musculoskeletal pain (MSP) is a common arising threat for cricketers and can occur in various ways while playing cricket. Rotation, flexion, extension, abduction rapidly repeated movements are performed by a cricketer and wrist joint, elbow joint, shoulder joint, hip joint, knee joint, ankle joint and many other joints are involving to complete a perfect function.[6] Prevention is always considered to be better than a cure but some cricketers are not getting sufficient physical training and ultimately result is disqualification from the cricket match.[4] Long time performance of high bowling workload is strongly increasing the risk factor of MSP for adolescent fast bowler.[7] MSP commonly is found mainly in the lower limbs (~49%) and partially to the upper limbs, back and trunk and soft tissue predominantly muscle is highly affected as well as joint, tendon and ligament in musculoskeletal injury.[8]

Proper designing of exercise therapy plays a vital role for adolescent cricketers. The design may consist of stretching and strengthening techniques in various joints and it can be active and resistive. Passive stretching helps to increase range of motion (ROM) especially for hip joint which is why clinicians and coaches prescribe passive stretching for improvement of individuals.[9] To make an effective treatment design for players, appropriate injury records and data collection is also important for reduction, prevention, treatment and rehabilitation.[10] Reduction and prevention methods are applied before or during game by different kinds of exercises and use of components for support like knee brace, elbow brace, arm band, kinesio tape and others. Pain killers (some injected), stretching, strengthening, sliding, and gliding techniques are applied initially during match and hospitalization if required. Hard and uneven ground can also responsible for sudden severe soft tissue injury.[11] Rehabilitation programs start after match which is decided on by the discussion of physicians, coaches and the injured players. Effectiveness of postural rehabilitation plays a vital role also in preventing MSP.[12] Prevention of interference with daily activities is most effective for the treatment of MSP.[13] Scientific studies help to measure common risk factors of adolescent cricketers. Prevention, reduction and preparation for MSP will be a future concern.

Considering all the aforementioned data, our research was conducted for the following objectives such as identification of the prevalence of MSP among adolescent cricketers with their socio-demographic factors, considering their received treatment during training and finally revealing whether there's any correlation between these factors and MSP.

Aim

This study aimed to find out the musculoskeletal pain at various anatomical sites and relationship between musculoskeletal pain and work load among adolescent cricketers.

Material and methods

Participants

The study was conducted on those groups of candidates who had started their yearly training before 1 month and will continue for 5 months without any rehabilitation protocol. Inclusion criteria of participating candidates, who were willing to participate in the study was an age group 10-19 years, a club player, and male adolescent crickets because female candidate were less in number and were not comfortable participating. Data were not taken from participants below 11 years old or above 19 years old avoiding those histories of musculoskeletal pain which lead to physical disability and/or disease condition. The study activity started with the random selection of 110 cricketers and an oral conversation with questioners. This included a blind interview process by the request of participants. The study was conducted from different cricket clubs including - Kolabagan Cricket Academy, Abahoni Club Dhanmondi and Uttara Friends Club Cricket Academy of Dhaka city.

Data processing

The research actively started with the random selection of 110 cricketers and their oral conversation with the given questioners as followed by physical testing, inspection (symmetry, swelling, muscle atrophy), palpation (warmth, tenderness, trigger points) and joint range of motion by the passive, active and resisted movement of flexion, extension, abduction, adduction

internal rotation and external rotation. Some special tests performed when collecting data were the empty can and drop arm test, external rotation lag sign, belly press and lift off test for t shoulder joint assessment, Cozen's test, golfer's elbow test, hook test,[19] Finkelstein's test, Tinel's sign, Murphy sign for the upper limb physical assessment, FABER (Patrick's) test, Trendelenburg sign, AB-HEER test, the prone instability test, HEER test, anterior drawer test and posterior drawer test, patellar grind test, Varus stress test and Valgus stress test for the lower limb physical assessment and neuro-dynamic test.[14-26] Musculoskeletal pain assessed by numeric pain rating scale where the intensity of pain was defined as mild, moderate and severe.[27]

Data analysis

After collection of data, all interview questionnaires were checked for completeness correctness and internal consistency to exclude missing or inconsistent data. Corrected data was entered into a computer. The data was analyzed by using SPSS (Statistical Package for Social Science) version 22 and Graph Pad Prism (version 5.0, Graph Pad Software, San Diego, CA, USA). Prior to data collection, permission from the ethical committee of the State College of Health Sciences was taken.

Results

Among the 110 participants, approximately 16.4% (n=18) were among the age group 10-15 and 83.6% (n=92) were among the age group 16-19. Among them, the second group has the highest mean age (16.94±1.191 years). Between them, more than half (54.5%, n=60) of the players were all-rounder, 26.4% (n=29) of the players were batsman, and 19.1% (n=21) of the players were bowlers according to the specialty of playing match. Among them, the All-rounder had the highest percentage and the lowest percentage was bowler. On the other hand, 23.6% (n=26) were left hand batsman and 76.4% (n=84) were right hand batsman and 22.7% (n=25) were left hand bowlers and approximately 77.3% (n=85) were right hand bowlers.

In the research, 51 candidate cricketers showed upper limb musculoskeletal pain (UL-MSP) with 33.3% shoulder joint pain, 21.6% elbow joint pain, 27.5% wrist joint pain, 17.6% hand joint pain. In contrast, 46 candidates were found in lower limb musculoskeletal pain (LL-MSP) category containing 19.6%, 30.4%, 30.4% and 19.6% hip joint, knee joint, ankle joint and foot joint MSP, respectively (Table 1). We found p value as chi-square test of R1,R2,R3,R4 and R5 in UL-MSP respectively 0.410, 0.725, 0.435, 0.263 and 0.166 on the other hand LL-MSP 0.537, 0.471, 0.359, 0.471 and 0.687, respectively (Table 1). According to numeric pain rating scale this research shows, Replication 1 showed UL-MSP with 31.4% (n=16) mild pain, 47.1% (n=24) moderate pain, 21.6% (n=11) severe pain. In contrast, LL-MSP containing 26.1% (n=12), 58.7% (n=27) and 15.2% (n=7) mild, moderate and severe pain, respectively. Replication 2 showed UL-MSP with 27.5% (n=14) mild pain, 54.9% (n=28) moderate pain, 17.6% (n=9) severe pain. In contrast, LL-MSP containing 19.6% (n=9), 65.2% (n=30) and 15.2% (n=7) mild, moderate and severe pain, respectively. Replication 3 showed UL-MSP with 35.3% (n=18) mild pain, 39.2% (n=20) moderate pain, 25.5% (n=13) severe pain. In contrast, LL-MSP containing 30.4% (n=14), 47.8% (n=22) and 21.7% (n=10) mild, moderate and severe pain, respectively. Replication 4 showed UL-MSP with 35.3% (n=18) mild pain, 47.1% (n=24) moderate pain, 17.6% (n=9) severe pain. In contrast, LL-MSP containing 30.4% (n=14), 60.9% (n=28) and 8.7% (n=4) mild, moderate and severe pain, respectively. Replication 5 showed UL-MSP with 27.5% (n=14) mild pain, 51.0% (n=26) moderate pain, 21.6% (n=11) severe pain. In contrast, LL-MSP containing 26.1% (n=12), 63.0% (n=29) and 10.9% (n=5) mild, moderate and severe pain, respec-

Table 1. Male Cricketers of different groups suffering from locus specific MSP

Replication of subjective data assessment	Locus of musculoskeletal pain (MSP)									
	Upper limb (UL)					Lower limb (LL)				
	*N= 51					*N=46				
	p Value	Shoulder joint	Elbow joint	Wrist joint	Joints of hand	p Value	Hip joint	Knee joint	Ankle joint	Joints of foot
R1	0.410	17	11	14	9	0.537	9	14	14	9
R2	0.725	16	11	13	11	0.471	8	13	15	10
R3	0.435	17	10	14	10	0.359	9	15	14	8
R4	0.263	18	12	13	8	0.471	10	15	13	8
R5	0.166	17	11	16	7	0.687	9	13	14	10
Average (%)		33.30	21.60	27.50	17.60		19.60	30.40	30.40	19.60

* *N* refers the number of candidates in all categories; R is for the replication/rounds of data collection and assessments from different player groups

** p value marked as chi-square test which indicated that there is no significant relationship between UL-MSP and LL-MSP.

tively. 13 cricketers were observed to be safe from all types of MSP in our research. The intensity of pain experienced by candidates was 27.2% mild, 53.2% moderate and 17.7% severe. 13 cricketers were observed to be safe from all types of MSP in our research.

In the research, 51 candidates showed UL-MSP with 15.7% (n=8) underweight and 84.3% (n=43) normal weight of BMI. In contrast, LL-MSP containing 19.6% (n=9) and 80.4% (n=37) underweight and normal weight, respectively (Figure 1). On the other hand, 23.1% (n=3) underweight and 76.9% (n=10) normal weight of BMI who has not found MSP.

In this study, 51 cricketers who were in UL-MSP, out of them 11 batsman played respectively (n=3) 2 hour per session (H/S), (n=3) 3 H/S and (n=4) 5 H/S between 3 session per week (S/W) also 4 batsman played (n=2) 2 H/S and (n=2) 5 H/S between 5 S/W (Figure 2*a*), 13 bowler played (n=8) 3 H/S, (n=3) 5 H/S between 3 S/W and (n=1) 2 H/S, (n=1) 3 H/S between 5 S/W (Figure 2*b*) and 24 all-rounder played (n=3) 2 H/S, (n=3) 3 H/S, (n=2) 5 H/S between 3 S/W on the other hand (n=5) 2 H/S, (n=5) 3 H/S and (n=6) 5 H/S between 5 S/W (Figure 2*c*). 46 cricketers who ware in LL-MSP out of them 10 batsman played respectively (n=2) 2 H/S, (n=2) 3 H/S and (n=1) 5 H/S between 3 S/W also (n=1) 2H/S and (n=4) 5 H/S between 5 S/W(Figure 2*d*), 13 bowler played (n=1) 2 H/S, (n=7) 3 H/S and (n=4) 5 H/S between 3 S/W also (n=1) cricketer played 2 H/S between 5 S/W (Figure 2*e*) and 23 all-rounder played (n=4) 2 H/S, (n=2) 3 H/S, (n=4) 5 H/S between 3 S/W also (n=3) 2 H/S. (n=4) 3 H/S and (n=6) 5 H/S between 5 S/W (Figure 2*f*). 13 cricketers who has no MSP out of them 7 batsman played (n=4) 2 H/S and (n=3) 3 H/S between 3 S/W also 1 bowler played (n=1) 2 H/S between 3 S/W and 5 all-rounder played (n=4) 2 H/S, (n=1) 3 H/S between 3 S/W.

Batsmen playing for 4 sessions or more per week were the main victims of UL-MSP, below 4 sessions

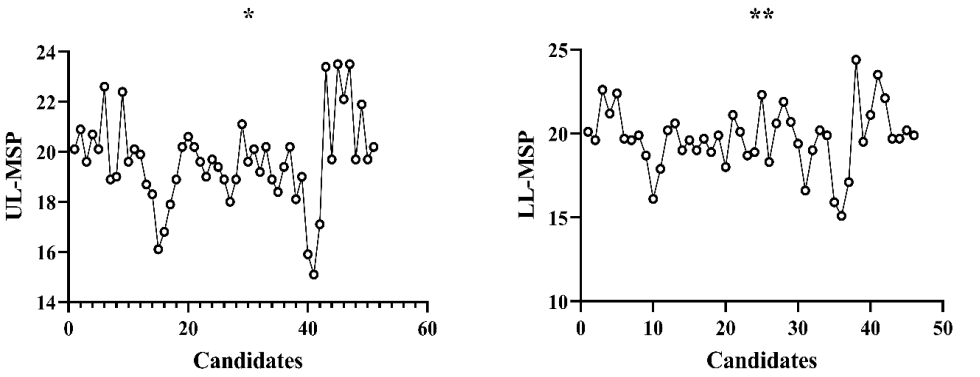

Fig. 1. Comparative analysis of the variance of BMI level between the UL-MSP (*) and LL-MSP (**) player groups

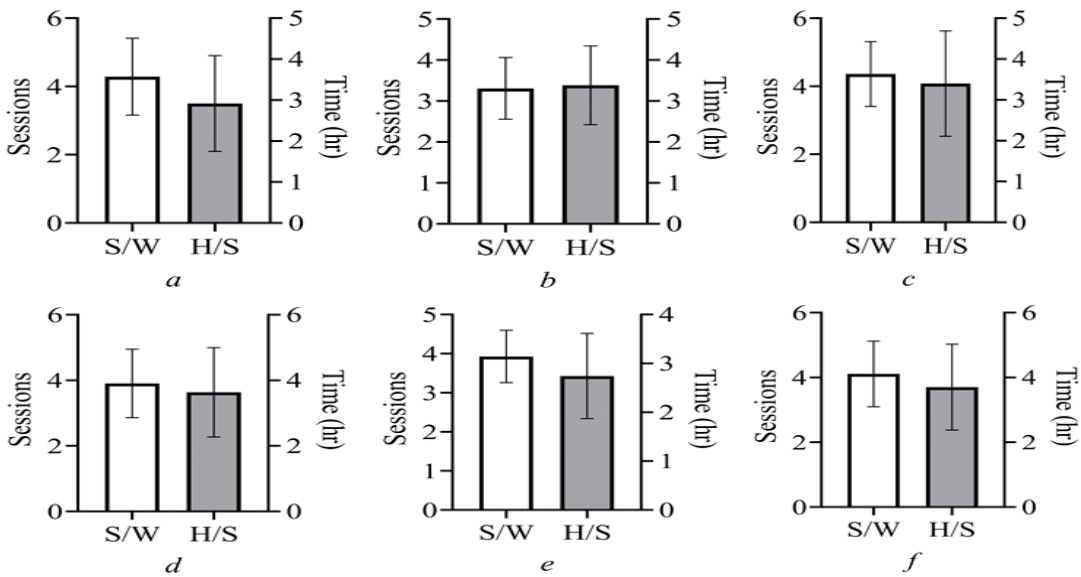

Fig. 2. Comparative analysis of upper limb and lower limb musculoskeletal pain between batsman, bowlers and all-rounders based on sessions and hours, S/W - session per week, H/S - hour per session

LL-MSP (Figure 2*a*; 2*d*) in our research. The bowlers performing 4 or more sessions per week are suffering from LL-MSP compared to the upper limb group (2*b*; 2*e*). Furthermore, the all-rounders playing more than 4 sessions have UL-MSP as compared to the all-rounders playing below 4 sessions per week (Figure 2*c*, 2*f*).

Discussion

In this research, 51 candidate cricketers showed upper limb musculoskeletal pain with 33.3% shoulder joint pain, 21.6% elbow joint pain, 27.5% wrist joint pain, 17.6% hand joints pain. In contrast, 46 candidates were found in lower limb musculoskeletal pain category containing 19.6%, 30.4%, 30.4% and 19.6% hip joint, knee joint, ankle joint and foot joint MSP, respectively (Table 1). That situation directly reflects the findings of Noorbhai.[4] Additionally, Saayman mentioned the front foot hip joint pain and lower back pain (LBP) of the fast bowlers which are highly concerning to the final team combination selection.[28] Ranges of treatment options have been implemented on the basis of injury level and players response to the injury. Few have suggested by Rao especially to sufferer from foot and ankle pain.[29]

Gregory stated that the fast bowlers experienced higher frequency of injury than the spin bowlers, where, the sustainability of injury was 8.6%, 11.4% and 2.3% higher.[30] In contrast, the spin bowlers were at ~7% higher risk of shoulder injury. High prevalence of moderate pain noticed in our study which is directly reflects the findings of Noorbhai.[4] 13 cricketers were observed to be safe from all types of MSP in our research.

BMI has found no significant effect on the typical upper and lower limb musculoskeletal pain formation (Figure 1) in the research which is directly similar with the findings of Das.[31] But Talupuru showed the evidence of BMI effect on overall bating performance for gripping and hard hitting.[32] Futhermore, BMI has found a tremendous relationship for returning to sports of an athlete. John stated a significant study between returning of sports of professional cricket players and armature cricket players. His research also showed those players with more come-back-chances who have less than 25 BMI.[33] For the adolescent players, BMI plays important role in their performance and sporting attitude.

Considering age and health condition like pain in lower limbs including knees and hips, BMI is considered as one of the main reasons of injury by Stovitz.[34] Age and BMI explained only 1.9% of the variance in pain generation of individuals as reported by Wright.[35] Which directly opposite to the aforementioned ideas of Stovitz,[34] Which is directly similar to our findings (Figure 1).

Orchard, Drew and many others report that workload is mainly responsible for musculoskeletal injury of adolescent cricketers which is directly similar to our findings.[36-38] In this study, mainly all-rounders are main victim of MSP rather then bowlers. These findings are dissimilar with a previous study on adolescent cricketers in KwaZulu-Natal, where injuries to all-rounders 28% and batsman 30% mentioned by Noorbhai.[4] On the other hand, Sathya and Parekh stated that all-rounders received maximum injuries of 70% and bowlers 60% which directly reflects our study.[39] Batsmen playing for 4 sessions or more per week are the main victims of UL-MSP which agrees with Hulin.[40] While, below 4 sessions are of LL-MSP (Figure 2*a*; 2*d*) in our research. Most of the adolescent participants usually suffer from LL-MSP over that of their UL-MSP conditions on a usual basis according to Garbenytė-Apolinskienė.[41] The bowlers performing 4 or more sessions per week are suffering from LL-MSP more than that of the upper limb group (2*b*; 2*e*). Furthermore, the all-rounders playing more than 4 sessions have UL-MSP as compared to the all-rounders playing below 4 sessions per week (Figure 2*c*, 2*f*). Sathya and Parekh stated that all-rounders are mainly suffering from LL-MSP which is not in agreement with our findings.[39] MSP is co-related to sporting attitude that's why it cannot be measured.

Shoulder pain has become very acute in that case when the participants fall into the category of 10 to 19 years or from the little league of sports observed by Drescher.[42] Things are quite different for the healthy adult players of either professional or non-professional cases mentioned by Dannecker and Koltyn.[43]

Conclusion

This study represents that male adolescent cricket players residing in Dhaka city have high prevalence of musculoskeletal pain. A more concise overview reflects the regional upper limb and lower limb musculoskeletal pain where the risk of upper limb injury is most common between all types of players. Shoulder joint and wrist joint from upper limb region relatively knee and ankle joint was mainly affected to musculoskeletal injury. This study also noticed a strong impact between musculoskeletal pain and workload. Mainly all-rounders followed by bowlers are main victims of lower limb musculoskeletal pain and batsman are the main victims of upper limb musculoskeletal pain due to workload. Parents, guardians and coaches should pay specific caution to reduce exacerbating factors causing musculoskeletal pain and also pay strong attention for the rehabilitation protocol.

Acknowledgements

The authors would like to extend their appreciation and gratitude to the state college of health sciences department of physiotherapy; Kolabagan Cricket Academy (KCA), Abahoni Club Dhanmondi (ACD) and Uttara Friends Club Cricket Academy (UFCCA).

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.5

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 315–325

ORIGINAL PAPER

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Prevalence of depression in Indian patients with type 2 diabetes mellitus and/or hypertension: DEPTH Study

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ABSTRACT

Introduction. Depression, a common psychiatric mood disorder, is a leading cause of disability and a significant contributor to the overall global burden of disease.

Aim. To determine the prevalence of depression in patients with controlled and uncontrolled type-2 diabetes mellitus (T2DM) and/or hypertension (HTN) in India. The association of depression with socio-demographic profile and clinical risk factors was also assessed.

Material and methods. In this cross-sectional epidemiological study, T2DM and/or HTN patients attending outpatient department at tertiary care hospitals and private clinics across 54 cities in India were enrolled. The primary outcome measure was to determine the prevalence of depression in T2DM, HTN and T2DM + HTN patients. Association of depression with patients' demography, socio-economic status, anxiety, and clinically diagnosed insomnia were also investigated.

Results. Of 1829 patients, the prevalence of depression in T2DM, HTN and T2DM+HTN cases were found to be 51.03%, 46.94% and 48.64%, respectively. A higher proportion of patients with uncontrolled T2DM and HTN reported depression (T2DM: 77.64% vs. 22.36%; HTN: 72.49% vs. 27.51%). There was a significant association between anxiety and severity of depression across all indications ($p<0.0001$). Depression was significantly associated with complications in T2DM ($p=0.0001$) and comorbidities in T2DM + HTN ($p=0.0023$) cases.

Conclusion. Depression is highly prevalent and has a direct significant association with various socio-demographic variables and anxiety in Indian patients with T2DM and/or HTN.

Keywords. comorbidity, diabetes, depression, hypertension; prevalence, type 2 diabetes mellitus

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 1.08.2019 | Accepted: 21.10.2019
Publication date: December 2019

Lalwani RK, Shah JD, Chatterjea T, Nadakuduru PR, Erande S. *Prevalence of depression in Indian patients with type 2 diabetes mellitus and/or hypertension: DEPTH Study.* Eur J Clin Exp Med. 2019;17(4):315–325. doi: 10.15584/ejcem.2019.4.5

Introduction

Depression, a common psychiatric mood disorder, is a leading cause of disability and a significant contributor to the overall global burden of disease. Worldwide, it affects around 350 million people with a lifetime risk of 7%.[1] It is estimated that by 2020, depression is projected to increase up to 5.7% of the total disease burden and would be the second leading cause of disability-adjusted life years.[2] In India, approximately 57 million people are affected by depression which contributes approximately 18% of the global estimate.[3] Multiple risk factors such as stressful life events, social or financial stresses, cultural and environmental factors, family history or enduring medical conditions play a critical role in the development of depression.

There is enough compelling evidence to demonstrate the strong bidirectional relationship between depression and chronic illnesses in the community.[4] Depression is a negative prognostic indicator in many chronic medical diseases. Hypertension (HTN) and type-2 diabetes mellitus (T2DM), two most common non-communicable chronic illnesses, are shown to serve as a risk factor for depression.[5] Data from previous studies indicate depression as a comorbidity in approximately 39-41% of T2DM and 15-40.1% of HTN patients.[6-10] The risk of depression was 1.8 times higher in diabetics compared to non-diabetics.[11] Major depression, also termed as major depressive disorder, was reported in one of eight individuals with diabetes, while one fifth may have a less severe form of depressive symptoms.[12]

Type-2 diabetes and HTN often co-exist due to substantial overlap with respect to their etiology, disease mechanisms and complications including microvascular and macrovascular disorders.[13] In the US, HTN occurs in approximately 50% to 80% of patients with T2DM, while in India, around 20.6% patients were co-existent with both diseases.[14] Both T2DM and HTN patients are more susceptible to psychological distress, especially depression, which may further lead to poor self-management and treatment compliance, alterations in behavioral, dietary and lifestyle habits, and escalation in the rate of morbidity and mortality.[15-25] This intermingling of the symptoms of diabetes and depression, termed as "diapression", needs to be addressed in an integrated manner to improve patient care.[12] In addition to depression, anxiety also tends to affect the outcome of these two diseases.[26] The American Diabetes Association (ADA) and the International Diabetes Federation recommends regular screening for depression in diabetics.[25,27] Furthermore, ADA recommends annual screening of depression in all patients with diabetes, particularly in those with a self-reported history.[27]

In view of the availability of limited data and association of depression with an increased likelihood of T2DM/HTN-related complications, the relationship between T2DM/HTN and depression requires careful deliberation. Hence, the present study assessed the prevalence of depression in these patients, given that cases of T2DM, HTN and their co-occurrence are rapidly increasing which may upsurge the overall rate of depression in Indian population. We also investigated the association of depression with socio-demographic factors, anxiety, and clinically diagnosed insomnia.

Material and methods

Patient and Setting

This was a cross-sectional, epidemiological, multi-centric study. Patients (between 18-60 years, both inclusive), with documented history of T2DM and/or HTN since ≥ 5 years, attending the outpatient department of a tertiary care set-up/private clinics were enrolled between May to Oct 2017 across 54 cities in India. This study recruited controlled and uncontrolled T2DM and HTN patients in the ratio of 30:70 within each primary disease group. Patients diagnosed with any other psychiatric disorders, alcohol dependence or drug abuse, severe cardiac, hepatic, neurological and renal diseases were excluded from the study. Additionally, pregnant or lactating women, patients with a history of any clinical evidence of malignancies, exacerbation of chronic illnesses, severe and acute infections, complicated infections or participation in any other intervention trial within 30 days prior to screening were also excluded from the study.

The study protocol was approved by Conscience independent ethics committee, Ahmedabad, India. The study was conducted in accordance with the principles of Declaration of Helsinki, International Conference on Harmonization Good Clinical Practice (ICHGCP) guidelines, and Indian regulatory guidelines (Indian Council of Medical Research and Indian GCP guidelines). All patients provided written consent in the patient authorization form to participate in the study.

Survey Method and Data Collection

Patients meeting the eligibility criteria were enrolled and a unique identification number was assigned to each patient. The demographic details, socioeconomic status (based on modified Kuppuswamy's scale)[28], and family history of patients were recorded at the day of enrolment. The duration and complications of T2DM and/or HTN, associated comorbidities and related complications, physical examination, vital signs, laboratory details (if available) were also recorded. Details of clinically diagnosed insomnia based on the past medical records and concomitant medications were captured. Study assessment tools like Patient Health Questionnaire-9 scale (PHQ-9) and Generalized Anxiety Disorder 7-item (GAD-7) (defined under "Study Assessment Tools") were administered to assess depression and anx-

iety, respectively. All the study-related observations were documented in case report form by the investigator or his/her qualified designee. The study monitors ensured that the data is complete and genuine and the patient's safety and rights are well-protected.

Study Definitions

Diabetic patients with glycosylated hemoglobin (HbA1c) ≥7% or postprandial blood glucose (PPG) ≥180 mg/dL were considered as uncontrolled and those with HbA1c <7% or PPG <180 mg/dL were considered as controlled patients.[27]

A subject's blood pressure was measured once, however, if elevated, a repeat BP was taken 5 min later. Patients with an average systolic BP (SBP) ≥140 mmHg or an average diastolic BP (DBP) ≥90 mmHg were considered as uncontrolled hypertensive patients. Patients with an average SBP <140 mmHg or an average DBP <90 mmHg, based on physician's discretion were considered as controlled hypertensive patients.[29]

Patients were categorized into obese and non-obese category based on their BMIs. A BMI of <17.5 kg/m^2 indicated underweight, 17.5-22.9 kg/m^2 as normal weight, 23-27.9 kg/m^2 as overweight and >28 kg/m^2 as obese (class 1).[30]

Study Assessment Tools

Prime MD PHQ-9: The PHQ-9 is used to screen, monitor, diagnose, and measure the severity of depressive symptoms. The 9-item questionnaire ranges from 0 to 27 and can be scored from 0 (not at all) to 3 (nearly every day). The present study assessed major depressive syndrome and other depressive symptoms in patients.[31] Patients were said to have not categorized depression if the symptoms did not fulfil the criteria of either major depressive syndrome and other depressive symptoms. A score of <5 indicates "no symptoms of depression", score 5-9 as "mild", 10-14 as "moderate depression", 15-19 as "moderately severe depression" and ≥20 as "severe depression".

Generalized Anxiety Disorder (GAD)-7: It is a self-reported questionnaire to identify probable cases of GAD and measure the severity of GAD symptoms. The GAD-7 items included: 1) nervousness; 2) inability to stop worrying; 3) excessive worry; 4) restlessness; 5) difficulty in relaxing; 6) easy irritation; and 7) fear of something awful happening. Response categories were "not at all", "several days", "more than half the days", and "nearly every day", scored as 0, 1, 2, and 3, respectively. The total score of GAD-7 ranges from 0 to 21. A score of 0-4 indicates "no symptoms of anxiety", score 5-9 as "mild anxiety", 10-14 as "moderate anxiety", and 15-21 as "severe anxiety".[32]

Study Outcomes

The primary outcome was to determine the prevalence of depression in patients with T2DM, HTN and both T2DM+HTN (controlled and uncontrolled cases). The other outcome measures were to identify the association between depression and patients' demographic profile, socio-economic status (based on modified Kuppuswamy's scale), lifestyle parameters, anxiety, clinically diagnosed insomnia, co-morbidities and complications.

Statistical Analyses

Assuming 27% prevalence rate of depression in patients with T2DM and HTN, the sample size was estimated to be 936 in both indications to estimate the prevalence rate with 3% error margin and 95% level of significance.[33,34] Descriptive statistics were used in the study. The continuous variables were analyzed by independent Analysis of Variance and categorical variables by Chi-square Test/Fisher exact test at 5% level of significance. All the data were analyzed using Statistical Analysis System (SAS)® version 9.4.

Results

Patient Disposition

A total of 1829 (97.70%), out of 1872 screened patients, completed the study (T2DM: 631 [33.71%]; HTN: 573 [30.61%]; T2DM+HTN: 625 [33.39%]). The remaining 43 (2.30%) patients were screen failures.

Table 1. Demographic details*

Variable	HTN (N=573)	T2DM (N=631)	HTN +T2DM (N=625)	Total (N=1829)
Gender				
Male, n (%)	295 (51.48)	315 (49.92)	319 (51.04)	929 (50.79)
Female, n (%)	278 (48.52)	316 (50.08)	306 (48.96)	900 (49.21)
Age (Years), mean±SD	50.31±8.00	49.95±8.25	53.12±6.51	51.14±7.75
BMI (kg/m^2), mean±SD	27.51±4.65	27.33±4.54	28.05±4.69	27.63±4.63
Marital Status, n (%)				
Married	560 (97.73)	609 (96.51)	614 (98.24)	1783 (97.48)
Single	4 (0.70)	7 (1.11)	5 (0.80)	16 (0.87)
Divorced	1 (0.17)	1 (0.16)	0	2 (0.11)
Widowed	8 (1.40)	14 (2.22)	6 (0.96)	28 (1.53)
Duration of Disease, n (%)				
5-10 years	542 (85.90)	474 (82.72)	475 (76.00)	1491 (81.52)
>10 years	86 (13.63)	93 (16.23)	55 (8.80)	234 (12.79)

* BMI, body mass index; HTN, hypertension; SD, standard deviation; T2DM, type 2 diabetes mellitus

A higher proportion of patients with T2DM and HTN had uncontrolled disease in comparison to controlled disease (T2DM: 77.64% vs. 22.36%; HTN: 72.49% vs. 27.51%). The mean ± SD age and BMI of the overall population were 51.14 ± 7.75 years and 27.63 ±

Table 2. Prevalence of Depression in T2DM, HTN and T2DM+HTN using Prime MD PHQ-9 (Score ≥5)

Depression Diagnosed	T2DM			HTN			T2DM+HTN
	Number (%) of Patients, 95% Confidence Interval						
	Control	Uncontrolled	Total (N=322)	Control	Uncontrolled	Total (N=269)	Total (N=304)
Major Depressive syndrome	6 (1.86), 0.39:3.34	14 (4.35), 2.12:6.58	20 (6.21), 3.57:8.85	8 (2.97), 0.94:5.00	16 (5.95), 3.12:8.77	24 (8.92), 5.52:12.33	52 (17.11), 12.87:21.34
Other Depressive symptoms	16 (4.97), 2.60:7.34	86 (26.71), 21.88:31.54	102 (31.68), 26.60:36.76	25 (9.29), 5.82:12.76	75 (27.88), 22.52:33.24	100 (37.17), 31.40:42.95	95 (31.25), 26.04:36.46
Not Categorized	50 (15.53), 11.57:19.48	150 (46.58), 41.14:52.03	200 (62.11), 56.81:67.41	41 (15.24), 10.95:19.54	104 (38.66), 32.84:44.48	145 (53.90), 47.95:59.86	157 (51.64), 46.03:57.26
Total	**72 (22.36), 17.81:26.91**	**250 (77.64), 73.09:82.19**	**322 (100.00), 100.00:100.00**	**74 (27.51), 22.17:32.85**	**195 (72.49), 67.15:77.83**	**269 (100.00), 100.00:100.00**	**304 (100.00), 100.00:100.00**

Table 3. Depression severity in HTN+T2DM cases

Depression Severity	Number (%) of Patients			
	Control HTN + Control T2DM (N=80)	Control HTN + Uncontrolled T2DM (N=109)	Uncontrolled HTN + Control T2DM (N=119)	Uncontrolled HTN + Uncontrolled T2DM (N=317)
None	51 (63.75)	49 (44.95)	76 (63.87)	145 (45.74)
Mild depression	19 (23.75)	35 (32.11)	33 (27.73)	109 (34.38)
Moderate depression	8 (10.00)	21 (19.27)	9 (7.56)	49 (15.46)
Moderately severe depression	2 (2.50)	4 (3.67)	1 (0.84)	13 (4.10)
Severe depression	0	0	0	1 (0.32)

4.63 kg/m^2, respectively. There was a similar distribution of men (50.79%) and women (49.21%) within the study. A significantly higher proportion of patients had T2DM and/or HTN since 5-10 years in comparison to >10 years (81.52% vs 12.79%, p< 0.0001) (Table 1). Four hundred and twenty-three (23.13%) patients (T2DM: 135; HTN: 161; T2DM+HTN: 127) had a significant family history of T2DM and/or HTN.

Prevalence of Depression

Approximately half (48.9%; 895/1829) of the overall population suffered from depression (score of ≥5 in the MD PHQ-9), including 322 (51.03%) patients with T2DM, 269 (46.94%) patients with HTN and 304 (48.64%) patients with T2DM+HTN. Majority of T2DM and/or HTN patients had not categorized depression. Of the other two categories of depression, a greater proportion of patients had "other depressive symptoms" compared to "major depressive syndrome" across all indications. In overall, a higher proportion of depressive patients with T2DM and HTN had an "uncontrolled" versus "controlled" nature of the disease. No statistically significant association was reported between different categories of depression (major depressive syndrome and other depressive symptoms) and controlled/uncontrolled T2DM or HTN (Table 2). A direct significant association was noted between severity of depression and controlled/uncontrolled T2DM and/or HTN cases (p<0.0001) (Table 3).

Association of Depression with Socio-demographic and Clinical Factors

Demographic profile

There was no significant association between gender and depression in T2DM and/or HTN patients. A significant association was observed between BMI and different types of depression in T2DM (p=0.0015) patients. The higher proportion of patients with T2DM and/or HTN with depression were overweight or obese (class 1). A significant association was reported between age and depression in patients with HTN (p=0.0386) while no such significant association was reported in T2DM or T2DM+HTN cases (Table 4).

Lifestyle parameters

There was a significant association between exercise and depression in T2DM+HTN patients (p=0.0243) but no such association was reported in T2DM and HTN patients. No significant association was reported between physical activity or alcohol consumption and depression. A significant association was reported between smoking habits and depression in patients with T2DM (p=0.0044) and T2DM+HTN (p=0.0169) (Table 4).

Socio-economic Status

There was a significant association between socioeconomic status and depression in patients with T2DM (p=0.0324) and T2DM + HTN (p=0.0006). The higher proportion of patients in the upper middle class reported depression across all indications (T2DM: 45.03%;

Table 4. Association of Depression with independent variables in HTN, T2DM and HTN+T2DM

	T2DM				HTN				T2DM+HTN			
Variable	**1 (N=20)**	**2 (N=102)**	**3 (N=200)**	**Total (N=322)**	**1 (N=24)**	**2 (N=100)**	**3 (N=145)**	**Total (N=269)**	**1 (N=52)**	**2 (N=95)**	**3 (N=157)**	**Total (N=304)**
Gender, n (%)												
Male	14 (4.35)	45 (13.98)	97 (30.12)	156 (48.45)	13 (4.83)	48 (17.84)	65 (24.16)	126 (46.84)	21 (6.91)	49 (16.12)	83 (27.30)	153 (50.33)
Female	6 (1.86)	57 (17.70)	103 (31.99)	166 (51.55)	11 (4.09)	52 (19.33)	80 (29.74)	143 (53.16)	31 (10.20)	46 (15.13)	74 (24.34)	151 (49.67)
Age (Years) Mean±SD	54.05 ±6.79	52.65 ±7.65	51.49 ±7.17	52.01 ±7.32	50.75 ±6.83	53.57 ±7.16	51.37 ±7.19	52.13 ±7.21	53.69 ±6.21	54.38 ±5.69	53.80 ±5.82	53.96 ±5.84
BMI (kg/m²), n (%)												
< 17.5	0	1 (0.31)	1 (0.31)	2 (0.62)	0	0	1 (0.37)	1 (0.37)	0	0	0	0
17.5 – 22.9	5 (1.55)	22 (6.83)	22 (6.83)	49 (15.22)	3 (1.12)	11(4.09)	18 (6.69)	32 (11.90)	2 (0.66)	12 (3.95)	15 (4.93)	29 (9.54)
23.0 – 27.9	7 (2.17)	54 (16.77)	76 (23.60)	137 (42.55)	12 (4.46)	44 (16.36)	70 (26.02)	126 (46.84)	23 (7.57)	46 (15.13)	65 (21.38)	134 (44.08)
>28	8 (2.48)	25 (7.76)	101 (31.37)	134 (41.61)	9 (3.35)	45 (16.73)	56 (20.82)	110 (40.89)	27 (8.88)	37 (12.17)	77 (25.33)	141 (46.38)
Lifestyle parameters, n (%)												
Does the patient exercise regularly												
Yes	3 (0.93)	27 (8.39)	47 (14.60)	77 (23.91)	8 (2.97)	36 (13.38)	34 (12.64)	78 (29.00)	27 (8.88)	36 (11.84)	87 (28.62)	150 (49.34)
No	17 (5.28)	75 (23.29)	153 (47.52)	245 (76.09)	16 (5.95)	64 (23.79)	111 (41.26)	191 (71.00)	25 (8.22)	59 (19.41)	70 (23.03)	154 (50.66)
Brisk walking	3 (0.93)	15 (4.66)	32 (9.94)	50 (15.53)	7 (2.60)	27 (10.04)	23 (8.55)	57 (21.19)	18 (5.92)	26 (8.55)	71 (23.36)	115 (37.83)
Cardio	0	0	1 (0.31)	1 (0.31)	-	-	-	-	1(0.33)	1 (0.33)	0	2 (0.66)
Jogg/running	0	5 (1.55)	9 (2.80)	14 (4.35)	0	5 (1.86)	6 (2.23)	11 (4.09)	2 (0.66)	2 (0.66)	5 (1.64)	9 (2.96)
Swimming	-	-	-	-	0	0	1 (0.37)	1 (0.37)	-	-	-	-
Heavy Weight	-	-	-	-	-	-	-	-	1(0.33)	0	0	1 (0.33)
Yoga	0	8 (2.48)	7 (2.17)	15 (4.66)	0	5 (1.86)	4 (1.49)	9 (3.35)	6 (1.97)	8 (2.63)	18 (5.92)	32 (10.53)
Cervical exercise	-	-	-	-	0	0	1 (0.37)	1 (0.37)	-	-	-	-
Cycling	-	-	-	-	1 (0.37)	0	1 (0.37)	2 (0.74)	0	0	2 (0.66)	2 (0.66)
Walking	0	1 (0.31)	1 (0.31)	2 (0.62)	0	1 (0.37)	2 (0.74)	3 (1.12)	2 (0.66)	1 (0.33)	2 (0.66)	5 (1.64)
Leg exerciser	-	-	-	-	-	-	-	-	0	1 (0.33)	0	1 (0.33)

Variable	T2DM				HTN				T2DM+HTN			
	1 (N=20)	2 (N=102)	3 (N=200)	Total (N=322)	1 (N=24)	2 (N=100)	3 (N=145)	Total (N=269)	1 (N=52)	2 (N=95)	3 (N=157)	Total (N=304)
Alcohol consumption, n (%)												
Yes	2 (0.62)	15 (4.66)	19 (5.90)	36 (11.18)	4 (1.49)	9 (3.35)	19 (7.06)	32 (11.90)	4 (1.32)	5 (1.64)	11 (3.62)	20 (6.58)
No	18 (5.59)	87 (27.02)	181 (56.21)	286 (88.82)	20 (7.43)	91 (33.83)	126 (46.84)	237 (88.10)	48 (15.79)	90 (29.61)	146 (48.03)	284 (93.42)
Smoking habits, n (%)												
Never smoked	20 (6.21)	77 (23.91)	177 (54.97)	274 (85.09)	16 (5.95)	78 (29.00)	123 (45.72)	217 (80.67)	46 (15.13)	72 (23.68)	138 (45.39)	256 (84.21)
Former smoker	0	8 (2.48)	12 (3.73)	20 (6.21)	4 (1.49)	9 (3.35)	13 (4.83)	26 (9.67)	4 (1.32)	9 (2.96)	13 (4.28)	26 (8.55)
Current smoker	0	17 (5.28)	11 (3.42)	28 (8.70)	4 (1.49)	13 (4.83)	9 (3.35)	26 (9.67)	2 (0.66)	14 (4.61)	6 (1.97)	22 (7.24)
Duration (Years), Mean±SD	-	20.71 ±7.28	18.64 ±8.10	19.89 ± 7.53	25.50 ±4.20	20.69 ±6.36	17.89 ±7.89	20.4 6±6.95	26.00 ±5.66	20.86 ±6.57	24.67 ±4.97	22.36 ±6.20
Socio-economic status, n (%)												
Lower	1 (0.31)	4(1.24)	30 (9.32)	35 (10.87)	3 (1.12)	11 (4.09)	19 (7.06)	33 (12.27)	3 (0.99)	2 (0.66)	3 (0.99)	8 (2.63)
Upper Lower	6 (1.86)	14 (4.35)	43 (13.3)	63 (19.57)	5 (1.86)	12 (4.46)	24 (8.92)	41 (15.24)	6 (1.97)	10 (3.29)	44 (14.47)	60 (19.74)
Lower Middle	3 (0.93)	19 (5.90)	32 (9.94)	54 (16.77)	7 (2.60)	18 (6.69)	31 (11.52)	56 (20.82)	16 (5.26)	27 (8.88)	39 (12.83)	82 (26.97)
Upper Middle	8 (2.48)	5 (18.01)	79 (24.53)	145 (45.03)	7 (2.60)	49 (18.22)	60 (22.30)	116 (43.12)	25 (8.22)	47 (15.46)	46 (15.13)	118 (38.82)
Upper	2 (0.62)	7 (2.17)	16 (4.97)	25 (7.76)	2 (0.74)	10 (3.72)	11 (4.09)	23 (8.55)	2 (0.66)	9 (2.96)	25 (8.22)	36 (11.84)

HTN: 43.12%; T2DM+HTN: 38.82%) (Table 4). Furthermore, a significantly greater proportion of depressive patients had monthly family income between 18,498 and 36,996 (in Indian National Rupees) in patients with T2DM (29.50%; p<0.0001), HTN (28.25%; p=0.0329) and T2DM + HTN (31.91%; p=0.0011) (data not shown).

Anxiety

There was a statistically significant association between severity of depression and anxiety scores across all indications (p<0.0001; Table 5).

Insomnia

A higher proportion of patients with depression had insomnia (versus no insomnia) across all the indications (T2DM: 5.23% vs. 4.12%; HTN: 10.12% vs. 3.49%; T2DM+HTN: 9.28% vs. 3.84%). However, the association was found to be non-significant (Table 5).

Comorbidities and Complications

Dyslipidemia was the most common comorbidity reported in patients with T2DM (24.22%), HTN (14.87%) and T2DM+HTN (27.30%). The proportion of dyslipidemic patients with other depressive symptoms was higher in comparison to patients with major depressive syndrome across all the indications (T2DM: 5.28% vs. 1.86%; HTN: 5.95% vs. 1.12%; T2DM+HTN: 9.54% vs. 5.59%).

Among patients with HTN and depression, the most common complications reported (> 10%) were angina (13.01%) and heart failure (11.15%). Diabetic neuropathy (13.35%) was the most common complications reported among patients with T2DM and depression. Depression was found to be significantly associated with complications in T2DM (p=0.0001) and comorbidities in T2DM + HTN (p=0.0023) cases. In addition, the complications of HTN have also shown a significant association with depression in T2DM + HTN patients (p=0.0246) (data not shown).

Discussion

The co-occurrence of depression in patients with T2DM and HTN is well-known and has been reported in wide array of literature.[11,26] Depression is usually associated with poor disease control, adverse health outcomes in patients with T2DM/HTN.[22,34]

In the current study, more than 45% of the patients with T2DM (51.03%), HTN (46.94%) and T2DM+HTN (48.64%) reported depression (score of ≥5 in the MD PHQ-9). In comparison to our results, previous studies have reported a lesser prevalence of depression in both T2DM (23.4% to 42.2%) and HTN (15% to 40.1%) patients.[6-10,35-38] Majority of the patients with depression had uncontrolled nature of the disease (T2DM: 77.64%; HTN: 72.49%); indicating an association between depression and poorly managed disease conditions. The higher proportion of patients did not fulfil the criteria of either "major depression" or "other depressive symptoms" across all indications. This might be due to fact that substantially larger proportion of patients in our study had none to mild severe depression (>70%). This study further reports that patients meeting the criteria of "other depressive symptoms" were higher in comparison to those with "major depression" across all clinical conditions (T2DM: 31.68% vs. 6.21%; HTN: 37.17% vs. 8.92% and T2DM+HTN: 31.25% vs. 17.11%). These findings were in concordance with the earlier published literature where a higher proportion of diabetics reported to have "other depressive symptoms" in comparison to "major depression" (20% vs. 12.5%).[39] A systematic diagnosis of depression using established screening tools and treatment initiation without commensurate increase in clinical duration should be devised for an improved therapeutic outcome in HTN and T2DM patients. Furthermore, a call for integrated clinical approach for assessment and management of major depression should be more emphasized in patients with both T2DM and HTN.

Socioeconomic status was significantly associated with depression in T2DM and T2DM + HTN cases. Our results were in agreement with earlier findings where socio-demographics was postulated as a risk factor for cases of depression in DM and HTN patients.[10,40] In the study, a significant association between BMI and depression was observed in T2DM patients (p=0.0015), however, the majority of patients across each indication were overweight or obese (class 1). Similar results were reported by Barnes et al (2015) in which depression was found to be significantly associated with elevated BMI.[41] Hence, the obesity status of T2DM and/or HTN patients should be documented and efforts should be made to identify the disease progress and initiate timely, and effective treatment for improved outcomes. In addition, poor lifestyle also serves as a predisposing factor for depression and worsens self-management of T2DM and HTN. In the present study, more than 70% of the patients with depression and T2DM/HTN did not exercise regularly, making them more susceptible to disease-related complications and comorbidities.

Depressive T2DM patients are more likely to develop comorbidities and complications than the non-depressive patients.[42] In our study, depression was significantly correlated with associated complications in T2DM patients (p=0.0001). Additionally, depression was significantly associated with comorbidities (p=0.0023) and complications of HTN (p=0.0246) in T2DM + HTN patients. This could be due to the fact that depression activates the hypothalamic-pituitary-adrenal axis, sympathetic nervous system, proinflammatory and proco-

Table 5. Association of Depression with Anxiety (GAD-7 scores) and insomnia in patients with HTN, T2DM and HTN+T2DM

Depression	Anxiety (GAD-Score)					Diagnosed with insomnia clinically	
	No Symptoms	Mild	Moderate	Severe	Total	Yes	No
HTN, n (%)							
None	254 (44.33)	45 (7.85)	4 (0.70)	1 (0.17)	304 (53.05)	12 (2.09)	3 (0.52)
Mild	59 (10.30)	132 (23.04)	15 (2.62)	1 (0.17)	207 (36.13)	28 (4.89)	11(1.92)
Moderate	2 (0.35)	23 (4.01)	22 (3.84)	2 (0.35)	49 (8.55)	13 (2.27)	3 (0.52)
Moderately Severe	0	2 (0.35)	6 (1.05)	2 (0.35)	10 (1.75)	4 (0.70)	2 (0.35)
Severe	1 (0.17)	0	0	2 (0.35)	3 (0.52)	1 (0.17)	1 (0.17)
Total	**316 (55.15)**	**202 (35.25)**	**47 (8.20)**	**8 (1.40)**	**573 (100)**	**58 (10.12)**	**20 (3.49)**
T2DM, n (%)							
None	275 (43.58)	32 (5.07)	2 (0.32)	0	309 (48.97)	8 (1.27)	3 (0.48)
Mild	89 (14.10)	151 (23.93)	13 (2.06)	3 (0.48)	256 (40.57)	16 (2.54)	14 (2.22)
Moderate	4 (0.63)	28 (4.44)	20 (3.17)	4 (0.63)	56 (8.87)	8 (1.27)	7 (1.11)
Moderately Severe	0	2 (0.32)	7 (1.11)	1 (0.16)	10 (1.58)	1 (0.16)	2 (0.32)
Severe	0	0	0	0	0	0	0
Total	**368 (58.32)**	**213 (33.76)**	**42 (6.66)**	**8 (1.27)**	**631 (100)**	**33 (5.23)**	**26 (4.12)**
T2DM+HTN, n (%)							
None	298 (47.68)	19 (3.04)	4 (0.64)	0	321 (51.36)	8 (1.28)	5 (0.80)
Mild	59 (9.44)	120 (19.20)	15 (2.40)	2(0.32)	196 (31.36)	19 (3.04)	11 (1.76)
Moderate	2 (0.32)	41 (6.56)	40 (6.40)	4 (0.64)	87 (13.92)	26 (4.16)	7 (1.12)
Moderately Severe	0	3 (0.48)	9 (1.44)	8 (1.28)	20 (3.20)	5 (0.80)	1 (0.16)
Severe	0	0	0	1 (0.16)	1 (0.16)	0	0
Total	**359 (57.44)**	**183 (29.28)**	**68 (10.88)**	**15 (2.40)**	**625 (100)**	**58 (9.28)**	**24 (3.84)**

agulation responses which in turn increases cortisol secretion, catecholamine release, cytokines and platelet/endothelial cell adhesion molecule-1. These mediators may play a vital role in disease progression in depressive patients with coexisting T2DM and HTN.[22] However, no significant association was reported between depression and comorbidities/complications in patients with HTN.

As stated, we noted substantially higher proportion of depressive patients in uncontrolled T2DM and HTN cases in our study. Previous studies have also shown that uncontrolled diabetes increases the risk of diabetic complications which in turn enhances depressive symptoms in T2DM patients.[43,44] On the other hand, depression was found to be a risk factor for poor blood pressure control in HTN patients.[45] There is still no consensus regarding the true nature of the relationship between depressive symptoms and uncontrolled nature of disease, which remains to be elucidated in well-designed studies. However, the current finding suggests that depression management may require an additional strategy of optimizing the disease control, which also reduces the risk of complications in both T2DM and HTN patients. A better understanding of the relationship may help clinicians to address the affective conditions that may lead to more comprehensive management of depression.

Quality of sleep plays a vital role in reducing the risk of DM and HTN.[46] Poor sleep quality and altered circadian rhythms increase depression and insulin resistance.[26,47] In our study, more than 5% of the patients with depression suffered from insomnia (T2DM: 5.23%, HTN: 10.12%; T2DM+HTN: 9.28%).

Anxiety, in addition to depression, serves an important role in the outcome of any disease.[26] A significant association was observed between depression and anxiety across all the indications ($p<0.0001$). Since, majority of our patients had none to mild depression, the prevalence of no or mild anxiety was reported in a higher proportion of patients.[48]

Clinical Implication

Depression has been identified as a cause and a consequence of T2DM and HTN. The presence of depression worsens the disease condition, deteriorate the symptoms and impacts self-care in patients with T2DM and HTN and vice versa. People with depression are more likely to be sedentary and eat diet that is rich in saturated fats and refined sugars and avoid fruit and vegetables, which may further aggravate the risk of developing T2DM and HTN.[49] Furthermore, depression significantly affects treatment adherence and patients tend to miss medical appointments, takes erratic diet, skips exercise and medications and avoid self-care; further deteriorating disease management and exacerbating the severity of depressive symptoms.[17,50] Clinicians and other healthcare professionals in T2DM and/or HTN setting(s) should be made aware of concurrent depression and must undertake subjective and objective assessments of depression. Patients with T2DM+HTN, who are at risk of complications or have predominant physical symptoms or an uncontrolled disease must undergo regular screening for depression. This would enable clinicians to have an adequate management of T2DM and HTN in patients with comorbid depression and offer them better disease control by preventing long-term complications. It is very critical to take care of depressive symptoms in patients with T2DM and/or HTN as psychological treatment of depressive symptoms would not only improve T2DM and HTN-related complications but would also improve anxiety and patient well-being.

Strength and Limitations

Our study has few strengths and limitations. This was a first of its kind study conducted across different geographical regions of 54 cities in India. The study highlighted the burden of depression in patients with controlled and uncontrolled T2DM and HTN and has identified the various risk factors associated with development or aggravation of depressive symptoms in the most common metabolic disorders. Validated and widely used questionnaires were used to assess the severity of depression (Prime MD PHQ-9) and anxiety (GAD-7), which ensured the credibility of our findings. There are few limitations of this study as well. Firstly, no comparator group was included to see the viability of the study results. Additionally, no longitudinal assessment was done to evaluate the changes in depression, anxiety and other factors from baseline. Nevertheless, this study provides the first nationwide data on the prevalence of depression in patients with T2DM and/or HTN. Further longitudinal studies are warranted to ascertain the long-term association of depression with these metabolic disorders and to assess its impact on disease progression and vice versa.

Conclusion

The current study indicates an increased prevalence of depressive disorders in patients with T2DM (51.03%), HTN (46.94%) and T2DM+HTN (48.64%) in conjunction with anxiety and sleep disturbances. There was a direct significant association between depression and socioeconomic status, education, occupation, physical activity in patients with T2DM and/or HTN. Depression care in patients with T2DM and/or HTN is critical as it worsens the disease condition and increases the T2DM and HTN-related complications, anxiety and deteriorates the patient well-being. Patients who are on high-risk should undergo regular screening for depression and management under comprehensive mental healthcare systems.

Acknowledgment

We thank Dr Jilani AQ and Dr Sanjay Kumawat for their expert review of this article. We also thank GCE Solutions for overall preparation of the manuscript.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.6

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 326–330

ORIGINAL PAPER

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Rating of the effectiveness of cleaning and disinfection procedures in a mass catering establishment

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ABSTRACT

Introduction. An increasing problem in maintaining the proper level of hygiene in food industry plants is microbiological hazards, affecting the quality and safety of produced food, which in consequence may lead to the creation of many negative health effects on consumers.

Aim. Determination of the degree of microbiological contamination of machinery and equipment as well as small production equipment before the commencement of production activities and rating of the effectiveness of the implemented procedures of cleaning and disinfection in a mass catering establishment.

Material and methods. The research material was the surfaces of machinery and equipment as well as small production equipment used in a mass catering establishment located in the Primary School in Przemyśl. Microbiological tests were carried out using the swab method in accordance with the recommendations of the Polish Standard PN-A-82055-19: 2000.

Results. The hygienic condition of the marked surfaces largely differed from the specific hygiene standards described in PN-A-82055-19: 2000. In most cases the degree of microbial contamination was insufficient. It is recommended to follow strictly defined washing and disinfection procedures every time after finishing work and if necessary before proceeding with production.

Conclusion. The obtained results showed that there was secondary microbiological contamination of the determined production areas subjected to cleaning and disinfection. It was found that the procedures of cleaning and disinfection of small production equipment, including parts of machines and devices, were properly developed, while there are discrepancies in their implementation.

Keywords. food safety, production hygiene, washing and disinfection

Introduction

Washing and disinfection play a very important role in maintaining an appropriate level of hygiene, ensuring safety and high quality of the food produced.[1,3]

The main purpose of carrying out cleaning and disinfection procedures is to remove or reduce all physical, chemical and, above all, microbiological contamination that accumulates on production surfaces, which is a major threat to the food being produced. The concept of production hygiene in the food industry includes both cleanliness of production rooms, machinery and equipment, as well as personal hygiene of personnel.[2,4,5]

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 23.03.2019 | Accepted: 25.08.2019
Publication date: December 2019

Sidor A, Preizner P. *Rating of the effectiveness of cleaning and disinfection procedures in a mass catering establishment.* Eur J Clin Exp Med. 2019;17(4):326–330. doi: 10.15584/ejcem.2019.4.6

In food production plants, the basis for hygiene is to perform cleaning and disinfection of all tools, machines and equipment as well as production rooms in accordance with the procedures and instructions developed.[6-9] The correct way to carry out cleaning and disinfection procedures with the use of appropriate equipment and devices, as well as the selection of appropriate cleaning agents, allows proper preparation of the rooms and production areas. The use of appropriate washing and disinfection systems should guarantee that the highest hygienic and sanitary standards, as well as health food safety, are met. [10] In food production, a very important stage is the monitoring of washing and disinfection processes, aimed at limiting potential hazards and controlling each stage of the production process, which has a decisive impact on the health quality of food.[11,12] Systematic control of the effectiveness of cleaning and disinfection processes by performing microbiological tests and analyses of both production rooms and all surfaces in contact with the produced food will allow to identify and locate the potential source of hazards, obtaining an actual picture of the hygienic condition of the devices.[13-16]

Material and methods

Research into this work was carried out on the premises of a collective catering facility located at the Primary School in Przemyśl.

The nutrition block located on the ground floor part of the school building has been in operation since 1978. The facility's activity is based on organized nutrition for children and youth as well as school employees. The food segment provides about 150 meals a day in the form of a two-course dinner and a dessert compote and fresh fruit. Meals are served every day from Monday to Friday from 11:00 a.m. to 1:00 p.m.

The preparation of meals takes place in a designated kitchen room, equipped with the necessary equipment as well as machinery and equipment.

After the end of the work, are treatments carried out for cleaning and disinfecting kitchen utensils, all production areas, floors, machinery and equipment and auxiliary equipment in accordance with the developed procedures and instructions.

The research material consisted of machine and equipment surfaces (a vegetable and fruit slicer, meat grinder and electric frying pan), as well as small production equipment (ladle, bowl, lid, a roasting pan, colander) used in a mass catering establishment.

Test samples were taken from the controlled areas for five days a week at 7:00 a.m., prior to the start of production at the plant, then transported to the laboratory for microbiological testing. The transport time did not exceed 60 minutes, the temperature was within 0-5°C.

Microbiological tests in this work were carried out using the swab method in accordance with the recommendations of PN-A-82055-19: 2000, to determine the degree of microbiological contamination of machinery and equipment and small production equipment based on the total number of mesophilic aerobic microorganisms.

Determination of microbiological contamination of machinery and equipment as well as small production equipment, carried out as part of the internal control of the plant, were carried out in the morning before production. Sterile tampons and a jar with 30 milliliters of Ringer's solution were prepared prior to the collection of the swabs. The jar with the sample taken was placed in an isothermal container, and then swabs were taken from the following surfaces, proceeding in the same way as described above.

During the day, four different surfaces were marked. After collecting the swabs from all four surfaces, the jars were put into an isothermal container with ice to keep the temperature between 0 ° C and 5° C, and within one hour of collection, they were transported to the laboratory for microbiological testing.

Before starting the work, the laminar table top was disinfected by rubbing with ethyl alcohol. Sterile tubes with 9 milliliters of Ringer's solution were then prepared and labeled accordingly. Prior to the dilution, a sample of the test material was shaken intensely for about 1 minute. Sterile 1 milliliters of the test sample was removed from the sterile pipette and transferred to a test tube with 9 milliliters of Ringer's solution. The first dilution obtained in this way (10^{-1}) was thoroughly mixed. Worn tips were placed in a beaker with cotton wool and alcohol. All operations were carried out under sterile conditions.

Two sterile Petri dishes were prepared for each dilution and labelled accordingly. Before seeding, the tubes with lavage were mixed thoroughly. The automatic pipette was made of the initial suspension and dilution of 10^{-1} inoculation of 1 milliliter into two parallel plates. Subsequently, 15 milliliters of cooled agar fluid (Nutrient Agar 70148-500G) was poured on each plate within 15 minutes of the culture. After complete solidification of the medium, inverted plates in the incubator were placed. Incubation was carried out at 30 °C for 48 hours.

After 48 hours incubation in the incubator, the number of colonies on the plates of two parallel replicate cultures from the washings (starting suspension) was read, due to the lack of the presence of CFU on the plates with a dilution of 10^{-1}.

The number of microorganisms located on 1 cm^2 limited by the surface template (25 cm^2) was calculated on the basis of PN-A-82055-19: 2000.

Results

After reading the number of colonies grown on Petri dishes, calculations were made to determine the total number of mesophilic aerobic microorganisms and on

Table 1. Summary of results from the microbiological contamination of machines and devices

The total number of microorganisms		Research object: Machines and devices							
		Vegetable and fruit slicer			Meat grinder			Electric frying pan	
		The term of research							
		30.05.	31.05.	01.06.	30.05.	31.05.	01.06.	02.06.	03.06.
The number of colonies on the plates(from the starting suspension)	I plate	63	23	21	35	29	18	34	23
	II plate	67	21	25	39	31	22	36	25
The average number of colonies		65	22	23	37	30	20	35	24
Standard deviation		±2,83	±1,41	±2,83	±2,83	±1,41	±2,83	±1,41	±1,41
The number of microorganisms on 25 cm^2 area [jtk/25cm^2]		$2,0\times10^3$	$6,6\times10^2$	$6,9\times10^2$	$1,1\times10^3$	$9,0\times10^2$	$6,0\times10^2$	$1,1\times10^3$	$7,2\times10^2$

Table 2. Summary of results from the microbiological contamination of small production equipment

The total number of microorganisms		Research object: Machines and devices					
		Roasting pan			Colander		
		The term of research					
		30.05.	31.05.	01.06.	30.05.	31.05.	01.06.
The number of colonies on the plates(from the starting suspension)	I plate	28	18	7	27	13	5
	II plate	24	20	7	29	17	3
The average number of colonies		25	19	7	28	15	4
Standard deviation		±1,41	±1,41	±0,00	±1,41	±2,83	±1,41
The number of microorganisms on 25 cm^2 area [jtk/25cm^2]		$7,5\times10^2$	$5,7\times10^2$	$1,0\times10^2$	$8,4\times10^2$	$1,5\times10^2$	$1,0\times10^2$

Table 3. Summary of results from the microbiological contamination of small production equipment

The total number of microorganisms		Research object: Machines and devices					
		Lid		Bowl		Ladle	
		The term of research					
		02.06.	03.06.	02.06.	03.06.	02.06.	03.06.
The number of colonies on the plates(from the starting suspension)	I plate	23	11	7	3	7	4
	II plate	21	13	5	3	11	6
The average number of colonies		22	12	6	3	9	5
Standard deviation		±1,41	±1,41	±1,41	±0,00	±2,83	±1,41
The number of microorganisms on 25 cm^2 area [jtk/25cm^2]		$6,6\times10^2$	$3,6\times10^2$	$1,0\times10^2$	$9,0\times10$	$2,7\times10^2$	$1,5\times10^2$

its basis to assess the degree of microbial contamination of machinery and equipment as well as small production equipment.

After calculations, the results are presented in two tables divided into machines and devices (table 1) and small production equipment (table 2 and 3).

Discussion

Microbiological hazards are a very serious problem in maintaining the proper level of hygiene in food industry devices. Exposure to biological agents may cause a decrease in the quality of food produced, as well as threaten its safety, which in turn may lead to many negative health effects on consumers.[17-20] Therefore, a prerequisite for maintaining the hygienic condition of the plant and full health comfort for consumers is to conduct systematic inspections, by performing tests and microbiological analyses assessing the degree of microbiological contamination and the effectiveness of the procedures of washing and disinfection implemented. The necessary measure to achieve the abovementioned objective is to develop appropriate standards and guidelines that are widely accepted and allow for correct interpretation of the results obtained.

In contrast to the majority of physical and chemical hazards, there are no universally accepted criteria for the assessment of microbial contamination, as well as acceptable normative values or methodological recommendations. Nevertheless, there are many standards, standards or proposals for limit values that are helpful in

interpreting the obtained test results. They are usually of relative or arbitrary nature.[17-20]

The assessment of microbial contamination of machinery and equipment as well as small production equipment on individual test days was made on the basis of the guidelines of PN-A-82055-19: 2000. Summing up, the obtained results from the entire research period (from 30/05/2016 to 03/06/2016) indicate that the hygienic condition of the marked surfaces to a large extent deviated from the specific hygiene standards described in the standard. In most cases the degree of microbial contamination was insufficient (above 100 cfu/25cm^2). During the whole research period, the surface of machines and devices was much more dirty than small production equipment. This may have been caused by the difficulty in maintaining a high degree of cleanliness requiring the dismantling of machinery and equipment components.

Conclusion

On the basis of conducted microbiological tests and a review of the applied washing and disinfection procedures in a mass catering establishment, the following conclusions and recommendations were formulated:

1. The hygienic condition of the marked surfaces largely differed from the specific hygiene standards described in PN-A-82055-19: 2000. In most cases the degree of microbial contamination was insufficient. It is recommended to follow strictly defined washing and disinfection procedures every time after finishing work and if necessary before proceeding with production.
2. At the beginning of the week, ie. 30/05/2016, during the inspection, the highest microbiological contamination of the tested areas was recorded, from the entire research period. It could be caused by a longer break in carrying out the cleaning and disinfection procedures, ie. from Friday to Monday.
3. During the whole research period, the surface of machines and devices was much more dirty than small production equipment. This may have been caused by the difficulty in maintaining a high degree of cleanliness requiring the dismantling of machinery and equipment components. For this type of surfaces, appropriate methods and techniques of cleaning and disinfection should be applied in accordance with the developed procedures.
4. Due to the lack of generally recognized microbiological purity criteria in the law, it is recommended that the operator of the food sector should develop its own limits of plant purity.
5. After analyzing the obtained test results, it was found that after at least 12 hours after the completion of the cleaning and disinfection procedures, there was a secondary infection of the tested production areas. It was found that there are slight discrepancies in the implementation of cleaning and disinfection procedures. It is recommended to perform initial rinsing of production equipment before starting the production process, in order to reduce the microbiological contamination of production areas and increase the efficiency of the implemented procedures of cleaning and disinfection in a collective catering establishment.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.7

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 331–333

REVIEW PAPER

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Multiparametric MRI and other imaging methods suitable to stage prostate carcinoma

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ABSTRACT

Introduction. The role of multiparametric magnetic resonance imaging (mpMRI) in staging prostate carcinoma has been increasing over the last years. It's high sensitivity is indispensable when diagnosing this disease. It is a very accurate imaging method that helps the physician choose the best treatment method for his patient.

Aim. Assessment of mpMRI which uses both anatomic and functional imaging techniques as a method to diagnose prostatic lesions. Advantages and disadvantages of staging prostate carcinoma with the use of biparametric MRI (bpMRI).

Methods. The literature search was performed.

Results. MpMRI can be used in pre-operative staging of prostate cancer. The technique is accurate in diagnosing and assessment of prostate carcinoma with Gleason Score (GS) of 7 and above. It is also recommended when planning a second biopsy of the prostatic gland.

Keywords: Prostate cancer, multiparametric MRI (mpMRI), biparametric MRI (bpMRI), staging

Prostate carcinoma

Prostate carcinoma is the second most frequent malignant tumor diagnosed in the male population worldwide.[1-3] Over 85% of cases are seen in patients over 65 years of age.[4] Pathologists in the United States diagnose prostate carcinoma in over 80% of patients in their 70's upon post mortem tissue examiantion.[5] Differentiating lethal from nonlethal disease is the number one issue due to controversies concerning the correct treatment.[6] Correct assessment of clinical stage and a pathologic stage are crucial.[7] No imaging can accurately confirm or exclude presence of a prostate carcinoma. Diagnosis relies on microscopic

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 16.09.2018 | Accepted: 29.11.2019
Publication date: December 2019

Osuchowski M, Aebisher D, Galiniak S, Bartusik-Aebisher D, Kaznowska E. *Multiparametric MRI and other imaging methods suitable to stage prostate carcinoma.* Eur J Clin Exp Med. 2019;17(4):331–333. doi: 10.15584/ejcem.2019.4.7

examination of prostatic tissue.[6] Diagnostic imaging can be used however for staging of local disease. While computed tomography (CT) isn't capable of reliable detection of prostate cancer, MR shows a lot of promise.[8,9] Guidelines for staging prostate carcinoma include not using CT or transrectal ultrasound in any risk group, not using additional imaging in low-risk group for staging purposes and using mpMRI in intermediate-risk and high-risk groups.[7] There are several ways to perform an MRI while attempting to stage prostatic carcinoma. Multiparametric MRI uses a combination of T_2 – weighted imaging, diffusion imaging, perfusion and spectroscopic imaging while biparametric focuses on morphologic T_2 – weighted imaging and diffusion-weighted imaging.[10-12] Associating T_2 – weighted imaging with at least one functional imaging technique (DWI, DCE, ^{1}H spectroscopy) has good sensitivity for the detection and localization of GS ≥ 7 cancers in expert centers. For a tumor volume less than 0.5 mL the sensitivity is 63% for GS = 7 and 80% for GS above 7. When evaluating larger lesions the sensitivity is higher – for tumor volume 0.5 – 2 mL 82-88% for GS = 7 and 93% for GS above 7 and for tumor volume over 2 mL 97% for GS = 7 and 100% for GS above 7.[13] A scoring system has been introduced to help reproduce good results in less-experienced centers. The first version of a system called PI-RADS has not proved an improved interobserver variability as compared with subjective scoring.[14] Currently, a second version of PI-RADS is being used and some authors suggest there is still room for improvement by, for example, adding ADC values to the equation.[15,16] Lots of studies performed in a single center suggest that multiparametric magnetic resonance imaging can reliably detect aggressive tumors with a negative predictive value (NPV) ranging from 63% to 98% and positive predictive value ranging from 34% to 68% .[17] Others show that even with the use of the PIRADS v2 scoring system, mpMRI inter-reader reproducibility is showing moderate specificity.[18-20] This fact limits its broad use outside expert centers. All this may lead to substantial patient mismanagement. This method of imaging is also not accurate enough to consistently grade tumor aggressiveness when planning focal therapy.[21,22] The upside is shown by the PROMIS study. It proved that mpMRI, used as a triage test before first prostate biopsy, could reduce unnecessary biopsies by 27%. It can also reduce over-diagnosis of clinically insignificant prostate cancer and improve detection of clinically significant cancer when compared with the standard pathway of TRUS-biopsy for all patients.[23,24] Pre-operative 3TmpMRI may even serve as a prognostic marker of treatment outcomes independently of biopsy GS or histological type of the carcinoma.[25] The most recent studies concentrate on reducing cost, time, and contrast exposure by eliminating the DCE phase of mpMRI. Scherrer et al. suggest that mpMRI can be replaced by biparametric MRI (bpMRI) without forfeiting valuable diagnostic information. Biparametric MRI and multiparametric MRI have similar cancer detection rates, particularly for clinically significant cases of prostate carcinoma.[26-28] When staging prostate carcinoma hybrid imaging devices such as single-photon emission CT/CT gamma cameras (SPECT) or positron emission tomography/CT cameras (PET) are often a necessity. These methods are primarily used to diagnose metastases. [29-31] With SPECT imaging bone metastases can be detected with very high sensitivity and specificity (over 79% and 82% respectively).[32] PET imaging using ^{11}C-choline or ^{18}F-choline as contrast agents can be used to diagnose lymph node and bone metastases. For the latter sensitivity is at 100% and specificity is around 86%.[33,34] Due to relatively low glucose absorption by prostate carcinoma the use of FDG-PET imaging method is very limited.[35-40]

Conclusion

In conclusion, an MRI can accurately stage prostate carcinoma. This type of imaging has high sensitivity and moderate specificity when diagnosing the disease[36-38] and can be very helpful prior to a second biopsy or when planning the correct curative approach.[39,40]

Acknowledgments

Dorota Bartusik-Aebisher acknowledges support from the National Center of Science NCN (New drug delivery systems-MRI study, Grant OPUS-13 number 2017/25/B/ST4/02481).

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© Wydawnictwo UR 2019
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.8

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 334–337

REVIEW PAPER

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Imaging studies of kidney cancer

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ABSTRACT

Introduction. In 2017 in the USA about 5% in men and 3% in women newly diagnosed cases of malignant tumors were kidney and renal pelvis cancer.

Aim. Kidney cancer in adults includes malignant tumors derived from kidney parenchyma and renal pelvis. The dominating types are kidney parenchyma, and mainly renal cell carcinomas

Material and methods. This review was performed according to systematic literature search of three major bibliographic databases (Scopus, PubMed, and Cochran).

Results. Imaging studies play a very important role in kidney cancer. They allow one to assess the clinical stage, justify the extent of surgery and have an impact on the prognosis.

Conclusion. The field for research involves the use of magnetic resonance and positron emission tomography in diagnosing kidney changes.

Keywords. Kidney cancer, MRI, PET.

Introduction

The neoplasms of the calyx - pyelone system originating from the transitional epithelium constitute less than 10% of all kidney cancers.[1] The majority of kidney cancers in children are germline (Wilms' tumor), and its frequency is around 1.1%.[2] Small papillary adenomas of the renal cortex (less than 0.5 cm), found in 40% of people, have no clinical significance.[3] It is estimated that in 2017 in the USA about 5% in men (40,610 cases) and 3% in women (23,380) newly diagnosed cases of malignant

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 25.08.2018 | Accepted: 29.11.2018
Publication date: December 2019

Przyczyna P, Aebisher D, Gustalik J, Galiniak S, Bartusik-Aebisher D, Kaznowska E. *Imaging studies of kidney cancer*. Eur J Clin Exp Med. 2019;17(4):334–337. doi: 10.15584/ejcem.2019.4.8

tumors were kidney and renal pelvis cancer.[4] Kidney cancer is characterized by a triad of symptoms: hematuria, palpable mass and pain in the side. Some patients may also experience anemia, weight loss, fever and varicocele.[5] Renal cell carcinoma in patients under 46 may indicate hereditary origins.[6] Due to the prevalence of imaging methods (especially computed tomography of the abdominal cavity and pelvis as well as ultrasound examination), the frequency of incidental detection of kidney cancer has increased.[7,8]

Material and methods

This article is based on an analysis of articles posted on three major bibliographic databases (Scopus, PubMed, and Cochran) and books.

Ultrasound

Incidentally detected kidney cancers are generally smaller, and are associated with a better prognosis than symptomatic tumors, regardless of grading and clinical stage.[9-10] Therefore, in recent times, interest in screening programs for this disease has increased.[11] In addition, the establishment of a screening program for abdominal aortic aneurysm in the United Kingdom, for men over 65 years, gave ideal conditions to verify the validity of this study. This is possible due to the fact that the risk factors and methods for detecting both diseases are similar.[12] Current data of the National Cancer Intelligence Network indicate that only 44% of patients with RCC are diagnosed in the first stage. About 10% of patients are diagnosed in stage II. Metastasis at diagnosis occurs in up to 25% of patients.[13] Meta-analysis suggests a positive shift in the severity of the population covered by the screening study.[14] Only 2% of patients had metastases or lymph node involvement at diagnosis. As many as 84.4% of tumors were detected in the T1-T2N0 stage and 13.7% in the T3-T4N0 stage. Ultrasound examination also has a dark side in the form of false positives. In one study, among 6,678 cases, 22 cases of kidney masses suspected of renal cell carcinoma were detected. However, despite additional CT examinations, only 15 of them had a positive histological diagnosis.[12] In addition, there are differences in the detection of kidney cancer depending on the geographical region.[15] Autopsy examinations of organ donors after the age of 65 showed renal cell carcinoma in 0.7-0.9%, which is more than in meta-analyzes.[14, 16, 17] Therefore, the incidence and histological evidence of kidney cancer may be underestimated. According to data among 1,000 patients examined, masses in the kidney will be detected in 4, of which at least one of them will be diagnosed with renal cell carcinoma.[12] For comparison, the NHS screening program of the abdominal aortic aneurysm shows 10 patients per 1000 examined patients with a change in size of 3 cm or more, of which only two undergo elective surgery.[18] The Bowel Cancer Screening Program in England shows 1.6 patients with colorectal cancer per 1000 people in the study[19] , and the Breast Cancer Screening Program has 8.3 patients with breast cancer per 1,000 women.[20] These numbers are much higher than in the screening project of kidney cancer, however, it is estimated that 15-25% of positive results in breast cancer screening are diagnosed.[21]

Computed tomography

Renal changes can be easily diagnosed by imaging tests and in many cases do not require histopathological verification.[22] However, complex cysts and cysts with a fixed component require more detailed characterization allowing for differential diagnosis, and then developing a therapeutic plan and prognosis.[23-26] In response to the above demand, in 1986, Bosniak developed a classification based on computed tomography. During the assessment, the following are taken into account: contours of change, content, presence of partitions and calcifications, as well as enhancement after giving contrast.[27-28] Changes in the kidneys are classified in terms of increasing malignancy as follows:

a) Bosniak I simple - the majority of changes detected in the kidneys. The changes qualified for this group are always mild, without the possibility of malignancy and do not require further diagnosis[27]

b) Bosniak II minimally complicated - these changes, like in the first category, are considered to be mild, but may have some disturbing features. However, during histopathological examination, changes in this category have been included in the group of potentially malignant or malicious changes.[29-30]

c) Bosniak IIF - minimally complicated follow-up - included in the classification in 1993.[31,32] These changes do not meet the criteria for inclusion in Group III, and at the same time are more complex than in Group II. Their differentiation is subtle and difficult, and also has a high degree of variability between the described research. However, taking into account variability in the clinical process, it is clinically relevant.[33-34]

d) Bosniak III indeterminate - this group contains lesions with mild and malignant differentiation, which cannot be reliably assessed by imaging. Therefore, there is a significant risk of malignancy. The histopathologically corrected lesions are classified as malignant in 31% to 100% of cases.[34]

e) Bosniak IV cystic neoplasm - the percentage of malignant tumors of these lesions ranges from 95% to 100%. Differentiation between categories III and IV can be difficult but is not essential, as both of these categories require surgical removal.[35]

Magnetic Resonance Imaging

In clinical practice, magnetic resonance imaging is used to assess lower vena cava infiltrate and clinical stage in contrast-sensitized patients with renal failure or metas-

tases.[36-37] The problem of using magnetic resonance imaging in kidney changes is the use of the Bosniak scale by radiologists, which was created to describe computed tomography images. In this test, additional baffles may be visible, otherwise reinforced with contrast, and the thickness of the walls may be different than in tomography. In some cases this leads to overstating the scale and differences in the proceedings[35]

Positron emission tomography

Currently, positron emission tomography alone is not normally used to assess the clinical stage or to look for recurrences in renal cancer.[38] Post-operative surveillance is also controversial because there is no level 1 evidence that early intervention improves survival.[39] On the other hand, it was shown that the initial value of F-18 fludeoxyglucose (FDG) uptake correlates with the forecast.[40-41]

Conclusion

The field for research involves the use of magnetic resonance and positron emission tomography in diagnosing kidney changes.

Acknowledgments

Dorota Bartusik-Aebisher acknowledges support from the National Center of Science NCN (New drug delivery systems-MRI study, Grant OPUS-13 number 2017/25/B/ST4/02481).

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© Wydawnictwo UR 2019
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.9

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 338–346

REVIEW PAPER

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Clinical application of monoclonal antibodies in targeted therapy

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ABSTRACT

Introduction. Recently, monoclonal antibodies (mAbs) have become powerful human therapeutics in the diagnosis and treatment of many diseases. Drugs based on mAbs are approved for the treatment of cardiovascular, respiratory, hematology, autoimmunology, and oncology diseases.

Aim. To present the current state of knowledge about the application of mAbs in the therapy of various diseases such as cancer, autoimmune and Alzheimer's diseases.

Material and methods. We conducted a thorough review of the scientific literature from the following databases: EBSCO, PubMed, Science Direct, and Springer Link.

Results. Currently, the Food and Drug Administration (FDA) has approved more than 50 therapeutic mAbs which are applied in various clinical trials. Action of mAb are based on various mechanisms, including directly targeting the cells, modifying the host response, recognizing and degrading molecules as well as delivering cytotoxic moieties.

Conclusion. Despite some limitations including side effects, and therapeutic challenges, monoclonal antibodies are an attractive option for the development of new therapies and molecular drug targets against a wide range of common diseases due to their specificity and flexibility. MAbs are considered as a great hope for medicine, and effective and safe drugs in the treatment of various diseases.

Keywords. cancer, inflammatory bowel diseases, Alzheimer's disease, immunotherapy, targeted therapy

Introduction

Monoclonal antibodies (mAbs) are specific antibodies that have the same specificity for an antigen and the same affinity for it. All antibodies are obtained from one B cell clone. Generally, monoclonal antibodies are IgG glycoproteins composed of two light and heavy polypeptide chains linked by a disulfide bridge. In each of the chain types there are variable antigen-binding fragments - Fab and Fc fragments, constant for all isotypes of the given isotype, the presence of which is associated with the activation of the immune system after the antibody binds with the antigen. The interaction of an antibody with an antigen most often inhibits the activity of the protein that it binds. Monoclonal antibodies are

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 31.01.2019 | Accepted: 15.05.2019
Publication date: December 2019

Superson M, Szmyt K, Szymańska K, Walczak K, Wnorowski J, Zarębski Ł. *Clinical application of monoclonal antibodies in targeted therapy*. Eur J Clin Exp Med. 2019;17(4):338–346. doi: 10.15584/ejcem.2019.4.9

used in many fields of medicine: oncology, dermatology, transplantology, cardiology, hepatology, immunology, and laboratory diagnostics (Fig. 1).[1]

Monoclonal antibodies can be divided based into groups on their origin as chimeric, chimeric/humanized, humanized, or fully human. Their use in oncology is associated with their selective interaction with a well-defined molecular target in cancer cells that leads to blocking oncogenesis pathways. Monoclonal antibodies affect cancer cells by activating an immune response in an antibody-dependent cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).[2] Cancer cell death may also occur as a result of the antibody's enhancement of apoptosis, modulation of the ligand-receptor reaction or blocking of a specific receptor for growth factor.[3] Targeted treatment is also directed at interfering with the angiogenesis within the tumor.[4] In order to increase the effectiveness of immunotherapy, antibodies can be combined with radioisotopes, toxins, cytostatics or cytokines that they would become antibody drug conjugates.[5] Currently, Food and Drug Administration (FDA) has approved more than 50 therapeutic mAbs which are applied in various clinical trials.

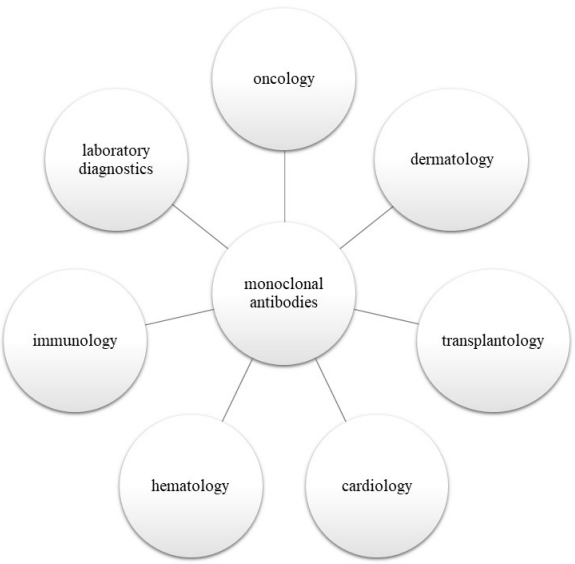

Fig. 1. Fields of application of monoclonal antibodies

Application of monoclonal antibodies in therapy

Cancer

For several years, a constant increase in the incidence of cancer has been observed around the world. High hopes for improvement of treatment results are associated with the implementation of therapy directed at specific molecular targets. One of the most promising therapeutic targets is the epidermal growth factor receptor (EGFR).[6] Blocking the EGFR by binding it to a specific antibody is therefore a validated method of targeted therapy in many cancers. Firstly, cetuximab (Erbitux™) is a chimeric, human-mouse monoclonal antibody of the IgG1 class, while panitumumab (Vectibix) is a completely human monoclonal antibody of the IgG2 class. MAbs against EGFR bind to the extracellular domain of EGFR in its inactive state. Mab against EGFR and EGFR compete for receptor binding by occluding the ligand-binding region, and thereby block activation of ligand-induced EGFR tyrosine kinase leading to subsequent degradation. The consequence of the action of cetuximab and panitumumab is the intensification of apoptosis by increasing expression of pro-apoptotic proteins, reduction of synthesis and secretion of proangiogenic factors, blocking in cancer cells repair of DNA damage caused by chemo-and radiotherapy, as well as inhibition of cell cycle progression.[6-9] Currently, evaluation of mutations in the KRAS proto-oncogene is considered essential for the selection for anti-EGFR therapy in colorectal cancer. In many published papers, it has been demonstrated that the mutation in the KRAS gene results in the abolition of the therapeutic effect of the drug aimed at inhibiting EGFR activity. The results of numerous retrospective and randomized phase II and III clinical trials suggest that the activating mutations in KRAS are recognized as a strong predictor of resistance to EGFR-targeted mAbs in colorectal cancer.[10-12]

On their basis, the assessment of the presence of mutations in the KRAS gene should be a standard element of the qualification of patients with advanced colorectal cancer for therapy with the use of cetuximab and panitumumab. Available data indicate that patients with the KRAS mutation should not be treated with anti-EGFR monoclonal antibodies because they not only do not benefit them, but their use may result in worse treatment results with simultaneous exposure to side effects such as skin toxicity and hypomagnesaemia.[13-15] Currently, cetuximab is also used for the treatment of patients with head and neck squamous cancer.[16] Another example of targeted therapy introduced for the treatment of patients with colorectal cancer is the use of bevacizumab (Avastin), a monoclonal antibody directed against the vascular endothelial growth factor (VEGF), which plays an important role in the process of angiogenesis. Bevacizumab is a recombinant humanized IgG1 monoclonal antibody produced by recombinant DNA. It binds VEGF selectively and neutralizes all its isoforms, which blocks the cell-induced VEGF-induced proliferation. This drug, binding the main factor responsible for neoangiogenesis, leads to inhibition of the formation of new vessels, regression of vessels already produced, and reduces the pressure inside the tumor, which causes that cytostatic drugs reach the cancerous tissues more effectively.[17] The use of bevacizumab does not increase the toxicity of chemotherapy and is well tolerated by patients.[18] In colorectal cancer, it is used in combination

with chemotherapy in the first wave of palliative disease. Bevacizumab is also currently being approved for the treatment of patients with non-small-cell lung cancer, ovarian cancer and glioblastoma.[19-21] Overexpression or amplification of the human epidermal growth factor receptor 2 gene (HER2) is found in 15-30% of cases of invasive breast cancer.[22] This feature is associated with a more aggressive course of the disease. Trastuzumab (Herceptin) selectively binds to the HER2/neu receptor present on the surface of cancer cells from breast cancer. Trastuzumab is a humanized monoclonal antibody directed against the HER2/neu receptor belonging to the EGF receptor family that by binding to the extracellular fragment of the receptor inhibits signaling to the cell nucleus while accelerating the internalization and degradation of the HER2 receptor.[23] This mAb is a potent activator of antibody-dependent cellular cytotoxicity and complement system. Reports revealed that mAb increases the effectiveness of chemotherapy both in the treatment of disseminated cancer and in adjuvant treatment after surgery.[24] Trastuzumab is approved for the first-line treatment of patients with metastatic breast cancer in combination with chemotherapy, in the palliative treatment of postmenopausal patients, positive for hormone receptors in combination with an aromatase inhibitor, and in monotherapy in patients who received so far, at least two treatment regimens due to the spread of the disease, involving anthracyclines and taxanes.[24,25]

The results of the conducted research allow to conclude that the use of trastuzumab in adjuvant treatment in patients with overexpression of the HER2 receptor reduces the relative risk of relapse of the disease.[26] The use of trastuzumab in adjuvant therapy is effective in eradicating axillary lymph node metastases and HER2 receptor overexpression.[27] Treatment of patients with trastuzumab have some side effect such as alopecia, nausea, diarrhea, and cardiotoxicity.[26,28]

Non-Hodgkin's lymphomas constitute a group of cancers of the lymphatic system that is diverse in terms of clinical course, treatment and prognosis. Almost 85% of lymphomas originate from B lymphocytes. The CD20 surface antigen is present on over 90% of B cell lymphoma cells and chronic lymphocytic leukemia. It does not peel off the cell surface, modulate or internalize.[29] The chimeric human-mouse monoclonal antibody directed against the CD20 antigen, rituximab (Rituxan) is widely used in the treatment of non-Hodgkin's lymphomas. It is the first monoclonal antibody registered in 1997 by the FDA in oncology. It is currently used in the treatment of follicular lymphomas and in the treatment of large cell lymphomas expressing CD20, as well as in chronic lymphocytic leukemias. The antibody, by binding to CD20 antigen on the cell surface, triggers cell lysis mechanisms via ADCC and CDC.[30] Rituximab also induces cell apoptosis. The CD20 antigen acts as a calcium channel. The increase in calcium concentration in the cytoplasm initiates apoptosis.[31] This drug, causing the breakdown of lymphoma cells, increases the presentation of tumor antigens by activating specific T lymphocytes. In monotherapy, rituximab elicited a response in more than 50% of B-cell indolent lymphomas. In combination with chemotherapy, however, it induced an answer in 90-100% of cases.[32] In patients with high nodal mass, the use of immunotherapy can cause tumor lysis syndrome.[33] Radioimmunotherapy is a method of cancer treatment in which the monoclonal antibody selectively destroys cells on whose surface a specific antigen is found, e.g. CD20, whereas the radiation emitted by the antibody-bound isotope destroys neighboring cells, including cells that are difficult to access or with insufficient expression of antigen. Lymphoma cells belong to very radioactive cells.[34] In the treatment of non-Hodgkin's lymphomas, two mouse monoclonal antibodies connected with radioisotopes: ibritumomab and tositumomab are registered. Ibritumomab Tiuxetan (Zevalin) is an immunoconjugate of a mouse antibody that recognizes a CD20 antigen on the surface of tumor-transformed B-lymphocytes with tiuxetan, a selective Indu-111 and Itru-90 chelator.[35]

In contrast, 131I-tositumomab (Bexxar) is a mouse IgG2a class antibody directly bound to radioactive iodine (131I) emitting beta and gamma rays. 90Y-Ibritumomab emits beta radiation with higher energy and greater penetration distance in tissues than 131I-tositumomab, additionally has a more favorable half-life.[36]

Currently, only one immunotoxin, gemtuzumab ozogamicin (Mylotarg), is used in anti-cancer therapy as drug against acute myeloid leukemia. This mAb is a combination of recombinant, humanized IgG linked to a cytotoxic derivative of calicheamicin.[37] The constant regions contain human sequences, while the variable regions are derived from a murine antibody that recognizes the CD33 protein. The immunoconjugate has been registered for the treatment of acute myeloid leukemia in patients over 60 years who were insensitive to therapy with other chemotherapeutic agents.[38] In the treatment of leukemia, new mAb is introduced - epratuzumab (EMab) - a humanized antibody directed against CD22 on B lymphocytes, which is an immunoregulator affecting the activation of the B cell antigen receptor. After binding to CD22, epratuzumab's predominant antitumor activity appears to be mediated through ADCC. Epratuzumab is safe in the dosing scheme in more than 85% of children affected by acute lymphoblastic leukemia.[39] Epratuzumab is also used for the treatment of systemic lupus erythematosus.[40] Monoclonal antibodies which are used in oncology are presented in Table 1.

Autoimmune diseases

Rheumatoid arthritis (RA) is a frequently occurring autoimmune disease that causes progressive limita-

Table 1. Monoclonal antibodies currently FDA-approved in cancer therapy

Name	Trade name	Type of antibodies	Molecular target	Main therapeutic application
Panitumumab	Vectibix	human IgG2	EGFR	colorectal cancer
Cetuximab	Erbitux	chimeric IgG1	EGFR	colorectal cancer
Bevacizumab	Avastin	humanized IgG1	VEGF	colorectal cancer
Trastuzumab	Herceptin	humanized IgG1	HER2	breast cancer
Rituximab	Rituxan	chimeric IgG1	CD20	non-Hodgkin's lymphomas
Ibritumomab-tiuxetan	Zevalin	murine IgG1	CD20	non-Hodgkin's lymphomas
Tositumomab-I131	Bexxar	murine IgG2a	CD20	non-Hodgkin's lymphomas
Epratuzumab	EMab	humanized IgG1	CD22	acute myeloid leukemia
Gemtuzumab ozogamicin	Mylotarg	humanized IgG4	CD33	acute myeloid leukemia

tion of mobility, extra-articular organ damage, premature death and socio-economic problems. Among the available preparations used in the treatment of RA are drugs based on monoclonal antibodies. Certolizumab pegol and infliximab are directed against tumor necrosis factor α (TNF-α), which is involved in the development of inflammation during disease. TNF-α plays a central role in the pro-inflammatory cytokine cascade and stimulates the liver to produce acute phase proteins, stimulates phagocytosis and attracts neutrophils into the joints. Certolizumab pegol (Cimzia) is a PEGylated Fab' fragment of a recombinant humanized antibody, what increases the plasma half-life of mAb.[41] Infliximab (Remicade) is a chimeric immunoglobulin G1, monoclonal antibody which contains a human constant region and a mouse-derived murine variable region.[42] Golimumab is the latest, second-generation mAb approved by the FDA as anti-TNFα drug with efficacy and safety in treatment of RA. It belongs to human IgG1κ monoclonal antibody class produced by a murine hybridoma cell line with recombinant DNA method.[43]

Rituximab is also used in RA therapy. There are two theories to explain the role of anti CD20 drugs in therapy. The first assumes that elimination of B lymphocytes prevents their transformation into plasmocytes, producing autoantibodies, which results in a decrease in the secretion of TNF-α by macrophages. According to the second theory, the lack of B lymphocytes, which are antigen presenting cells, reduces the activity of T lymphocytes, which leads to the reduction of synovitis related to them. The use of rituximab in therapy is quite safe, however, it is problematic because the treatment with these drugs is long-lasting and might cause small risk of serious events such as infection and hypogammaglobulinemia.[44,45] Next, mavrilimumab (CAM-3001) is a high-affinity, immunoglobulin G4 monoclonal antibody (mAb) against the granulocyte macrophage colony-stimulating factor (GM-CSF) receptor-α chain. Clinical trials in RA patients treated with mAb have shown benefit outcomes with respect to both efficacy and safety with no serious side effects.[46]

In patients with RA, mAbs reduces the clinical signs and symptoms of active disease and inhibits the progression of structural joint damage. However their use may be associated with serious side effects such as serious infection or reactivation of chronic infections, such as Herpes Simplex Virus, hepatitis C and hepatitis B virus.[47]

Crohn's disease (CD) is a chronic disorder that affects the functioning of the digestive system, and thus the quality of life of the patient. The pathogenesis of this disease is unknown, but clinical and experimental evidence suggests that uncontrolled activation of T lymphocytes causing inflammation is the main cause. Therapy with drugs that inactivate pro-inflammatory factors, i.e. TNF-α, in many cases results in remission of the disease. Such medicines include Infliximab, a chimeric monoclonal antibody that is designed to inactivate TNF-α, and may also induce apoptosis of T lymphocytes in the intestinal mucosa.[48]

A relatively new generation of vedolizumab (Entyvio), a humanized monoclonal antibody, can be used in the absence of a response to TNF-α inactivators. It recognizes the α4β7 integrin - a glycoprotein found on the cell membrane of some T and B lymphocytes. A4β7 interacts with MAdCAM-1 - a molecule present in the intestinal vascular network responsible for lymphocyte adhesion. Vedolizumab blocks the migration of lymphocytes into the gastrointestinal tract, while not stopping the migration of white blood cells into the central nervous system.[49] Studies show that the therapy is effective, but may have side effects such as serious infections and adverse events such as nasopharyngitis, headache, joint pain, nausea, and fever leading to hospitalization.[50]

Adalimumab (Humira®) is next mAb which found application in treatment of inflammatory bowel diseases and has positive impact on endoscopic mucosal healing.[51] It is an IgG1 monoclonal antibody that targets TNF-α which was approved by FDA in 2002 to is used in treatment of moderate to severe cases of CD for symptom control and inducing and maintaining clinical remission.[52]

Multiple sclerosis (MS) is a chronic inflammatory-degenerative disease of the central nervous system, characterized by multifocal inflammatory changes and the accompanying demyelination, which leads to axonal damage and loss. The pathogenesis of MS is unclear. In the treatment of MS, drugs based on mAbs are also used. Alemtuzumab (CAMPATH) is a humanized monoclonal antibody directed against the CD52 differentiation molecule, which is approved from 2013 for treatment of relapsing multiple sclerosis.[53] The mechanism of mAb application in MS involves immunomodulation by the depletion and repopulation of lymphocytes. After binding of alemtuzumab to lymphocytes, mAb results in the rapid, but long-lasting depletion of circulating CD52-positive cells, and the mechanism of lymphocyte depletion includes ADCC, CDC and apoptosis induction.[54] Natalizumab (Tysabri) is the only mAb currently approved from 2004 for relapsing-remitting form of MS. It acts by targeting lymphocyte migration across the blood-brain barrier, an early stage in MS lesion development. Report shows that about 6% of patients treated with natalizumab developed persistent antibodies which result in reduced efficacy.[55] Rituximab has also been used in a phase II trial in primary progressive form of MS. Ocrelizumab (Ocrevus®) is a next humanized anti-CD20 monoclonal antibody approved for the treatment of adults with relapsing or primary progressive MS. Simultaneously, mAb is considered as valuable new treatment option for delaying progression in early MS and first approved antibody for secondary progressive form of MS.[56]

Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that leads to the death of the patient. It is characterized by cognitive impairment, loss of long-term memory, language difficulties and aggression. The exact cause of AD is unknown, but two major theories that explain the molecular basis of AD are now widespread. One of them explains that AD is caused by the accumulation of toxic fibrillar β-amyloid deposits (Aβ), which is a toxic form of the protein responsible for impaired calcium cell metabolism and the induction of its apoptosis. The second hypothesis explaining the cause of AD is that pathological forms of tau protein initiate a cascade of disease. Hyperphosphorylated tau protein fragments combine to form neurofibrillary tangles inside the pericarion of nerve cells. It is possible that both Aβ deposits and pathological forms of tau protein affect cognitive functions and memory, causing AD.[57]

However, current studies on passive immunization of AD patients using mAbs allow to think about the actual inhibition of the progression of neuropathological changes in AD.

MAbs that can potentially be used in the treatment of AD are divided into two classes: human (e.g., gantenerumab, aducanumab) and humanized (e.g., bapineuzumab, solanezumab, crenezumab). Monoclonal antibodies used in the treatment of AD are shown in table 2. MAbs are designed to be associated with specific Aβ epitopes, contributing to its degradation. The mechanism of action of mAbs is not fully understood, however it is assumed that after the mAb crosses the blood-brain barrier, it connects to specific Aβ epitopes and then the effector Fc fragment.

After that the complement system and microglia cells are activated, which phagocytose Aβ, reducing its amount in the brain. However, it is uncertain whether it will be necessary to get mAbs into the brain at all, because the other proposed mechanism of action of mAbs is the peripheral sink hypothesis, according to which the antibody does not go to the brain but binds to free Aβ in the peripheral blood, lowering its concentration. This changes the balance of Aβ across the blood-brain barrier, with the result that Aβ flows away from the brain into the peripheral blood, striving to equalize Aβ concentrations on both sides of the blood-brain barrier.[58]

Hypothetically, there is a decrease in Aβ concentration in the brain and inhibition of neuropathological changes caused by its accumulation. However, although in clinical trials monoclonal antibodies such as ponezumab and solanezumab have been shown to act by this mechanism and a reduction in serum Aβ40, no significant clinical effects have been observed with ponezumab and solanezumab treatment.[59]

In general, the safety and tolerability profile of mAbs is acceptable. The only side effect observed, which should be given special attention, is amyloid-related imaging abnormalities (ARIA). There are two types of ARIA - ARIA-H and ARIA-E. ARIA-E refers to brain edema that is noticeable in an MRI scan caused by the breakdown of tight endothelial connections in the brain blood barrier and, as a consequence, accumulation of cerebrospinal fluid at this site. ARIA-H is characterized by excessive accumulation of iron contained in proteins (hemosiderosis), which is considered to be the cause of microhemorrhage, which is also representative of ARIA-H.

The etiology of ARIA remains unclear, although vascular Aβ is thought to be a factor that increases the permeability of blood vessels, which causes symptoms indicative of ARIA. In search of the relationship between ARIA and the use of mAbs, it was observed that with increasing the dose of bapineuzumab, the incidence of ARIA-E increased. It was also found that not all mAbs may contribute to the emergence of ARIA. Probably this effect is caused only by mAbs binding to the N-terminal section of fibrillar Aβ - bapineuzumab, gantenerumab and aducanumab. In clinical trials, mAbs such as crenezumab or BAN 2401 ARIA were not observed at all, and in case of solanezumab, only 1% of subjects (11 patients

Table 2. Monoclonal antibodies tested in AD therapy

Monoclonal antibodies	Type of antibodies	Mechanism of action	Results of clinical trial
Bapineuzumab (AAB-001)	huminized IgG1	Recognizes the N-terminal part of Aβ, leads to the degradation of excess fibrillar, soluble form of β-amyloid. It binds to the. MAb stimulates phagocytic microglia and cytokine production.	Clinical trials were unsuccessful due to the lack of proven clinical benefits and serious adverse effects in AD patients.[63]
Solanezumab (LY2062430)	humanized IgG1	Recognizes monomeric and soluble Aβ and leads to its sequestration and shifts the balance between the various forms of Aβ. MAb removes small, soluble forms of Aβ, which are directly toxic to the functioning of the synapses.	Clinical trials were unsuccessful - no improvement in cognitive or memory status in AD patients was noted. In patients with a mild form of AD inhibition of disease progression was observed.[64]
Gantenerumab (RO4909832, 1G1450)	human IgG1	Recognize the N-terminal and central part of monomeric, oligomeric, and fibril Aβ. Degrades amyloid deposits by recruiting microglia and activating phagocytosis.	Clinical trials have shown a reduction in amyloid deposits in PET. Phase III studies are currently underway.[64]
Crenezumab (MABT5102A, RG7412)	humanized IgG4	Recognizes monomers and aggregated forms of Aβ with a 10-fold–higher affinity for oligomers. MAb removes excess Aβ by stimulating its phagocytosis. It inhibits the release of pro-inflammatory cytokines, counteracting cerebral edema.	Crenezumab has been declared safe for humans. In the second phase of clinical trials, PET scans showed a reduction in β-amyloid accumulation, however, mAb did not cause improve cognition.[65]
Ponezumab (PF-04360365)	humanized IgG2δA	Recognizes C-terminal part of monomeric forms of Aβ. It reduces the deposition of Aβ in the cerebral blood vessels, improving their functioning.	MAb was generally safe and well tolerated. In mild-to-moderate AD subjects, no changes in Aβ were found. There was also no improvement in brain amyloid burden and cognition.[66]
Aducanumab (BIIB037)	human IgG1	Recognizes conformational epitopes of aggregated β-amyloid forms.	A dose-dependent clinical response and a reduction in brain Aβ plaques were observed in PET.[67]
BAN2401 (mAb158)	humanized IgG1	Recognizes soluble Aβ protofibrils. MAb protects neurons, reducing the toxicity of Aβ in the brain and cerebrospinal fluid.	Clinical trials confirmed the safety and good tolerance of mAb15 by the human body.[68]
BIIB092 (BMS-986168, IPN007)	humanized IgG4	Recognizes the N-terminal domain of tau and neutralizes its toxicity. Reduction in the amount of free tau in the cerebrospinal fluid was noted.	BIIB092 is safe and well tolerated.[69]
C2N-8E12 (ABBV-8E12)	humanized IgG4	Recognizes aggregated, extracellular form of pathological tau. Reduction in brain tau, microglial activation, and tau--seeding activity detected in brain lysates. improving also cognitive deficits.	Preclinical studies have demonstrated the improvement of cognitive deficits. Phase I trials confirmed the medicine's safety.[70]

treated with solanezumab and 5 who received placebo) confirm that this result is not statistically significant. The positive feature of ARIA is that it is easy to control, because stopping the administration of mAbs results in the resolution of side effects.[58-62]

Current clinical trials for the treatment of AD using mAbs, despite promising theoretical foundations, have produced quite disappointing results. Tests on bapineuzumab and solanezumab - so far, the largest projects taking part in the third phase of clinical trials did not give clinically positive results. This could be due to low doses administered to patients, too advanced disease or poorly designed mAbs, targeting the wrong kind of Aβ.

This indicates the limitations associated with the use of mAbs. However, in the absence of effective AD therapy, screening for passive immunization should continue. Currently, many new mAbs targeting different Aβ epitopes are tested and clinical studies are performed.

Conclusion

Due to the huge number of current research, the potential therapeutic use of monoclonal antibodies is rapidly increasing, but it is still relatively rare and still largely belongs to unconventional methods of treatment. Nevertheless it is a rapidly growing branch of medicine with a huge and unused therapeutic potential should still be studied and developed because the use of mAbs in therapy in many cases turns out to be more effective and safer for the patient. Moreover, monoclonal antibodies may turn out to be a "miracle" drug for many incurable diseases such as many type of cancer including colorectal, breast cancer, lymphomas and leukemia, rheumatoid arthritis, inflammatory bowel diseases, multiple sclerosis as well as Alzheimer's disease.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.10

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 347–350

REVIEW PAPER

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Pelvic Exenteration: An Updated Mini-Review from 1948 to 2020

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ABSTRACT

Introduction. Pelvic exenteration (PE) is a curative or palliative radical surgical procedure applied for advanced or recurrent pelvic or perineal cancers. From 1948 to date, improvements in surgical techniques, including urinary conduits and pelvic reconstruction, have improved its morbidity and mortality.

Aim. The present study reviews the evolution of PE, indications, complications and current results.

Material and methods. Large case series and studies on PE were searched in PubMed, covering all years available, and recent applications of PE were reviewed.

Results. Indications of PE are primary or locally advanced tumors (cervix. rectum. vulva. bladder), recurrence after radiotherapy (cervix), recurrence after primary resection (vulva, vagina, cervix, rectum) and palliative treatment for advanced tumors or pubic fistulas. Contraindication are distant metastases, involvement of iliac vessels, pelvic side-wall or para-aortic lymph nodes and invasion of sacrum proximal to S1/S2 or sciatic foramen. However, recent studies have reported more radical resections, including side-wall and vessels. Patient's health condition and fitness are also important in decision-making.

Conclusion. PE can be the last chance of cure or improving quality of life for advanced or locally recurrent pelvic cancers. 5-year survival rates with PE are better, but complications of such a radical surgery are still high, and should be improved.

Keywords. complications, indications, morbidity, mortality, pelvic exenteration

Introduction

Pelvic exenteration (PE) was first performed in a patient with advanced cervical carcinoma by Brunschwig in 1948, and he described the operation as 'the most radical surgery for pelvic cancer so far'.[1] First series of PE included 22 cases with 5 cases died during early postoperative period.[2] In his series, there was no survival advantage, but patients benefited from aggressive surgery and the quality of life (QoL) improved. From 1948 to date, improvements in critical care, use of antibiotics, hyperalimentation and prophylaxis for thromboembolism, advances in surgical instruments such as staplers and in surgical techniques, including urinary conduits and pelvic reconstruction, have improved morbidity and

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 26.09.2019 | Accepted: 3.11.2019
Publication date: December 2019

Unal E, Yıldız A, Yuksekdag S, Fırat A. *Pelvic Exenteration: An Updated Mini-Review from 1948 to 2020.* Eur J Clin Exp Med. 2019;17(4):347–350. doi: 10.15584/ejcem.2019.4.10

mortality. Recent rates of morbidity and perioperative mortality are 30-85% and 3-5%, respectively.[3-5] However, there are reports presenting higher ratios of morbidity.[6] The 5-year survival rate of PE is now between 20 and 75% with an improved QoL.[7] The discrepancy in these numbers can be explained by the differences in patient selection criteria, presence of positive margins and extent of surgery.

Pelvic Exenteration

PE is first described for recurrent or locally advanced cervical cancer. Other gynecologic malignancies such as endometrial, vulvar, vaginal and ovarian cancers benefit from PE, especially in locally invasive or recurrent cases. Since the first operations undertaken to now, locally advanced or recurred bladder and ano-rectal cancers were also included in the indications of PE.[8] Moreover, extent of PE has widened from pelvic side-walls to the sacrum.[9,10]

In pelvic carcinomas, according to the location of the tumor, anterior (resection of bladder) or posterior (resection of rectum) PE operations have been described.[11,12] However, the most recent publications suggest that the goal of PE is total exenteration (removal of all pelvic organs), including the rectum, bladder, and whole reproductive organs.[13] Total PE targets R0 resection.[14] Even some reports state that R0 resection is not possible in case of pelvic side-wall involvement (external iliac vessels, sciatic foramen, obturator nerve or bone invasion); in many clinical series recently published, one can see the resections of sacral bone, sacrifications of sciatic nerve, and even use of vessel grafts.[9,15-17] For example, in 2017, Sasikumar et al. published a review suggesting PE with en bloc sacrectomy, for recurrent rectal adenocarcinoma.[14] The authors have claimed that R0 (>1-mm resection margin) resection was achieved in 78% of patients. Disease-free survival associated with R0 resection was 55% at a median follow-up period of 33 months; however, none of the patients with R1 (<1-mm resection margin) survived this period.[14]

Indications of PE can be summarized as primary but locally advanced tumors (cervix, rectum, vulva, bladder etc.), recurrence after radiotherapy (cervix), recurrence after primary resection (vulva, vagina, cervix, rectum etc.) palliative treatment for pubic fistulas (rectovaginal, vesicovaginal etc.).[2] Contraindication of PE are distant metastases, involvement of common or external iliac vessels, metastases to para-aortic lymph nodes, involvement of sacrum proximal to S1/S2, tumor extension through sciatic foramen and pelvic side-wall involvement.[2,3] Clinical triad of leg edema, ureteral obstruction, and leg pain is pathognomonic for pelvic side-wall involvement and considered as a contraindication to surgery. However, recent studies have reported an even more radical resection, the laterally extended resection, including striated muscle and vessels.[3,18] Patient's health condition and fitness are also important in decision-making. However, intensive care units (ICU) with adequate equipment can make it easier to choose candidates for operation. If operation will supply an improvement in QoL, even in metastatic tumors, palliative surgery can be undertaken, as well. Age and ureteral obstruction are not absolute contraindications anymore.[7]

Currently, there are many articles presenting PE series where the authors prefer to use robotic or laparoscopic techniques to minimize the morbidities.[11,13,19-21] Most of them report superior results in comparison with classical open operations.

Preoperatively, all candidate patients should undergo a thoroughly physical examination such as lymph node palpations, digital rectal exam, etc. Tumor marker measurements might help. Distant metastases should be excluded by radiographic scans, computerized tomography (CT), magnetic resonance imaging (MRI) and/or positron emission tomography (PET) scans. Rigid rectosigmoidoscopy and colonoscopy should also be performed.

Cystoscopy can be necessary at the beginning of operation, to assess bladder metastasis and to place ureteral stents. On modified-lithotomic position, after xiphoid to pubis midline incision, all viscera are evaluated for metastases. All adhesions should be taken down very carefully, and thick or suspicious adhesions should be excised for histopathology. Liver, paraaortic lymph nodes, pelvic side-walls, sacro-coccygeal bone, region lateral to the obturator fossa and external iliac vessels should be evaluated for possible metastases.[1-5] Then exenteration starts with total or modified techniques.

Total PE is defined as the excision of the rectum, distal colon, bladder, reproductive organs, draining lymph nodes, and pelvic peritoneum. After exploration of the abdomen, the pelvic dissection is begun at the level of the aortic bifurcation.[8,13] Anterior PE is used for anterior pelvic tumors involving cervix, vagina or bladder. It is the removal of pelvic peritoneum, lower part of ureters, reproductive organs, bladder, and lymph nodes. Posterior vaginal wall and uterus are the margins of resection.[11,22] In posterior PE, the uterus, adnexa, cervix, posterior wall of vagina and rectum are removed. Bladder is preserved. In middle or upper rectum cancers, primary anastomosis can be created. Tumors below the level of levator ani muscle require permanent colostomy as sphincters are excised.[12,23] Lastly, another modified PE, composite PE is the removal of bony resections, including portions of the sacrum-coccyx, ischium and symphysis pubis. In composite PE, one should remember S1/2 level, as above this level is unresectable.[24,25]

Following resections, reconstruction procedures such as urinary condiut or colostomy, pelvic floor or vaginal reconstructions start. Urinary conduit can be

done by isolating a distal ileal segment and anastomosing the ureters here and then taking this segment under the skin.[26] Indiana pouch is made from ascending colon and ileocecal valve.[27] In Kock's pouch, all the pouch, valves and outlet are made from terminal ileum.[28] Mitrofanoff pouch includes an outlet from appendix.[29] Miami pouch is a popular continent urinary reservoir because of 90% overall long-term continence rates.[5,30] Transverse colon is anastomosed to the ascending colon in a U-shaped fashion to create the colonic reservoir. Anti-refluxing uretero-colonic anastomoses are then created, and ileum is anastomosed at the level of the ileocecal valve, then exteriorized as a stoma for self-catheterization. Pelvic dead space filling to prevent fistula or abscess formation or bowel obstructions can be done by myocutaneous flaps (e.g. rectus abdominis, gracilis or gluteus maximus), synthetic meshes or omentum.[5,31] Neovagina construction can be done by gracilis or rectus abdominis muscles.[32]

Postoperative complications of a PE will depend mainly on what was removed and the patient' s overall health. Reconstructions may also be problematic for the patients. In general, the most common major complications are intraabdominal collections and wound infections.[3,33] Major complications are related to gastrointestinal tract (fistula or obstruction), urinary tract (fistula, infection, or obstruction), or wound (abscess, dehiscence/necrosis, or hemorrhage). Patients receiving pelvic radiotherapy prior to exenteration may have a much higher complication rate.[34] Reconstruction of the irradiated pelvis after exenteration by omental flap or myocutaneous flaps decreases the complications.[31,32] In most cases, a new local recurrence or distant metastasis is inevitable. Therefore, adjuvant chemo-radiotherapy can be necessary later on.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.11

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 351–355

REVIEW PAPER

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Applicability of Cardiopulmonary Exercise Test

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ABSTRACT

Introduction. Cardiopulmonary Exercise Testing (CPET) test is a test that allows an integrated response to the physical effort of the cardiovascular system, respiratory system, nervous system, skeletal muscles and metabolism. The growing awareness of better correlation between health condition and exercise tolerance than with resting measurements is the key importance. The range of clinical applications is expanding to assess impairment of physical capacity with an unclear cause and to objectively determine functional capacity.

Aim. The aim of this review was to discuss the applicability of cardiopulmonary exercise test in diverse branches of clinical and non-clinical use.

Material and methods. This review was performed according to latest literature. We mainly searched PubMed and Google Scholar to look for previous cases of CPET applicability.

Results. CPET has a very wide range of applications for the diagnosis of physical productivity as seen through athletes and amateurs in their own right. This medical diagnostic practice allows us to map out the inconsistencies the blood, respiratory and bone systems/structures.

Conclusion. Cardiopulmonary exercise test is a safe method of different disease assessment. It can be use in various cases, to rate reaction of an organism to physical effort.

Keywords. cardiopulmonary exercise test, CPET, stress test

Introduction

Physical activity is something that involves all of your muscles, which leads to an increased energy expenditure, higher than that of an individual resting state. It is an integral part of an organisms functionality, while also having a positive impact on an individual health and lifestyle.[1] Physical activity is crucial in overall development, height, as well as the physiological functioning

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 29.09.2019 | Accepted: 6.11.2019
Publication date: December 2019

Wołoszyn F, Pańczyszyn-Trzewik P, Ziajor S et al. *Applicability of Cardiopulmonary Exercise Test*. Eur J Clin Exp Med. 2019;17(4):351–355. doi: 10.15584/ejcem.2019.4.11

of cells. Physical activity counteracts obesity, fat gain, while halting almost entirely the development of diabetes. Regular physical activity has a positive effect on an individual blood network, bone structure, and overall respiratory function.[2] By examining a training structure, we can point out the effect that physical activity has on the body, and in the case that something bad happens (i.e some form of injury), we can deduce which system in our body is most likely responsible.[3]

Physical tests can be split into two groups: simple and complex. To the first group we add the stair test, where an individual climbs a set of stairs, as well as a simple walking exercise, of which the most meaningful is a 6 minutes walk test – 6MWT. However, the main representative of complex test is the cardiopulmonary exercise test CPET.[3] The methods of measuring physical activity can also be split into two groups—subjective and objective. To the first one we add a diagnostic survey, in which the studied individual relays to use their typical day to day routine of physical activity. To the objective methods we distinguish instead; indirect, which focuses on kinematic analysis as well as measuring blood pressure during physical activity, on the other hand the direct, which takes advantage of the metabolic criteria, for example human heat expenditure, carbon dioxide, and oxygen levels.[4]

In clinical applications the most meaningful is cardiopulmonary exercise test CPET, which belongs to the direct and objective group of examination for the measurement of physical activity. The test is a combination of attempts at exercise along with measured gases found within your respiratory system, allowing us to check the functionality and overall status of the cardiovascular, respiratory, neurophsycological, hemopoietic, and muscular systems in their reaction to controlled physical activity. The tests are performed either on a treadmill or stationary bicycle, and the results are mapped out in the following order: maximal oxygen uptake ($VO2_{peak}$), carbon dioxide emission (VCO2), minute ventilation (VE), ventilatory threshold (VT), ventilatory equivalents for oxygen (VE/VO2) and carbon dioxide (VE/VCO2), respiratory exchange ratio (RER, VCO2/VO2), forced expiratory volume in 1 second (FEV1), peak expiratory flow (PEF), saturation (SatO2), ECG, blood pressure (BP), heart rate (HR).[5, 6]

CPET has a very wide range of applications for the diagnosis of physical productivity as seen through athletes and amateurs in their own right. This medical diagnostic practice allows us to map out the inconsistencies the blood, respiratory and bone systems/structures.[6, 7]

Aim

The main goal of the work, was to find and describe the wide use of the CPET test in various fields of clinical and non-clinical research. The focus was on the description of the practical application of the test, proceedings, test variables and its use in clinical and non-clinical studies.

Material and methods

Google Scholar, PubMed, Science Direct and available literature from the publications were searched to find information about the test. Selected words like CPET, CPX, Cardiopulmonary Fitness were used to search for test data.

CPET-Praxis

Cardiopulmonary Exercise Testing CPET has many significant clinical and research appliance.[8-10] Cardiopulmonary exercise test offers the researcher the opportunities to study in same time, the cardiovascular, the ventilator and the cellular systems responses under circumstances of accurately controlled metabolic stress.[11] Spiroergometry is used for the evaluation of endurance in the physical effort, which intensity is determined on the basis of the physical effort on treadmill or cycle-ergometer.[12] On cycle-ergometer the patient rotates the pedals at 60 turnovers per minute. The pedalling rhythm may be controlled by using the speedometer or metronome.[13] The most common protocol used in treadmill and cycle-ergometer is the Bruce protocol. The protocols with smaller, gentler load increments (Cornell, Naughton) or ramp type of protocols, in which the load grows constantly, continuously are also recommended.[14] Myers and Bellin, claim that individualizing protocol of exercise, including personalized growth of work, demands some wide knowledge of the patient's exercise capacity prior to the test, and it has been one of the obstacles in the test.[15] The test consists in an electrocardiographic exercise test with the measurement of the blood pressure, monitoring of cardiac function to show the occurrence of conduction disorders and rhythm. The subject is wearing a mask that measures the exhaled air in terms of determining the vital capacity of the lungs and detecting possible lung obstruction. The oxygen and the carbon dioxide concentrations in the exhaled air are also measured. Owing to the fact, we can analyse the metabolism that occurs during exercise in the body.[14,16]

Applicability

Previously, the physical capacity was described as a value based on the maximum amount of work obtained on the treadmill or cycle-ergometer. The CPET test was limited to pulmonary medicine and physiologists. Today its usage is wider. Researchers increasingly appreciate the precision and additional information obtained from the test. They provide parameters related to assess the inheritance and degree of cardiovascular disease and stratifying risk.[17] CPET test in cardiology is used in patients with cardio-vascular diseases such as heart failure or congenital heart disease. It could be also used in assessment of heart performance and to prognosticate the health condition of patients with chronic health failure.[18] Stress test helps to qualify patients for heart transplantation and to assess the effectiveness of cardiologic rehabilitation.[19] The cardio-

vascular shortage has a close impact on different systems and organs, such as the renal, the skeletal and the pulmonary. CPET is helpful in adult congenital heart disease patients who have had a limited exercise performance and adjust to increasing symptoms of heart failure by reducing their functional level.[20,21] Cardiopulmonary exercise test is regarded as "gold standard" for the functional assessment of patients with congestive heart failure CHF, using prognostic and diagnostic data coming from direct measurement of different parameters such as VO2, VCO and VE.[22] Examples of using the CPET test conducted on people with CHF was mainly used to obtain forecast data.[23] The CPET test was also used to optimize exercise tolerance in patients with chronic heart failure by examining the effect of placebo on physical performance.[24] Cardiopulmonary exercise test can also be used to calculate the maximum heart rate. This is one of the method that characterizes the maximum effort of a person. An example is a study which has been done to optimize the heart rate in beta-blocked heart failure patients. Information derived from CPET test may be useful in rehabilitation programs and ischemic test, or to find criteria which determines maximal oxygen uptake.[25] Ramp-incrementation rate remarkably effects the RER during exercise testing in patients with congestive heart failure.[26] Confirmation of the usability of the test can also be found in the comparison of test results obtained from the 6-minute test or step test. The range of the results received evaluates efficiency and its prognosis is significantly wider.[27] The use of this research tool made it possible to compare the likelihood of death in patients with CHF. Studies have shown that comparing each of the parameters with the exception of RER, was meaningly different.[28] Using VO2 peak as an final point, coherent changes in mortality and VO2 peak were seeming for interventions such as cardiac resynchronization therapy (þ/þ for change in VO2 and improvement in mortality, respectively.[29,30] On the other hand Barocco et al. assesses the leg muscle oxygenation during effort in patients with heart failure.[31]

In another study the CPET test is used to assess the toleration of the physical effort and in diagnostic of dyspnoea during effort.[32] In pulmonology also CPET is used in assessment of patients with pulmonary diseases, to evaluate transplanted lung or heart-lung and to assess the effectiveness of respiratory rehabilitation.[33] The study showed that the CPET test is relatively safe in patients with inoperable lung cancer and may be the basis for planning further stages of rehabilitation for these patients.[34] Like in cardiovascular disease in the lung cancer treatment, we are able to investigate the curative intent and prognosis.[35,36] CPET in studies of respiratory diseases is also used in a wide spectrum. The trial was carried out on pulmonary hypertension. The test helped in determining the degree of functional impairment, the severity of the disease, assessment of interventional efficacy and prognosis of the disease.[37] In the study of patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis , the test was also used.[38] Holverda et al. characterized whether the existence of pulmonary hypertension is associated with cardiovascular parameters and gas exchange results during cardiopulmonary exercise testing.[39]

Cardiopulmonary exercise test helps in adjusting physical activity also in other civilization diseases such as obesity or diabetes. CPET is directed ahead of exercise intervention in adolescents with obesity to estimate medical safety of exercise and physical performance, or to assess exertional dyspnoea in obesity.[40-42] It was observed that in a child with obesity it is hard to individualize the variables obtained from the CPET test, because the fat contained in the organism "virtually metabolically inactive during exercise" can hide the result of the metabolically active muscle tissue when cardiopulmonary exercise test is standardize to body mass.[43] Most often referring to Faria et al, exercise test is carried out to assess the influence of obesity to pulmonary function or heart failure.[44,45] The test can be carried out among adults, children and adolescents. Examples of this are the study of children with type 1 diabetes (T1DM) .The safety and need to conduct research on a wider scale is confirmed by latest International Society for Pediatric and Adolescent Diabetes ISPAD guidelines. It is necessary tool to cope with diabetes and to widen the management with this disease.[46] Adolfson et al. performed research on group of adolescents with diabetes mellitus type 1. Group of investigators found no differences which were significant. It shows that for this group of patients CPET is safe and they can participate in physical activity.[47,48] CPET on patients with diabetes type 2 can also be executed.[49] The influence of smoking can be also taken into consideration.[50]

In the study of Przednowek et al., the predictive statistical model of VO2max was made. It opens a wide range of mathematic and statistic cooperation. This research was made on 80 male physical education students.[51] It presents that also conducting study on healthy people is very important. We are able to diagnose a broad scope of healthy participants and find risk factors of presumable complications during physical activity.[52,53] Referring to Salvati et al., the cardiopulmonary exercise test is a crucial method to rate fitness, endurance and performance in sportsmen.[54]

Conclusion

Cardiopulmonary exercise test under controlled conditions can safely assess the body's response during dynamic exercise of increasing intensity. CPET is used not only in scientific research. The performance of this functional non-invasive test is increasing systematically in everyday clinical practice.

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© Wydawnictwo UR 2019
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.12

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 356–363

REVIEW PAPER

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Current recommendations for treatment and diagnosing of xerostomia in Sjögren's syndrome

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ABSTRACT

Introduction. Xerostomia is one of the most common and disturbing adverse effects of systemic diseases and their therapies. This complication markedly increases the risk for dental caries, difficulties with chewing, swallowing and sleep disorders with a significant impact on the patient's quality of life. Sjögren's syndrome (SS) is a systemic autoimmune disease that primarily affects the exocrine glands, resulting in dryness of the mouth due to lymphocytic infiltration of the salivary glands.

Aim. The aim of this paper is to present the current recommendations in diagnosing and treating SS-related xerostomia.

Material and methods. Analysis of literature

Results. For the assessment of SS-related xerostomia, only an unstimulated salivary flow with rates of 0.1 mL/min is included in the current SS classification criteria. Saxon test, sialography, ultrasonography of salivary glands play supporting function. Treatment of SS -related xerostomia includes an application of secretagogues and the implementation of specific dental prophylaxis measures. Adjuvant therapies include herbal remedies, photobiomodulation, and acupuncture.

Conclusion. Treatment of SS requires multidisciplinary care. There is no fully effective treatment of xerostomia that provides immediate and long-lasting results.

Keywords. saliva, Sjögren syndrome, xerostomia

Introduction

Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by periductal mononuclear cell infiltrate in the salivary and lachrymal glands, autoimmunization, injuries to endothelial cells and their subsequent apoptosis. The periductal mononuclear cell infiltrates cause damage to glandular tissue in the salivary glands and results in decreased salivation. Typical features of SS are severe xerostomia and xerophthalmia, which are basic SS diagnostic criteria.[1-9] The presence of both dry mouth and dry eyes classified patients with 93% sensitivity and 97.7% specificity. The rate of dry mouth in SS ranges from 41% at initial diagnosis to 84% 10 years after diagnosis.[10] Diagnosis of primary SS, as approved in 2016 by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR), is based on the weighted sum of 5 items.[11,12] According to this classification, an unstimulated salivary flow rate of 0.1 mL/minute in sialometry gives a score of 1 to the weighted sum of 5 items,

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 29.09.2019 | Accepted: 6.11.2019
Publication date: December 2019

Zablotskyy O, Tomczyk M, Błochowiak K. *Current recommendations for treatment and diagnosing of xerostomia in Sjögren's syndrome.* Eur J Clin Exp Med. 2019;17(4):356–363. doi: 10.15584/ejcem.2019.4.12

according to the current EULAR/ACR criteria. Decreased salivation markedly affects oral health and very often inhibits normal functioning. Xerostomia is a severe medical problem. There is a 20% prevalence of dry mouth complaints of different origins in the general population.[7] The loss of saliva causes serious oral consequences, manifesting as an uncomfortable feeling of dry mouth and presenting numerous signs and symptoms mainly in the mucous membranes, lips, tongue, salivary glands and teeth.[9] The oral cavity deprived of saliva and its natural lubricative, protective and antibacterial properties is prone to a number of unfavourable consequences. It may include exacerbation of diseases affecting hard dental tissues and the periodontium, as well as a predisposition to opportunistic infections and pathologies of the oral mucosa. Dry mouth correlates with numerous clinical and psychosocial problems. Clinical problems are common in patients with hyposalivation and include rampant caries, gingival inflammations, fungal infections, rhagades, limited denture retention, or limitations in swallowing, eating, and speaking.[8] Moreover, xerostomia has a significant impact on the quality of life of patients who are affected.[1-5,13,14] Gaining knowledge about the causes, symptoms and treatment of xerostomia is very important and useful for both general practitioners and dentists.

Aim

The aim of this paper is to present the current recommendations in diagnosing and treating SS-related xerostomia.

Diagnosing of xerostomia

Typical saliva production has been measured at 0.5-1.5l per day in the healthy adult.[7] Percentage contributions of the different salivary glands during unstimulated flow are as follows: 20% from parotid, 65% from submandibular, 7% to 8% from sublingual, and less than 10% from numerous minor salivary glands. Stimulated high flow rates drastically change percentage contributions from each gland, with the parotid contributing more than 50% of total salivary secretions.[15] Stimulated saliva is produced in response to a mechanical, gustatory, olfactory, or pharmacological stimulus, contributing to around 40-50% of daily salivary production.[16,17]

There are different causes of dry mouth known so far, such as post radiation of head and neck cancer treatment and salivary gland hypofunction. Moreover, the common cause is the use of medications with potential xerostomic effects, mainly anticholinergic, sympathomimetic, sedative, hypnotics, opiates, antihistamines and muscle relaxants.[9] Decreased salivation gives similar clinical symptoms and can be detected by the same tests. However, in SS the detection of xerostomia is an integral part of a comprehensive, tentative diagnosis and SS confirmation. In SS, xerostomia is a part of the diagnosis, not only the consequence of disease. We need more precise and objective oral tests and procedures that can be used for the diagnosis and clinical decision making in SS. It has been recommended that unstimulated whole saliva measurement, the Saxon test, sialography, ultrasonic examination of salivary glands are useful for diagnosing SS-related xerostomia.[16]

Salivary flow measurement (sialometry) is widely applied in diagnosing xerostomia. Several methods for collecting saliva have been reported so far.[18] It is the most common, objective diagnostic test of xerostomia and it uses two parameters to assess saliva secretion: Salivary Flow (SF) index and Salivary Flow Rates (SFR). SF is a parameter allowing stimulated and unstimulated saliva flow to be classified as normal, low or very low (hyposalivation).[17] In adults, normal total stimulated SF ranges are 1–3 mL/minute, and low ranges are 0.7–1.0 mL/minute, while hyposalivation is characterized by a stimulated SF <0.7mL/minute.[17] SF index is a basic, cheap diagnostic tool, which may be available in every physician's office. SF and SFR can be used in both unstimulated and stimulated whole sialometry. Unstimulated sialometry is performed by the accumulation of saliva on the floor of the mouth, without swallowing for 60s. Then, the accumulated saliva is collected into a tube graded in millilitres (mL) with the aid of a laboratory glass funnel. It is repeated 4 more times for a total of 5min. Stimulated sialometry is performed by stimulation with 2% citrate solution to the dorsolateral borders of the tongue, with a cotton tipped applicator, 5 times over 2 min (0, 30, 60, 90, and 120s). Next, all the retained citrate solution in the mouth are eliminated. The steps of saliva collection and SFR assessment are the same as for unstimulated sialometry.[19] A more precise diagnostic test of SS-related xerostomia is an unstimulated whole sialometry, which lasts for 15 min. Unstimulated whole sialometry lasting for 15min is performed 2h after a meal using the standardized collection procedure. Saliva is collected in a graduated tube via a funnel every 2min. Volumes of up to ≤1.5mL/15min are marked as abnormal; volumes between 1.5 and 2.5mL/15min are marked as intermediate and volumes of ≥2.5mL/15min as normal. Volumes <2.5mL/min are considered abnormal and used for estimating sensitivity for correlation with all scintigraphic parameters.[20] According to the final classification criteria of SS, which was approved by the ACR and the EULAR in 2016, an unstimulated salivary flow rate of 0.1 ml/minute gives a score of 1 to the weighted sum of 5 diagnostic items. Moreover, sialometry can be divided into the whole saliva technique, which is the combined secretion of all salivary glands, and into the collection directly from a specific salivary gland. The reduced rate of secretion of unstimulated whole saliva has the highest diagnostic value in SS. On

the other hand, many changes in flow rate, not seen or less obvious when using whole saliva, have been reported in patients with SS tested with the separate glandular saliva technique. Differences in separate saliva secretion from major salivary glands in SS can depend on the severity and time of the disease. Among the salivary glands, in patients with SS, the parotid is the last one that is affected. Separate saliva collection by sialometry is considered a valuable diagnostic test.[21]

Another useful diagnostic method for xerostomia is the Saxon test. This test can complement stimulated whole sialometry and can be used for patients wearing dentures who are unable to masticate. It is implemented by chewing a folded sterile gauze sponge for 2 minutes and collecting the stimulated whole saliva. Salivation is quantitated by weighing the sponge before and after chewing. Normal control subjects produce ≤2.75 gm of saliva in 2 minutes. The Saxon test is a simple, reproducible and low-cost test for xerostomia and is treated as an equivalent of Schirmer's test in the labial glands.[22] This method can be modified by the different time of saliva collection and the use of different sizes of gauge sponge. In addition to measuring saliva collection, there are a few supplementary methods for quantitative analysis of salivary gland secretion function in SS. Commonly used imaging tests that will facilitate the diagnosis of xerostomia include radiograph sialography, salivary scintigraphy and various magnetic resonance imaging (MRI) techniques. They are mainly performed for the examination of the parotid glands. Major salivary glands scintigraphy is a nuclear imaging technique that through radioactive tracer infusion (Technethium-99 pertechnetate) permits to study glandular function by evaluating the distribution and speed of elimination of the radio-tracer after a secretive stimulation. Positive scintigraphy is defined as a test characterized by delayed uptake, reduced concentration and/or delayed secretion of the trace. The specificity and sensitivity of salivary gland scintigraphy are described as around 50% and up to 89%, respectively. Salivary gland scintigraphy is not a part of the recent classification criteria for SS. However, it is possible that this technique, monitoring salivary gland functioning over time, might still have some potential indications during patients' follow-up to objectively evaluate changes in their secretory function after treatment. Sialography is a traditional radiographic exam based on the cannulation of the main salivary ducts and the subsequent injection of an iodinated contrast medium, allowing the visualization of the architecture of the entire ductal system of the major salivary glands. Although sialography is considered a reliable and accepted method for SS diagnosis, it has limitations in terms of invasiveness and radiation exposure. In the current recommendation, the conventional techniques of radiograph sialography and salivary scintigraphy are replaced by various techniques of MRI and MR sialography. MRI is non-invasive, radiation-free, and sensitive to the morphological and signal changes of the parotid glands, and MR sialography is widely used to evaluate the parotid ductal system without using any exogenous contrast agent. Salivary gland scintigraphy is a safe and sensitive method for assessing the functions of salivary glands. Furthermore, not only the location and morphology of salivary glands can be obtained, but quantitative parameters can also be calculated. MRI is a technique that provides high-resolution images of the parotid glands, as well as great internal contrast of the parotid gland ducts and acinus, due to its high sensitivity to the protons in saliva. Two-dimensional (2D) sequences have been applied in the functional evaluation of the parotid glands. Furthermore, three-dimensional (3D) MRI is a potential modality for both the functional evaluation and morphological imaging of the salivary glands. The secretion function of the parotid glands can be assessed successfully in dynamic MR sialography by the time-dependent volume change ratio curve of the parotid gland duct. Compared with healthy volunteers, SS patients demonstrate a slower and more subtle curve of time-dependent volume change ratio, resulting in a significantly lower $slope_{1st}$ value, peak value, and total saliva secretion post-stimulation. In this method, the $slope_{1st}$ can be used as a quantitative indicator to differentiate normal salivary secretion in healthy people and salivary hypofunction in SS patients.[23,24]

Salivary gland ultrasonography (SGUS) plays a supporting role in the diagnosis of SS-related xerostomia. Salivary glandular damage is the cause of dryness in SS patients, and ultrasonographic imaging of the amount of glandular damage could be essential for evaluating the xerostomia. In SS, the damage could be found particularly in glandular tissues, mainly in the salivary glands, and fibrosis is the most common consequence of tissue damage. The list of parameters explored in SGUS includes echogenicity, homogeneity, number of hypo or anechoic areas, measurement of the biggest hypo or anechoic area, location of the hypoechoic areas in the gland, calcification, posterior border and measurement of the gland.

Among the tests presented for the assessment of SS-related xerostomia, only an unstimulated salivary flow with rates of 01mL/min is included in the current SS classification criteria. Other methods play an additional supporting function and can be used for detection xerostomia induced by other factors.

Treatment of patient with dry mouth

Treatment of xerostomia in SS is difficult. It is an autoimmune disease with systemic manifestations, thus a multidisciplinary management team has been recommended.[25] Treatment of xerostomia includes the resto-

ration or stimulation of saliva secretion and reduction of harmful symptoms of decreased salivation. There are different therapeutic methods to restore the lost functions, alleviating symptoms, preventing and correcting the possible consequences of the lack of natural saliva. They can be divided into endogenous and exogenous approaches. The endogenous approach involves the replacement or enhancement of the salivary gland function through pharmaceutical or genetic modifications and mechanical stimulation. Typically, such modifications are intended to stimulate the secretion of water, electrolytes as well as macromolecules, or preventive protection against harmful factors. The exogenous approach involves the topical application of saliva substitutes to replace lost or enhance the existing function of natural saliva, drinking water and the application of moisturizing preparations. Although there are various pharmaceutical compositions for managing the xerostomia, there is currently no fully effective treatment that provides immediate and long-lasting results.[25] General measures such as air humidification of the environment, namely of the bedroom, caries prevention and smoking cessation play an important role.[26] Frequent sips of oral solutions can be helpful, with options ranging from water to artificial saliva.[27] In SS, recent advances in how to assess changes in disease progression and activity objectively (via repeated biopsies of salivary glands, sialometry, sialochemistry, biomarkers, secretion and composition of tears, EULAR Sjögren's Syndrome Disease Activity Index: ESSDAI) and subjectively (EULAR Sjögren's Syndrome Patient Related Index: ESSPRI) have opened new ways to reliably assess the outcome of a particular treatment, although some final validation studies have to be completed before these tools can be generally applied in primary SS.[25] When looking at more organ-specific tools, sialometry, sialochemistry, ultrasound and repeated biopsies are proper tools to assess salivary gland functioning and regeneration.[25]

Endogenous approaches

Current first-line treatment in SS-related xerostomia is an application of secretagogues that promote the secretion of saliva. In patients with moderate-to-severe oral dryness and with residual salivary gland function, oral muscarinic agonists, such as pilocarpine or cevimeline, are the treatment of choice in the absence of contraindications.[26] Commonly reported adverse effects include sweating, warmth and flushing sensation, increased urinary frequency, headache, blurred vision, diarrhea and abdominal discomfort.[26,28] Moreover, these drugs are often contraindicated in patients with cardiac or respiratory disorders, so use is mainly restricted to patients with severe dry mouth due to SS.[28] Pilocarpine is a parasympathomimetic agent that functions primarily as a muscarinic agonist with mild β-adrenergic activity.[29] This alkaloid causes pharmacologic stimulation of exocrine glands in humans, resulting in diaphoresis, salivation, lacrimation, and gastric and pancreatic secretion.[29] The low doses of pilocarpine sodium alginate improve intraoral xerostomic conditions and quality of everyday life in SS patients with dry mouth through increasing saliva secretion.[16,29] Pilocarpine is prescribed for its acute and short-term effect on inducing salivary fluid secretion. Long-term pilocarpine administration in patients with xerostomia is effective for restoring salivary flow and relieving symptoms. This suggests that, in addition to its transient effect on stimulating salivary secretion, pilocarpine has long-lasting beneficial activity against salivary gland dysfunction. However, the underlying mechanism of this potential beneficial effect is not understood.[30] The patients usually receive 5 mg of pilocarpine hydrochloride three times a day and assuming a patient's weight of 60kg, the dose will be 8.3μg/100g, but the pilocarpine dose used in the long-lasting therapy is approximately 10 times higher than that used clinically in short-lasting therapy.[30] Another recommended secretagogue is cevimeline. Although the clinical practice guideline committee recommends the use of cevimeline to improve salivary secretion, to reduce dry mouth, and oral mucosal abnormalities, cevimeline can induce adverse events.[16] However, there are no reports assessing its use in long-lasting therapy.

Biological treatment

A small number of biological therapies have been tried in primary SS with mixed successes. The preliminary data on rituximab and eprattuzumab are promising, but the efficacy of IFN-α is unclear and TNF-α blockade has been shown ineffective.[31] Xerostomia in SS is not an indication for biological treatment, but the effectiveness of this therapy can reduce the severity of xerostomia.

Several other biological therapies targeting other immune pathways relevant to primary SS pathogenesis may also be useful, such as agents targeting T-cells, IFN-α, IL-6 and other cytokines, co-stimulation/adhesion molecules, B-cell growth factors and Toll-like receptors (TLRs).[31]

Clinical trials of other biological therapies in SS are warranted, but the appropriate outcome measures and patient selection for such clinical studies must be carefully considered when designing the clinical trials.[31]

Adjuvant therapies and adjuvant medical support

Xerostomia can be modified by accompanying systemic diseases and drugs. Pharmacological treatment of both xerostomia and systemic diseases requires the modification of doses of the drugs, the time of the drug application and the choice of the type of drugs in order not to worsen the symptoms of dry mouth. Moreover, ad-

juvant therapy and medical support can be used. These new therapies and preventive methods for dry mouth include antioxidants. It has been suggested that oxidative stress is one of the causes of age-related diseases. Ubiquinol has antioxidant activity and also stimulates ATP production, suggesting that promotion of saliva secretion by ubiquinol in previous studies may have been attributable to these two effects. Ubiquinol is synthesized by humans, but its production has been shown to decrease with aging, and this decrease in ubiquinol is possibly associated with reduced secretion of saliva.[29] Therefore, it is possible that maintaining a higher ubiquinol level after middle age might prevent dry mouth.[30] It has been suggested that tissue damage due to oxidative stress caused by oxygen radicals directly impairs salivary gland function, so protection against oxidative damage may be important for maintaining adequate production of saliva. Some randomized control trials showed thyme honey's positive effects on the management of xerostomia.[25] They also provided evidence that better management of xerostomia can improve patients' quality of life.[25] A diet rich in antioxidants reduces the consequences of xerostomia. Despite its limitations, randomized control trial showed thyme honey's positive effects on the management of radiation-induced xerostomia in head and neck cancer patients.[25] It also provided evidence that the better management of xerostomia can improve patients' quality of life.[25] Some randomized control trials showed thyme honey's positive effects on the management of xerostomia in head and neck cancer patients.[25]

Some research showed a significant increase in salivary flow rate after treatment with herbals.[16] Herbal medicines potentially improve salivary function and reduce the severity of dry mouth in cancer patients, and they are relatively safe. The most common single herbs used for SS are Scrophularia ningpoensis, Ophiopogon japonicus, raw Rehmannia glutinosa, Trichosanthes kirilowii, Scutellaria baicalensis, Lycium barbarum, Rheum tanguticum, Chrysanthemum morifolium, Salvia miltiorrhiza and Dendrobium chrysanthum. They can be administered in a single prescription or in combinations of formulae, usually in patterns for two and three herbals.[32] These items may have effects on antioxidant capacity, anti-inflammatory function and dry eye or dry month improvement. Furthermore, they have an immune modulation or a tissue fibrosis alleviation function. However, methodological limitations and a relatively small sample size reduce the strength of the evidence of their effectiveness. In the future, more high-quality trials reporting sufficient methodological data, more clinically homogenous trials and further evidence of safety are warranted to draw definitive conclusions concerning the effectiveness of herbal medicines.[33] Zeng Ye decoction is extracted from figwort, Ophiopogon japonicus and Rehmannia glutinosa Libosch. The Zeng Ye decoction consists of 30g figwort, 24g Ophiopogon japonicas and 24g Rehmannia glutinosa Libosch, decocted for ~30min to produce the solution of 1g raw herbs per 1ml decoction. In the field of traditional Chinese medicine, Zeng Ye decoction, as an important Chinese medicinal agent, is widely used for relieving constipation due to body fluid deficiency. The potentially effectiveness of this agent is based on the theory of 'increasing body fluid for curing constipation', which was proposed by the famous medical scholar JuTong Wu with regard to epidemic febrile diseases.[34] Zeng Ye decoction has a significant curative effect on SS via upregulation of the levels of aquaporin-1 and -5. The high yield of these water channel proteins in the salivary glands facilitates the secretion of fluid, and is beneficial to recovery from SS. This result may be associated with the different immune mechanisms of the different ingredients of Zeng Ye decoction in the early development of SS. Another recommended Chinese herbal composition is a combination of Radix Pseudostellariae 30g, Radices Paeoniae Alba 12g, Schisandrae Chinensis Fructus 10g, Fructus Ligustri Lucidi 15g, Polygonatum SibiricumRed 15g, Smoked Plums 12g, Fructus Mori 15g, Glabrous Sarcandra Herb 15g, Rhodiola Sachalinesis15g, Artemisia Apiacea 15g. The Chinese medicine decoction is given in two packs a day. This composition has the effect of nourishing, supplementing and activating the blood. The two packs are given after breakfast and lunch respectively and the course of treatment usually lasts for 3 months.[34] Chinese herbal medicine for nourishing, supplementing and activating blood can alleviate the disease activity of SS by regulating the immune balance of Th1/Th2.[35,36,37]

Furthermore, some non-pharmacological therapies can be very helpful in treating xerostomia. They improve the quality of life and reduce the consequences of decreased salivation. Acupuncture as part of therapy for xerostomia can improve patients' subjective symptoms. A study evaluating the preventive effect of acupuncture for xerostomia showed positive changes in both unstimulated and stimulated salivary flow rates and dry mouth related symptoms. Acupuncture treatment is well tolerated by all patients and no severe adverse effects are seen.[38] Another non-pharmacological treatment of xerostomia is photobiomodulation. Photobiomodulation therapy is defined as a form of light therapy.[39] Visible, infrared and near-infrared light is absorbed by endogenous chromophores, triggering biological reactions that are not thermal or cytotoxic, through photochemical or photophysical events, leading to physiological changes.[39] Photobiomodulation supports the physiological function of salivary glands. There are few reports regarding the effectiveness of this therapy and possible side-effects. However, treatment of xerostomia is extremely

difficult in patients who wear dentures. Dentures may exacerbate symptoms of xerostomia. Moreover, denture stabilization is limited. Wearing removable dentures in worse salivary conditions can promote oral lesions formation. On the other hand, dentures can be used in the treatment of xerostomia. Some modified removable dentures were described in the therapy of xerostomia, and a removable dental denture was fabricated with inbuilt sensors to help in the management of xerostomia.[39] A micropressure sensor is incorporated into the prosthesis to detect dry mouth. On detecting a dry mouth, tongue pressure ejects artificial saliva from a capsule inside the sensor. The addition of a small sensing unit helps detect dry mouth. A saliva substitute is released according to the patient's requirements and unnecessary dispensing and frequent replacement of artificial saliva is avoided.[40]

Oral moisturizing agents

Oral moisturizing agents can improve dry mouth and oral mucosa abnormalities, and has virtually no side effects. However, oral moisturizing gel and artificial saliva have adverse events related to digestive symptoms.[16] Moreover, it has been shown in the literature that some oral moisturizers may have erosive potential due to their acidic pH, which is below the critical pH of dentin and enamel.[38] Clinicians should therefore be aware of this erosive potential of the products and make recommendations to manufacturers for future formulations avoiding acidic pH.[41] For this reason, care should be taken to formulate products with safe pH values for both enamel and root dentin which, based on the specific formulation, should be around 6.7 or higher.[41] Recent studies have concluded that there is great variation in the pH values among the most common oral moisturizers on the market and that there is a strong correlation between the pH values and the erosive potential of these products.[38] Thus it would seem reasonable for practitioners to take care in recommending oral moisturizing agents with a safe formulation for their patients.[41,42] Saliva substitutes, lubricating agents and mechanical stimulation by chewing sugar-free gum are usually employed in patients with mild hyposialia.[26] Topical fluoride and fluoride toothpaste to prevent caries are also strongly recommended.[26] A multicenter randomized controlled trial has shown that mild intraoral electrostimulation can alleviate oral dryness and had no adverse effects.[26,42,43]

Prophylaxis

In every case of xerostomia, the mechanism of oral dryness should be individually determined, since there are different ways to prevent sicca symptoms. It may be possible to keep to the strategy of limited intake of certain kinds of medicine during the treatment of other conditions or switching to another drug that does not cause oral dryness, if this is possible. Those measures may include salivary gland protectors, such as amifostine, hyperbaric oxygen and the use of intraoral stents during head and neck cancer radiotherapy.[5,33,42,43] In terms of SS, we have not found many prevention mechanisms, due to its complexity as an autoimmune disorder. Moreover, at present the only thing that a person diagnosed with this disease can do is to reduce the severity of the condition. As stated above, patients with SS must be observed by a multidisciplinary team and such conditions as malignant lymphoma (the features of which should be noted in diagnosis or other serious complications, including hematological malignancies, liver diseases and cardiovascular disease) can negatively affect the prognosis of patients with SS.[16]

Conclusions

To sum up, we have to conclude that nowadays there are a lot of different ways to manage xerostomia or sicca manifestations of SS. Some of these are limited (hydroxychloroquine, herbal medicine) and more randomized control trials need to be conducted to prove their effectiveness. In the systemic treatment of SS, promising results have been shown by some biological therapies and oral muscarinic agonists in improving dry mouth abnormality. However, one must remember that as of today, there is no fully effective treatment of xerostomia that provides immediate and long-lasting results.

There is currently no cure for SS that can restore gland secretion, although with recent advances in treatment modalities, there is a hope that the management of this autoimmune disease will soon improve. Moreover, in the treatment of patients with xerostomia, due to causes other than SS, careful evaluation is important to define the cause and appropriate treatment.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.13

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 364–367

CASUISTIC PAPER

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Laparoscopic partial cystectomy for bladder endometriosis – case report

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ABSTRACT

Introduction. Endometriosis is defined as a presence of endometrial glands and stroma outside the uterus. Urinary track endometriosis is a rare occurrence (1-2%) usually associated with bladder involvement (85%).

Aim. The diagnostic evaluation is not complicated but can be delay because of the lack of specific symptoms.

Description of the case. We present a case of 20-years old female with bladder endometriosis localized on the posterior wall. The patient was effective treated with laparoscopic partial cystectomy

Conclusion. The patient was effective treated with laparoscopic partial cystectomy

Keywords. CT, endometriosis, MRI, ultrasonography

Introduction

Endometriosis is a common gynecological entity and affects up to 15–20% of women of reproductive age.[1] Depending on extension, three main forms are recognized: superficial peritoneal endometriosis, ovarian endometriosis and deep infiltrating endometriosis (DIE). DIE is the most severe, particular type that penetrating more than 5 mm under the peritoneal surface and occurs in approximately 1% of cases. Urinary track endometriosis (UTE) is a rare (about 1–2 %) but potentially devastating disease affecting quality of life.[2] It can also cause significant morbidity such as progressive renal function loss. The UTE incidence increases up to 19-53% among patients with DIE.[3] In cases of urinary involvement, bladder endometriosis (BE) is the most frequent type (85%) and is defined as a presence of endometrial glands and stroma in the detrusor muscle but the vesical mucosa is not always involved by disease.[3] The most often affected part of the bladder is the base and the dome.[4] From a clinical standpoint BE should not be consider as an independent entity because in the vast majority (87,9%) of patients the presence of concomitant nonvesical lesions has been documented.[5] Accurate and early diagnosis is crucial for the prognosis but the assessment may be difficult when specific symptoms are lacking.

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 6.08.2019 | Accepted: 20.09.2019
Publication date: December 2019

Godlewski D, Pszczółkowski P, Góra T, Futyma Ł, Fedus T, Aebisher D. *Laparoscopic partial cystectomy for bladder endometriosis – case report.* Eur J Clin Exp Med. 2019;17(4):364–367. doi: 10.15584/ejcem.2019.4.13

Primary clinical presentation consists of dysuria (up to 69%), frequency, urgency and suprapubic pain. These symptoms may have a cyclical manifestation depending on menstruation. Hematuria is not a common symptom (0–35%) because the lesion rarely ulcerates the mucosal layer. Clinical manifestation may be attributed to recurrent cystitis, bladder carcinoma or interstitial cystitis, so a series of complementary tests are needed to confirm the diagnosis.[3,6,7]

Ultrasonography (USG) performed either transvaginally or transabdominally is the ideal first line examination. It is also a reliable method for planning the most appropriate treatment. The USG performed with the full bladder enables to evaluate the size and location of the endometrial lesion and estimate the distance from ureteral orifice. BE appears as a heterogeneous iso/hypoechoic, intraluminal, conical lesion. The protrusion, usually localized on the posterior vesical wall or the dome, is covered by a narrow rim of hyperechoic submucosal and serosa layer. Typical endometrial nodule is not vascularized, spherical or comma-shaped with regular contours.[1,3]

Cystoscopy visualize usually red or bluish cyst with non-ulcerated urothelium and provide access for biopsy which is important to rule out carcinoma, varices, papilloma or angioma. Nevertheless, it should be bear in mind that biopsy at cystoscopy is often nondiagnostic for endometriosis.[8]

MRI and CT scans usually do not contribute more detailed data to USG, cystoscopy and should not be routinely performed.[9]

Aim

The diagnostic evaluation is not complicated but can be delay because of the lack of specific symptoms.

Description of the case

20-years old female with symptoms of chronic bladder pain, painful menstruation and dyspareunia, but no he-

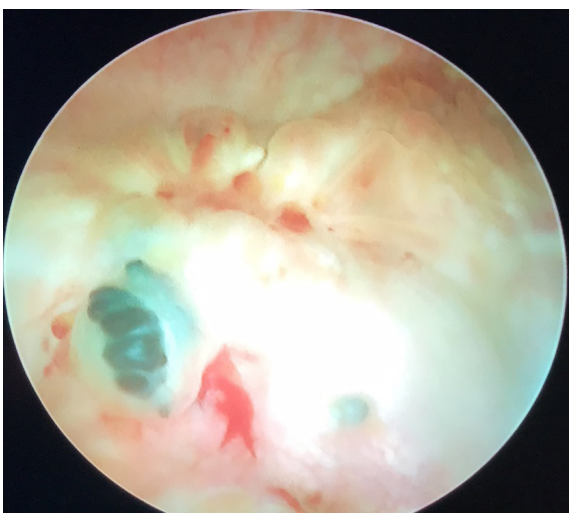

Fig. 1. Cystoscopic view

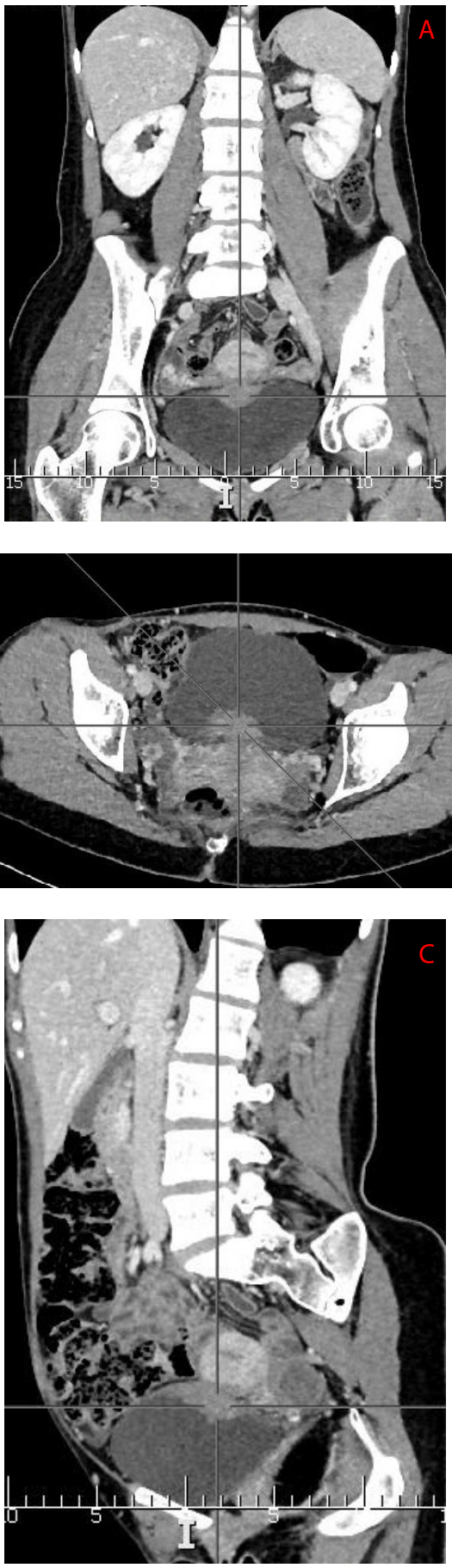

Fig. 2. Abdominopelvic CT scan

maturia was referred to urologist by gynecologist. In bimanual examination palpable mass in bladder was revealed. Transvaginal ultrasound (TVUS) developed pathological mass on posterior bladder wall. No former surgery was performed. Cystoscopic view (Fig.1) showed 3 centimeter orange-claret tumor behind bladder trigone with no clear margin from normal mucosa.

Abdominopelvic CT scan (Fig.2A, 2B, 2C) developed pathological area 62x40x20mm diameters adhering to uterus and cervix. Neither distant lesions nor ureterohydronephrosis and lymphadenopathy were observed.

Consecutive time patient was admitted to hospital for laparoscopic treatment. Before surgery ureteral double J stents were inserted for better control of ureters due to lesion localization (Fig.2B) and Foley catheter 20 Fr was left in bladder. In Trendelenburg position, we used three working trocars (1x10Fr, 2xFr) and one optical (10Fr) to enter peritoneum. The posterior bladder wall was tightly adherent to uterus so harmonic knife and scissors were used for sharp dissection. Than bladder was opened and lesion was resected with margin of unchanged bladder wall using harmonic knife. One layer running suture was applied for bladder closure. Than with 200 ml saline tightness was proofed. Redon drain was left. Blood loss was insignificant. Operation time was two hours.

Postoperative period was uncomplicated. Drain was removed on the second day. Patient was released from hospital on the third day with Foley catheter and double J stents, that were removed after 14 days.

Dienogest was administered by gynecologist. No preoperative symptoms appeared. After 4 months patient got pregnant. Pregnancy period was uncomplicated.

Discussion

Hormonal therapy should be regarded as primary treatment to control the symptoms of BE.[10] However medical therapy is effective in temporarily suppressing but not ensure complete excision of lesions. This treatment is ineffective or interrupted due to adverse effects in about 30%.[11]

Two main approaches are proposed for surgical management of BE: cystoscopic (TUR surgery) or abdominal. Only entirely removal of pathological tissue guarantee long term relief of urinary symptoms and pain. From a pathogenic point of view TUR is not an appropriate approach, as vesical nodule develops in external layer and later infiltrates the vesical wall. The excision of the whole lesion is unachievable or associate with bladder perforation. [3]

Partial cystectomy is the most commonly used method for the BE treatment that can be performed via laparoscopy or laparotomy. Preoperative, preventive ureteral catheterization may be advisable when the distance between the caudal margin of the endometrial lesion and the interureteric ridge is less than 2 cm.[1,3] The success rate increases to 100% among lesions localized in the dome.[12] Segmental bladder resection does not carry a high risk of complications. Abundant vascularization ensure appropriate suture healing and prolonged urine drainage (7–10 days) prevents fistula formation.[1,12]

Conclusion

We find laparoscopic partial cystectomy safe and radical method for treatment bladder endometriosis, when applied by experienced surgeon. Cooperation between urologist and gynecologist is essential for excellent outcome.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.14

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 368–370

CASUISTIC PAPER

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Difficulties in diagnosis of carcinoma of the tongue

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ABSTRACT

Introduction. Oral cancer is the second most common malignancy and there is an epidemic alert by WHO for oral cancers projected for 2030. The tongue remains the most common intraoral site for oral cancer worldwide.

Aim. To present a case report.

Description of the case. A 56-year-old patient was suffering from carcinoma of the tongue. He developed metastases in the lungs and upper part of the vertebral column. The PET scan report revealed the presence of hypermetabolic cells in the metastatic tissue. The biopsy of the lesion on the upper part of the back did not show neoplastic cells, epithelioid cells and giant cells. Radiotherapy was given for 25 cycles. Both the lungs were affected by metastases. Lastly the patient expired due to cardio-respiratory failure.

Conclusion. Tobacco is the most important known risk factor for the development of tongue cancer. The tumors in their early stage with complete excisional treatment have good prognosis. There is usually a history of long standing leukoplakia or erythroplakia. Ideally, imaging should take place prior to biopsy. Surgical procedures such as hemiglossectomy can cause functional defects in speech and swallowing. Difficulty in diagnosis results in inappropriate treatment.

Keywords. hemiglossectomy, leukoplakia, PET-CT scan, radiotherapy

Introduction

Oral cancer is the second most common malignancy and there is an epidemic alert by WHO for oral cancers projected for 2030.[1] The tongue remains the most common intraoral site for oral cancer worldwide.[2] In contrast to other sites of oral cancer, the incidence of the tongue carcinoma is increasing in the younger age group.[3] Speech, swallowing and breathing are associated with the integrity of the reconstructed tongue muscles after surgical resection.[4]

Description of the case

The patient was a smoker and tobacco chewer for 20 years. He developed leukoplakia and subsequently erythroplakia. The oral mucosa and the right side of the tongue became indurated and red. He felt difficulty in eating, swallowing food, and in speaking normally. Burning sensation was felt while taking spicy food. The affected portion of the tongue gradually became more indurated and red. Mastication became difficult. The patient did not agree for biopsy of the lesion in spite of repeated advice by the oncologist. He adopted oth-

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 8.07.2019 | Accepted: 25.10.2019
Publication date: December 2019

Panda DK. *Difficulties in diagnosis of carcinoma of the tongue.* Eur J Clin Exp Med. 2019;17(4):368–370. doi: 10.15584/ejcem.2019.4.14

er systems of therapy for a long period. Ultimately, the growth increased in size and metastasized. Biopsy was done in very late stage. It was finally diagnosed as squamous cell carcinoma (SCC), moderately differentiated type (Fig. 1).

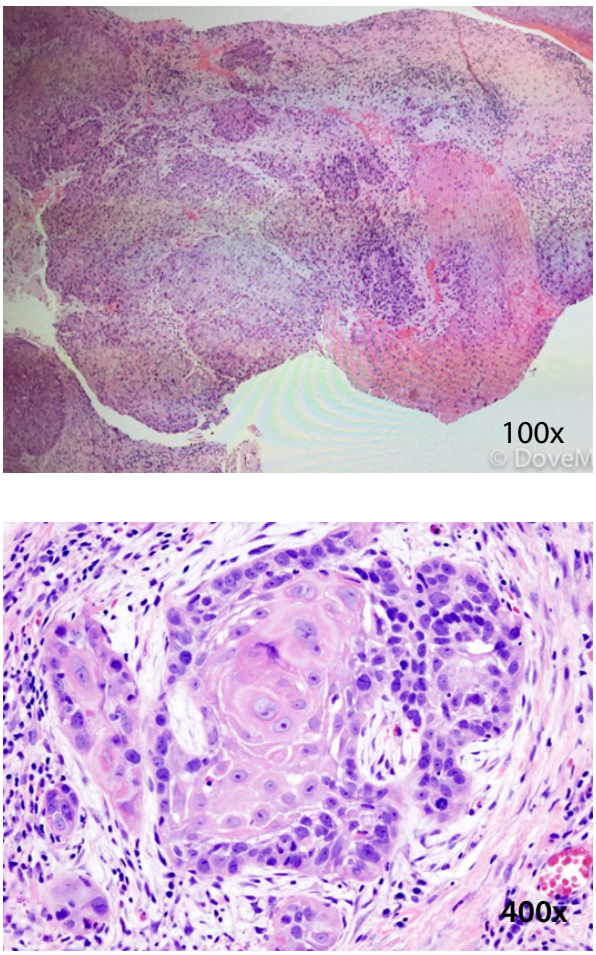

Fig. 1. Infiltrative squamous cell carcinoma of tongue

The patient was admitted to a reputed cancer hospital for treatment. After completing the required investigations, hemiglossectomy was done along with reconstructive surgery. But PET-CT scan was not done before surgery to obtain information regarding metabolic changes in other organs of the body. The surgical wound healed gradually. The patient was discharged. Radiotherapy was instituted for 25 cycles. During this period, the patient had copious expectoration of thick phlegm. Sputum culture was sterile. Malignant cells were not found. The patient suffered severe pain which did not respond to injectable analgesics and opium tablets. Kyphosis developed. The patient was again readmitted in the same hospital for checkup. PET-CT scan was done. The report revealed hypermetabolic cells in right paravertebral soft tissue mass with infiltration of D9-D10 vertebrae; metastatic intraspinal extension at D8, D9-10 level with fracture of right 9th rib (Fig. 2). Hypermetabolic metastatic right pleural based soft tissue deposit eroding right 6th rib was noticed. Biopsy was repeated. It was suggestive of reparative myofibroblastic proliferation. The report did not show neoplastic cells. A group of doctors decided on detecting *Mycobacterium tuberculosis* from the tissue. It came out as negative. The patient fell down in the bathroom and developed paraplegia. Therefore, the hospital discharged the patient for palliative treatment. Expectoration of phlegm, pain and fever continued. Again he was admitted, this time in another hospital. The mass on the back of upper chest was operated to release compression on the nerves and to confirm malignancy by repeating biopsy. No neoplastic cell was detected. The result of the tuberculosis tests (PCR/sputum-AFB) was negative. No improvement was noticed as regards paralysis of legs. Bowel and bladder reflex was lost. Indwelling catheter was inserted in the urethra for urination and repeated enema was given for fecal evacuation. The patient was discharged. His condition did not improve. Fever and pain increased. Respiratory distress developed. He was again admitted in the ICU in emergency and expired due to cardio-respiratory failure.

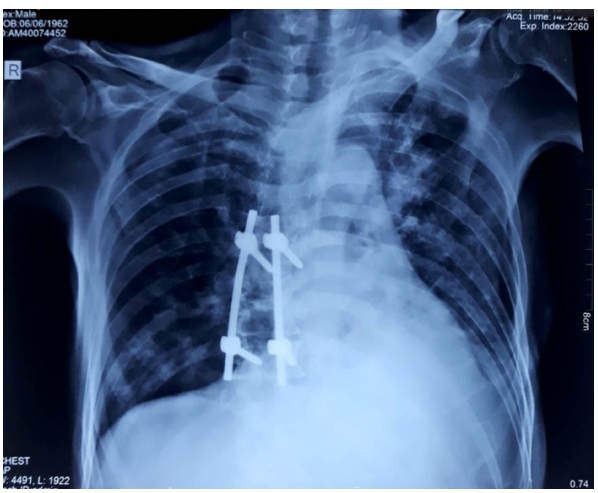

Fig. 2. Lungs and Intraspinal Metastasis

Discussion

The repeated exposure of the mucosa of the upper aero digestive tract to the carcinogenic effects of tobacco, alcohol, or both is the cause of carcinoma of tongue. The multiple primary and secondary tumors in this "condemned mucosa," are a phenomenon described as "field cancerization".[5]

It is highly probable that healed tuberculous granulomas that are culture and AFB negative for *M. tuberculosis* will sometimes be positive for its DNA.[6] Thus, when *M. tuberculosis* DNA is found in tissue specimens, all other laboratory and clinical data must be analyzed before a final diagnosis is made.[7] The PCR test for *M. tuberculosis* will increase diagnostic accuracy resulting in improved and timely care for patients.[8]

The various treatment options for the carcinoma of tongue include surgery, radiotherapy, chemotherapy and combined modalities.[9]

Combined positron emission tomography (PET-CT) scans may add accuracy in evaluating the extent of the primary tumor. It may aid in target delineation if definitive radiation therapy (RT) is being considered.[10] PET scanning may help to identify pathologically involved lymph nodes. Integrated PET-CT has greatly replaced other tests for detection of distant metastases.[11]

Improved imaging techniques, including functional or molecular-based studies prior to surgery, may eventually prove useful in selecting patients for neck dissection. Sentinel lymph node biopsy is an emerging technique that may help neck dissection in patients with intermediate-thickness tumors.[12,13]

An en bloc partial glossectomy with negative margins can preserve speech and swallowing for most stage I and II lesions of the tongue. The choice of reconstruction and the intensity of rehabilitation shall determine the ultimate functional outcome.[14] Excellent overall survival and swallowing have been reported using TLM (Trans oral laser microsurgery) as the primary treatment for advanced stage tongue cancer.[15]

Conclusion

Tobacco (smoked and smokeless) is the most important known risk factor for the development of tongue cancer. The tumors in their early stage with complete excisional treatment have good prognosis. There is usually a history of long standing leukoplakia or erythroplakia. Ideally, imaging should take place prior to biopsy. Surgical procedure such as hemiglossectomy can cause functional defects in speech and swallowing. Difficulty in diagnosis results in inappropriate treatment.

Learning points

- Carcinoma of tongue mostly occurs due to continuous use of tobacco in the form of chewing, snuff or smoking.
- Early diagnosis and treatment prolongs the life span of the patient.
- Successful microsurgery followed by radiotherapy raises the hope of survival.
- Improper and delayed diagnosis leads to metastases and serious complications, resulting in painful death.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.15

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 371–377

CASUISTIC PAPER

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Osmotic demyelination syndrome in a patient with slowly equalized severe hyponatremia – a case report

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ABSTRACT

Introduction. Osmotic demyelination syndrome (ODS), or central pontine myelinolysis (CPM), is a complication of severe and prolonged hyponatremia, particularly when hyponatremia is corrected too rapidly. However, even slow correction of hyponatremia can result in ODS.

Aim. In this paper, we describe a patient who developed ODS following slow correction of hyponatremia.

Description of the case. This article describes a case of chronic hyponatremia occurring in the course of alcoholism. The patient was admitted in severe condition with extremely low sodium level. Electrolyte supplementation was carried out according to the European Renal Best Practice (ERBP) guidelines; however, there was a rapid increase in sodium level leading to the development of symptomatic osmotic demyelinating syndrome. Following several weeks of rehabilitation and supplementation of B vitamins, the patient's condition gradually improved.

Conclusion. Sodium deficiency should be equilibrated very carefully, especially in patients with chronic hyponatremia in the course of alcoholism. Even small doses of sodium administered in accordance with the guidelines in chronic hyponatremia can cause a rapid increase in serum sodium level resulting in osmotic demyelination syndrome.

Keywords. alcoholism, osmotic demyelination syndrome, sodium

Introduction

Osmotic demyelination syndrome (ODS) is a complication of severe and prolonged hyponatremia, particularly when treated with rapid correction. The pathogenesis of ODS is not completely understood. During chronic hyponatremia, osmotically active substances and water are lost from brain cell. These solutes cannot be replaced quickly enough with a rapid correction of the hyponatremia. The rapid shifts of intracellular, extracellular, and intravascular water, sodium, chloride, and organic osmolytes produce relative glial dehydration, myelin degradation, and/or oligodendroglial apoptosis.[1-3]

Demyelination typically occurs in areas of the brain that are the slowest to uptake osmolytes, which most commonly include the central pons (central pontine myelinolysis – CMP), but can be found in the cerebellum, lateral geniculate body, hippocampus, cerebral cortex, thalamus, caudate nucleus, internal capsule,

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Received: 27.02.2019 | Accepted: 3.11.2019
Publication date: December 2019

Rudnicka-Czerwiec J, Bartosik-Psujek H. *Osmotic demyelination syndrome in a patient with slowly equalized severe hyponatremia – a case report*. Eur J Clin Exp Med. 2019;17(4):371–377. doi: 10.15584/ejcem.2019.4.15

midbrain, and mammillary body as well.[4-6]

CPM occurs in 30%-50% of patients, demyelination of extrapontine sites only were found in 20%-50%, and both the central pons and an extrapontine area in 30%-50%.[4,7]

Comorbidities that are highly associated with incidence of CPM include dialysis, liver failure and transplantation, advanced lymphoma, carcinoma, cachexia, severe bacterial infections, acute hemorrhagic pancreatitis, chronic alcoholism, and pellagra.[8,9] The clinical presentation of CPM is heterogeneous, including confusion, quadriplegia, pseudobulbar palsy, and coma.[10]

Rapid correction of hyponatremia is a known risk factor for the development of ODS. However, even a slow correction of hyponatremia can result in ODS. In this paper, we describe a patient who, with a slow correction of hyponatremia, developed ODS.[11]

Description of the case

The 51-year-old female patient, who had not previously received treatment on account of chronic diseases with a history of alcoholism, was transported in an ambulance directly from home to the Emergency Department. The patient's daughter found her lying unconscious on the floor at her home. Based on the information provided by the family, the patient had been vomiting, had had diarrhea and had fallen several times in four days preceding the admission. Before that the patient had also consumed alcohol. The family reported that the patient had been temporarily confused, had spoken incomprehensibly, had gibbered and had been excessively sleepy.

Physical exam on admission revealed: body temperature of 37.4°C, blood pressure 160/100 mmHg, heart rate 80 BPM. The patient was in deep sleep, she only opened her eyes slowly when stimulated with pain, unable to maintain verbal contact. Symptoms were observed in the following areas: meningeal (neck rigidity up to 4 fingers, positive Kernig sign); cranial nerves (the eyeballs periodically turned towards left, the pupils slightly dilated); limbs (the patient defended herself symmetrically with two upper limbs when stimulated with pain, the muscle tonus slightly decreased in lower limbs, the deep reflexes were symmetrical and there were no abnormal signs).

Lab test performed at the Emergency Department revealed very low sodium level — Na 99 mmol/L with reference range of 136–146 mmol/L. Results of all lab tests performed on admission were presented in Table 1.

At the Emergency Department the patient received 150 mL of 3% of NaCl i.v. due to severe hyponatremia.

After 4 hours additional 500 mL of 0.9% NaCl with 1.5 g of KCl i.v. and 2 g of $MgSO_4$ i.v. were administered.

A CT scan of the head was performed (Fig.1). Lumbar puncture was abandoned due to brain edema. The patient was admitted to the Department of Neurology.

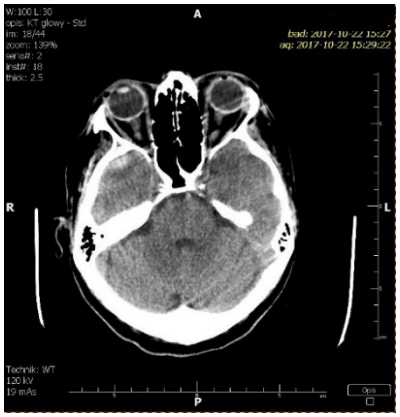

Fig. 1. A CT scan of the patient's head

At the base of the right temporal region, there is a hyperdense pericerebral hematoma measuring 15 × 7 mm in the plane of the scan. Furthermore, in this region there is a small hyperdense intracerebral hematoma surrounded by a region of edema. There is a small hypodense region in the right cerebellar hemisphere. There is a minor swelling of both cerebral hemispheres.

At the Department, according to the recommendations of a consulting specialist in internal diseases, another 150 mL of 3% NaCl i.v. were administered. During the first two days of hospitalization frequent electrolyte tests were performed. Changes in electrolyte levels were presented in Fig. 2.

In the following days, the patient's condition im-

Table 1. Laboratory tests results at the day of admission

ethanol	-		
blood count	WBC: 13,58*10^3/mcl (4,00-11,00*10^3/mcl) RBC: 4,29*10^6/mcl (4,2-5,4*10^6/mcl) Hgb: 14,2 g/dl (12,0-16,0 g/dl) PLT: 314*10^3/mcl (150-400*10^3/mcl)	acid-base balance	pH: 7,51 (7,35-7,43) pCO2: 15,0 mmHg (45-50 mmHg) pO2: 64,0 mmHg (33,0-53,0 mmHg) HCO3-: 20,7 mmol/l (22,0-26,0 mmol/l)
electrolites	Na: 99 mmol/l (136-146 mmol/l) K: 3,9 mmol/l (3,5-5,1 mmol/l) Cl: 67 mmol/l (101-109 mmol/l)	alanin aminotransferase	67 U/L (0,00-35,0 U/L)
urea	10 mg/dl (17-43 mmol/l)	aspartate aminotransferase	86 U/L (0,00-35,0 U/L)
creatinine	0,32 mg/dl (0,55-1,02 mg/dl)	CRP	23,5 mg/l (0-5 mg/l)

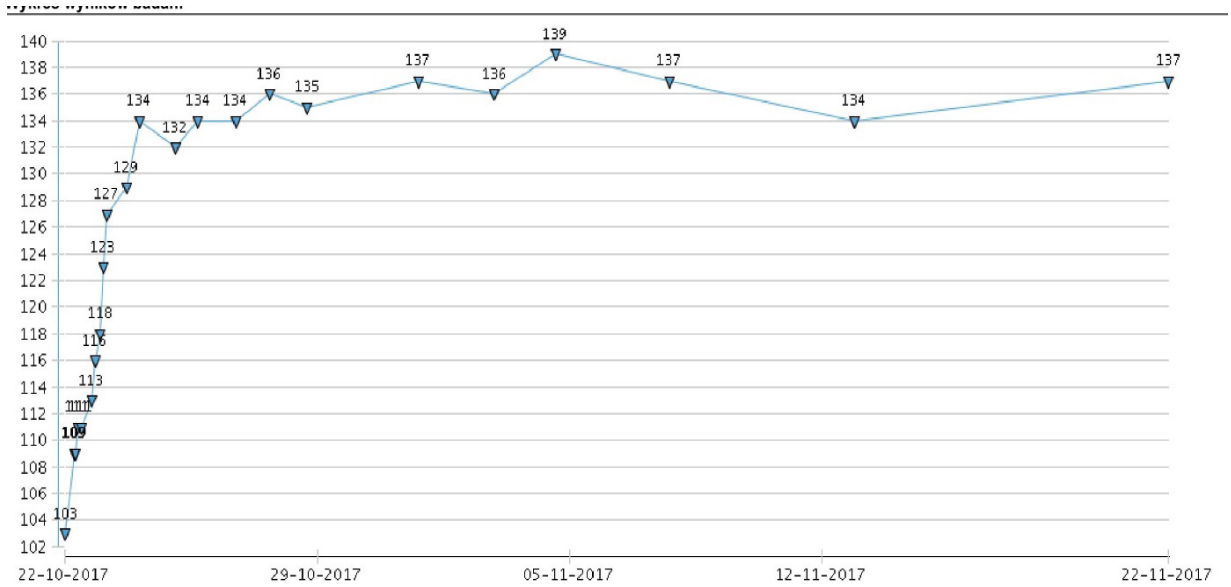

Fig. 2. Changes in the patient's blood sodium during hospitalization

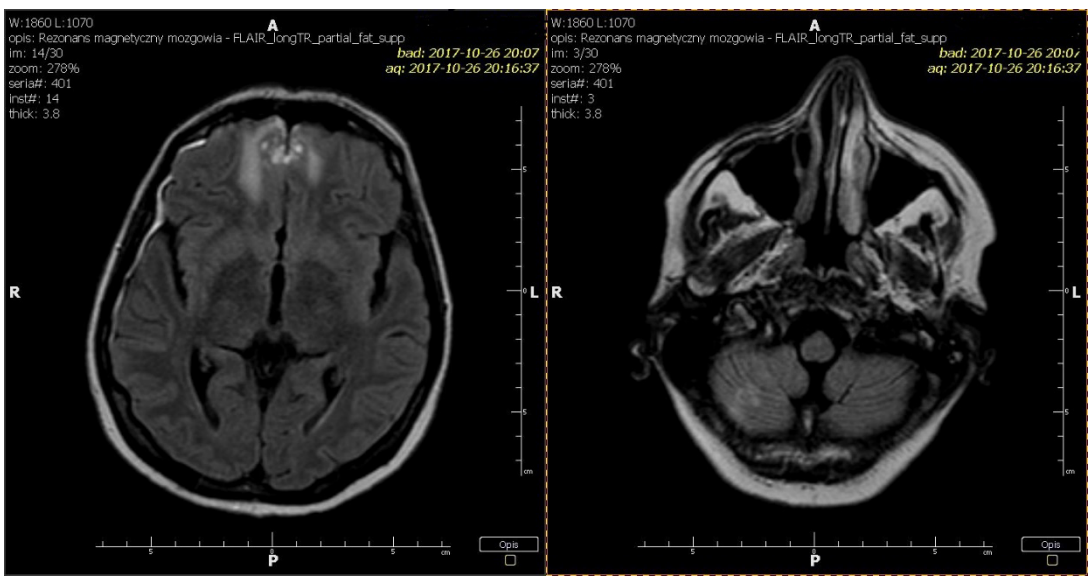

Fig. 3. A MRI of the patient's brain

proved. She was conscious, well oriented and able to walk without assistance. The patient denied alcohol abuse. She was also able to maintain a basic verbal contact and spoke consistently. However, the patient processed information more slowly and showed a decreased flexibility of thinking. Neuropsychological exam revealed cognitive deficits in short-term memory with preserved long-term and autobiographic memory. In addition, the patient had difficulties in executive, as well as visual and three-dimensional performance. Regarding abstract thinking, the patient presented the tendency to concretize. Understanding, praxis and visual gnosis of objects were all preserved.

The patient received dexamethasone, mannitol, ceftriaxone and probiotic - lacidofil.

A MRI of the brain was performed 4 days after the admission (Fig. 3).

On the right side, in the frontal, temporal and parietal region there is a cerebral hemorrhage. There are regions of brain contusion with evolving hematomas within them, in the right temporal region and adjacent parts of frontal lobes, located parasagittal, surrounded by a region of minor edema. In the right cerebellar hemisphere, there are ill-limited foci of increased signal in FLAIR and T2-weighted images, with contrast enhancement – blood-brain barrier damage within the foci of brain contusion.

In order to follow up the post-traumatic changes, according to the indications of a neurosurgeon, a CT scan of the head was performed 6 days after the admission. The imaging revealed regression of hematomas (Fig. 4).

On the tenth day of hospitalization the patient's condition deteriorated. She was conscious, drowsy, unable to

maintain verbal contact or to follow instructions. A neurological examination revealed: an increased muscular tension on the left side, deep reflexes more pronounced on the left side, lack of plantar reflexes, positive Babinski's sign on the left side. Meningeal symptoms were negative.

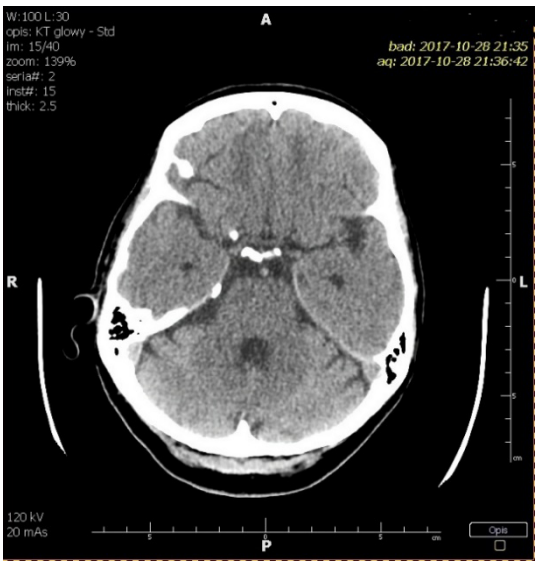

Fig. 4. Regression of hematomas. At the base of the frontal regions, bilaterally there are irregular regions of poorly decreased density – probably post-contusion. There is a similar, small focus in the right temporal lobe

An urgently performed CT scan of the head revealed no progression of post-traumatic changes or new abnormal foci (Fig. 5).

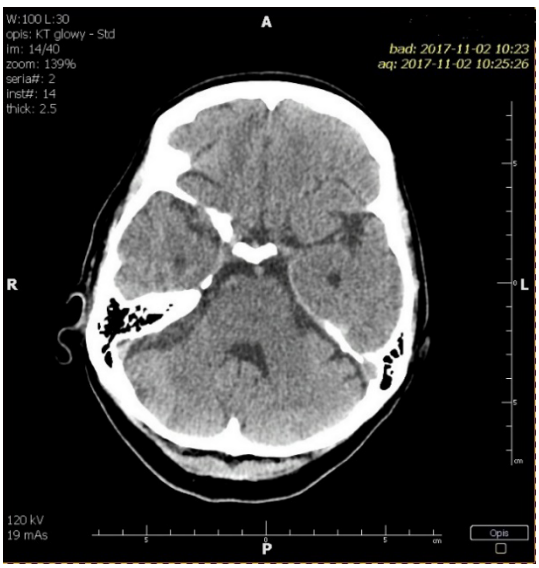

Fig. 5. There is no acute blood visible intracranially. At the base of the frontal regions, bilaterally there are irregular regions of poorly decreased density – probably post-contusion. There is a similar, small focus in the right temporal lobe and peripherally in the right cerebellar hemisphere

An EEG revealed a slowed basic activity.

Osmotic demyelination syndrome was suspected. Brain MRI confirmed the diagnosis and pontine and extrapontine demyelination was found. (Fig. 6).

Low molecular weight heparin was administered in order to prevent thromboembolism. The patient also received vitamin B1 and B12. Physical and cognitive rehabilitation was administered. After two weeks of treatment the patient was able to maintain logical verbal contact, was fully autopsychologically oriented and partially allopsychologically oriented. She was emotionally vulnerable, tend to cry and she was able to understand incoming information and execute instructions. Generalized psychological deficits were observed – psychomotor and verbal slowdown, spontaneity of action, prolonged reaction time, fatigability, attention deficits, disturbances of short-term memory and conceptual thinking.

The neurological examination revealed: negative meningeal symptoms; for cranial nerves: asymmetric grin on the left side; for limbs: minor weakening of the left limbs, no plantar reflexes, no abnormal signs, assisted gait.

The patient was transferred to the Department of Rehabilitation. in order to improve her performance and she stayed there for four weeks.

After three months control exam was performed. The patient was slightly distracted regarding orientation in time and space. The orientation regarding herself was preserved. Logical contact was preserved; she responded adequately to questions, her activity was slightly increased and her affect – elevated. There were no visible abnormalities in perception and content of thoughts. The course of thinking was slightly slowed. The patient maintained criticism regarding her health condition, but denied alcohol abuse. She presented deficit in lingual skills and verbal fluency, as well as difficulties in concentration. The patient exhibited memory disorders, especially regarding delayed rendering, perception and memorizing new information. The neurological examination revealed asymmetric grin on the left side, minor weakening of the left limbs, no plantar reflexes, no abnormal signs and normal gait.

A further MRI revealed regression of demyelination changes (Fig. 7).

At present the patient continues to improve her mobility and cognitive functions participating in environmental rehabilitation.

Discussion

The European Renal Best Practice (ERBP) of 2014 regarding treatment of hyponatremia with severe symptoms recommend that a 3% solution of NaCl i.v. should be administered immediately at the dose of 150ml within 20 minutes. This should be repeated until the sodium

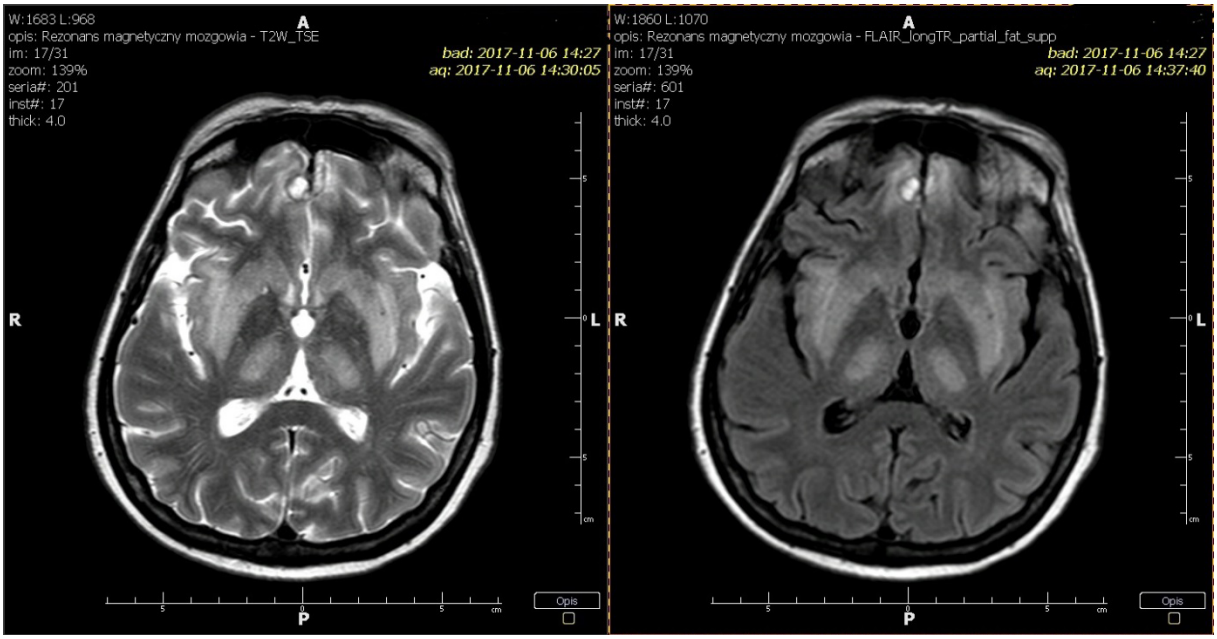

Fig. 6. There are regions of increased signal in T2-weighted images and sequences SEQ Flair bilaterally in the subcortical nuclei, in thalami, centrally in the pons and there are smaller foci in the subcortical white matter of both hemispheres – most likely resembling acute osmotic demyelination

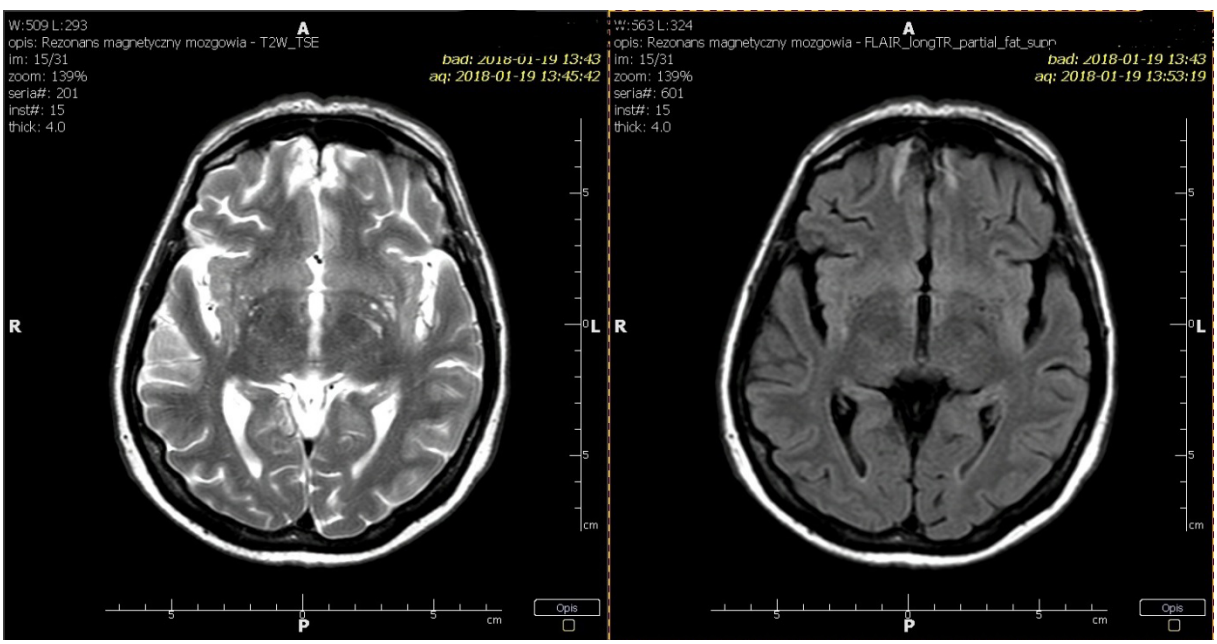

Fig. 7. The size of the foci and regions of demyelination within the basal nuclei and the pons decreased. Signs of edema relieved. There is mostly malacia surrounded by bands of astrocytic gliosis

level has increased by 5 mmol/L and the clinical condition has improved. Then, it is indicated to perform a slow infusion of 0.9 % NaCl i.v. until administration of casual treatment. An increase in sodium level in the first 24 hours must not exceed 10 mmo/L, and in the following days – 8 mmol/L. In cases of an increased risk of acute osmotic demyelination, as in the alcoholic patient described above, the increase in sodium level within the first 24 hours of supplementation should not exceed 8 mmol/L.[12]

In the presented case, the level of sodium was increased too rapidly, despite following the guidelines. On the first day of treatment, the sodium concentration increased by 10 mmol/L, and on the second – by as much as 16 mmol/ L, which is twice as high as the recommended and safe level.

The osmotic demyelination syndrome is considered to be an iatrogenic condition, caused by a too rapid administration of sodium in treatment of hyponatremia.[13] Chronic hyponatremia often occurs in patients

with alcoholism, especially in those abusing beer. This syndrome is called beer potomania.[14] It is described as the excessive intake of alcohol, particularly beer, together with poor dietary solute intake that leads to fatigue, dizziness, and muscular weakness.[15] In a literature review by Sanghvi et al., 18% of patients with beer potomania developed osmotic demyelination syndrome.[16] Beer potomania patients have a long-term history of beer intake, as well as a poor diet. Beer has trace amounts of sodium and almost negligible protein content. In addition, beer has calories that prevent muscular proteolysis, resulting in a dramatic decrease in urea generation. Thus, these patients have a very low osmolar load since dietary protein breakdown is the main component of osmolar load, in addition to small amounts from sodium and potassium. The presence of inadequate solute in the kidneys eventually causes dilutional hyponatremia, secondary to reduced clearance of excess fluid from the body.[17-19]

The described patient denied alcohol dependence. However, based on the information provided by the close family, she had been abusing alcohol, mostly beer, for several months. Therefore, the patient had supposedly had hyponatremia for a long time, which led to symptoms observed by the family such as drowsiness, irritability, muscle twitching, nausea and vomiting.

Alcoholism is a predisposing factor for osmotic demyelination syndrome not only because of co-existing low sodium levels. Feng et al. described the case of a patient who had been abusing alcohol for two months. She developed neuropsychiatric symptoms including drowsiness, tremors, as well as visual and auditory hallucinations. Blood tests showed no abnormality in electrolyte levels. An MRI scan showed changes characteristic of the osmotic demyelination syndrome. Treatment involved supplementation with vitamins B1 and B12, which resulted in significant clinical improvement.[20]

After the onset of osmotic demyelination symptoms, vitamins B1 and B12 were administered in an appropriate dosage. After three months of vitamin supplementation and rehabilitation, the patient's condition improved and the abnormalities in magnetic resonance imaging decreased.

Conclusion

This article presents a case of chronic hyponatremia in the course of alcoholism. The patient was admitted in a severe condition with extremely low sodium levels. Electrolyte supplementation was performed according to the ERBP guidelines; however, there was a rapid increase in sodium serum level leading to the development of symptomatic osmotic demyelinating syndrome. Following several weeks of rehabilitation and supplementation of B vitamins, the patient's condition gradually improved.

Sodium deficiency should be equilibrated very carefully, especially in patients with chronic hyponatremia in the course of alcoholism. It should be noted that even a small dose of sodium administered in accordance with the guidelines in chronic hyponatremia may cause a rapid increase in sodium level leading to osmotic demyelination syndrome.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.4.16

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 378–380

CASUISTIC PAPER

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Granular cell tumor of the neurohypophysis – a case report

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ABSTRACT

Introduction. The granulomatous tumor (GCT) is formed from the posterior pituitary (neurohypophysis) or its pedicle. The location of such a tumor in the region of the Turkish or supra saddle is a very rare matter.

Aim. To present a case report.

Description of the case. This article describes the case of a 39-year-old man admitted to the Department of Neurosurgery with a MR-diagnosed head tumor in the suprasellar area growing out of the pituitary funnel. The tumor appeared to be an epileptic fit.

Conclusion. Herein we described a clinical case of granulomatous tumor. GCTs in the pituitary nerves are benign tumors, which makes treatment dependent on the individual case. After the operation, the H-P study showed a granulomatous tumor (GCT) of the posterior pituitary gland.

Keywords. granulomatous tumor, pituitary gland, MRI, Turkish saddle

Introduction

Pituitary tumors are a frequent abnormality, often noticed accidentally in routine imaging using magnetic resonance. These tumors are largely mild, but they can cause clinical symptoms related to the effect of mass, opacity of the visual junction, excessive hormone secretion or pituitary insufficiency.[1] Tumors of the Turkish saddle and its surroundings are a very heterogeneous group of changes of various origins. The endocrine symptoms, which are often the first and longest manifestation of the disease, are specific for this location. The neurological symptoms are dominated by visual and ocular disorders, therefore patients usually go to an ophthalmologist. Due to slow growth, the symptoms are small or underestimated, and at the time of diagnosis the tumor is already large.[2] Therefore, the recognition and treatment of these tumors requires a coordinated interaction between a neurosurgeon, endocrinologist, ophthalmologist, neurologist.[1] Pituitary tumors are classified in four ways: on the basis of size, endocrine

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 13.06.2019 | Accepted: 8.09.2019
Publication date: December 2019

Dziedzic R, Leksa N, Aebisher D, Bartusik-Aebisher D. *Granular cell tumor of the neurohypophysis – a case report.* Eur J Clin Exp Med. 2019;17(4):378–380. doi: 10.15584/ejcem.2019.4.16

function, clinical symptoms and histological structure. With regard to size, it is assumed that the microadenoma is a tumor with a diameter of less than 1 cm. If microadenomas cause clinical symptoms, it is usually due to excessive hormone secretion, whereas macroadenomas they usually cause a symptom through the compression of normal secretory or nervous structures. The second axis of classification is hormonal activity or its lack. According to this, tumors are divided into secreting or non-secreting ones. Usually, the first two characteristics are used from the general classification because most of these tumors are histologically mild and clinical symptoms generally reflect the size of the tumor and its endocrine function.[1]

The granulomatous tumor (GCT) is formed from the posterior pituitary (neurohypophysis) or its pedicle.[3] The first described the tumor of Boyce and Beadles in 1893.[4] GCT operated under different names - pituicytoma, infidibuloma or choriostoma because its origin was unknown.[5,6] Currently, after the new classification in 2016, the WHO classification of central nervous system (CNS) included pituicytoma and GCT tumors as separate types of OUN tumors of the Turkish saddle region.[8] GCT is a cancer that is very rare in adults, occurs more often in women, in the 4th or 5th decade of life, and is rarely a symptomatic tumor.[7] It consists of large cells rich in cytoplasm containing eosinophilic PAS-positive granules and centrally located small nucleus. Cancer cells are characterized by the expression of NSE, S-100 proteins, while they are GFAP- and cytokeratin-negative. The mitotic index is very low. 1 The granulomatous tumor of the posterior pituitary gland is defined as a low-grade tumor.[8] In previous studies and reports, there are no definite specific images in radiological studies that can distinguish GCT from other cancers in the Turkish saddle region.

Description of the case

A 39-year-old man admitted to the Department of Neurochirugia with a tumor diagnosed in the MRI of the supra-horn area and an ambiguous change that strengthens after contrast in the left temporal lobe. In the history of alcohol dependence syndrome, congenital glaucoma, facial-cranial trauma. A tumor diagnosed in July 2010 during hospitalization in the Department of Neurology, where he was ill after the first epileptic seizure. He did not consent to surgical treatment at that time. In February 2011, another epileptic seizure. Control magnetic resonance imaging of the head without differences compared to the previous one. He consented to surgery. On the day of admission to the Department of Neurosurgery in neurological examination the patient: conscious, in logical contact, meets the right eye blindness (residual state after congenital glaucoma), field of vision and eye bottom in the norm, without limb paresis, walking alone. The profile of hormonal tests in the field of norms.

On February 14, 2011 the patient was operated on: the right-sided pseudorabic cordiotomy was subtotalously removed from the suprasellar area. The tumor had a purse, it grew from the pituitary funnel, did not grow into the Turkish saddle. The tumor fragments joined together with the internal carotid artery were left. There was no association with the change depicted in the magnetic resonance imaging seen in the left temporal lobe. After the surgery, the patient woke up properly, and in the neurological examination it was found as before the procedure. In the postoperative course, symptoms of diabetes insipidus and hypothyroidism. In histopathological diagnosis (preparations contain fragments of tumor formation; whose microscopic image indicates granular cell tumors of the neurohypophysis (GI WHO). Endocrine deficiency supplements were included after endocrine consultation. Written home in good general condition with control recommendations in Neurochirugal, Endocrine and Outpatient Clinic ophthalmology.

Discussion

Although the GCT tumor is rarely described in the medical literature, it can occur quite often undiagnosed. The 1999 work of Tomita and Gates, based on a post-mortem examination of the pituitary, revealed in 100% of cases in 9% of cases of a small GCT tumor, without clinical symptoms during life.[9] Therefore, they are rarely detected in the general population if they do not cause clinical symptoms which results in tumor removal and confirmation in the H-P study of the final diagnosis.[9,10] These data suggest slow tumor growth and the emergence of clinical symptoms only at the final stage of growth.[11] The GCT neurohypofisis tumor is derived from pithycytes which are neurohypofisis and pituitary gliocyte transformed gliocytes. Expression of TTF-1 (thyroid transcription factor-1) in normal pituicytes, pituicytoma and GCT neurohypofisis suggests a common piciicytic line.[12] The best way to distinguish between the H-P pituicytoma and GCT is the lack of Rosenthal filaments and eosinophils in the case of pituicytoma.[9] The change in GCT in CT is isodense or slightly hyper-insoluble compared to gray matter before contrast administration, contrast absorption is typical, change is usually homogeneous, calcification is extremely rare. The MR image is isointense in T1 and hypointense T2, the contrast may be uniform or heterogeneous with moderate intensity.[4,5,10] In our case, the patient started radiological diagnostics of the head after the first epileptic seizure. In the MR study of the head with contrast, a 3x2.5 cm tumor with the pressure of the third brain chamber and the optic nerve intersection was strongly amplified after contrast, in the left temporal lobe not associated with the tumor of the Turkish saddle, the change was

also strengthened after 2 x 1 cm contrast. After surgical treatment and H-P score, a granulomatous tumor (GCT) emerged from the pituitary fungus. The most common symptoms of GCT neurohypophysis are visual disturbances, poluria and polysempsia, headaches and dizziness.[8] Epileptic seizure in the case of tumors near the Turkish saddle occurs in a small number of cases. Kawasaki's work shows in 4% of tumors of the pituitary seizure.[7] No data in the literature on the coexistence of tumor GCT neurohypophisis. The operation remains the treatment of choice in the case of a tumor that appears clinically or shows progression in subsequent radiological examinations, the tumor remaining in the observation.[1-10] Due to the very good blood supply to the tumor, access from craniotomy is more preferred than transcranial access.[1-18] In the case of our patient, tumor removal by craniotomy was justified taking into account tumor size and clinical symptoms.

Conclusion

The occurrence of symptomatic granulomatous tumors (GCT) of the posterior pituitary gland is very rare. Despite the fact that tumor histology is well known, histogenesis and nomenclature remains to be more precisely specified, as is the uniqueness of traits in radiological studies allowing precise determination of tumor type in the case of demonstrating a change in the area of the Turkish saddle to accurately plan the surgical procedure. Surgery remains the treatment of choice in the case of a tumor manifesting clinically or showing progression in subsequent radiological studies of the tumor remaining in the observation.

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ISSN 2544-1361 (online); ISSN 2544-2406

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European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (4): 381–384

Instructions for Authors

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1. The Institute of Physiotherapy, University of Rzeszow, Poland
2. Centre for Innovative Research in Medical and Natural Sciences', Medical Faculty of University of Rzeszow, Poland

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