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LETTER TO THE EDITOR

Viroj Wiwanitkit (ADFG)

Stem cell therapy for renal failure: present considerations

Adjunct professor, Joseph Ayobabalola Univeristy, Ikeji-Arakej, Nigeria

Dear Editor, renal failure is an important problem in clinical nephrology as it is usually difficult to manage patients with chronic renal failure. At present, effective ways to manage a patient are limited. Dialysis is widely performed, but this is a long term process. Also, it cannot return a patient to a normal physiological status. Renal transplantation might be the best way to successfully manage the patient with chronic renal failure and end stage renal disease although usually there is a lack of donated organs to allow for transplantation. There have been many attempts to develop new alternative management for the patients.

Stem cell therapy is a new approach in clinical medicine. Stem cell therapy makes use of stem cells for treatment of many diseases. As a new approach, it is still under investigation in several clinical trials in medical centers around the world. Focusing on renal failure, there are some reports on using stem cell therapy for management of the patients. Since mesenchymal stem cells possess the ability to differentiate into tissues of mesodermal lineages, it is widely mentioned for its usefulness in the management of renal ischemia.[1,2] Further applications to cases of chronic renal failure and end stage kidney disease are also proposed. Nevertheless, there is still no supportive evidence from clinical trials in human subjects regarding the usefulness and safety of stem cell therapy for management of renal failure.[3] A recent case report from India details interesting new evidence. This is a case report concerning a patient receiving stem cell therapy for neurological problems. In this case, the patient also has chronic renal failure. After treatment with stem cells, the renal function of the patient improved.[4] There is also another case report from China on using combined renal transplantation and stem cell therapy for management of the patients with chronic renal failure. An improvement could be observed in this case, however, it cannot confirm that the improvement is due to transplantation or stem cell therapy.[5] Yun and Lee noted that the actual effects of stem cell therapy on survival rate as well as recovery of destroyed renal tissues are still inconclusive.[5] However, Swaminathan et al. found that stem cell therapy could help decrease the time required to return to normal renal function in cases of post cardiac surgery acute renal injury.[6] The rat model study on this specific issue is still interesting basic research.[7] At present, there are many ongoing registered clinical trials on stem cell therapy for management of renal failure (such as those described in clincialtrial.gov). It is expected that the results from these trials, after completeness in the near future, will be useful and result in a great step forward in development of stem cell therapy for renal failure.

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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

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While thr effectiveness of stem cell therapy is still questionable, an important consideration is raised regarding ethics in using stem cell therapy, a novel treatment without confirmation at present, for management of the patient.[3] In fact, there are some reports from around the world regarding the danger of using stem cell therapy for patients with chronic renal failure. Sometimes, adverse effect can be seen and in the worst case fatality. At present, illegal and unethical attempts to use stem cell therapy can be found in several countries around the world. The news of the death of a renal failure patient after receiving uncontrolled stem cell therapy in Thailand is the best example (https://www.bbc.co.uk/news/10339138).

Conclusively, stem cell therapy is a promising method and has potential for use in the treatment of renal failure. Nevertheless, the risk associated with applied stem cell therapy is still too high to utilize this method for clinical practice at present. It requires further accumulated evidence from ongoing studies and further medical development to establish proper standard guidelines for using stem cell therapy for renal failure.

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ORIGINAL PAPER

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Dynamics of changes in the level of IgA in patients with bronchial asthma against the background of excessive body weight or obesity

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ABSTRACT

Introduction. Among patients with asthma, a lot of attention is being given to, at the present time, to such comorbidity as excessive body mass (EBM) or obesity.

Aim. To evaluate the level of IgA in patients with bronchial asthma against the background of excessive body weight or obesity and to evaluate the effects of drug on the bacterial lysate and inosine pranobex.

Material and methods. According to the design, the study was conducted in two stages: the first stage – examination of 105 patients with asthma. 105 patients with a basic diagnosis of asthma were examined whose average age was 41.19 ± 1.05 years, 75 patients were found to have EBM or obesity (BMI 31.67 ± 0.53) who were included in the main group and 30 patients with NBMI (BMI 22.13 ± 0.32), which were the comparison group.

Results. The patients in the main group with a severe course had significantly lower serum IgA values than the patients in the comparison group ($p<0.05$), but the statistically significant difference between this index in the patients with a severe course in the main group and the control group was not revealed. The patients in the main group had a significant increase in the level of secret IgA against the background of the use of treatment-and-prophylactic complex (TPC) with the inclusion of a preparation of bacterial lysate in combination with inosine pranobex against the background of training in asthma school, receiving the basic treatment ($p<0.05$).

Conclusion. Patients with asthma who have large BMI have a more severe course of bronchial asthma. A Correlation relationship was established in the group of patients with bronchial asthma and with excessive body weight or obesity between the level of sIgA and the severity of the asthma course; there is a direct strong correlation. Patients in the main group had a significant increase in the level of sIgA against the background of the use of TPC with the addition of a basic treatment by the preparation of bacterial lysate together with inosine pranobex.

Keywords. bronchial asthma, excessive body weight, obesity

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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

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Introduction

Advanced studies of the 21st century have achieved significant success in understanding and treating bronchial asthma (BA). A deeper understanding of the mechanisms of the disease that was made possible by fundamental scientific investigations over the past twenty years has led to the development of highly specific methods of treatment. At the same time, publication of the results of clinical studies opened our eyes to the diversity of etiological, and pathogenetic mechanisms of development and the course of asthma. Due to the more advanced views on the pathophysiological mechanisms of asthma, the mechanisms that are not limited to allergic reactions and immune reactions of type II began to be studied.[1]

In the modern world, both the physician and the patient often need to solve the issues of comorbidity of pathologies. Among patients with asthma, a lot of attention is being given to such comorbidity as excessive body mass (EBM) or obesity.[2]

The study on adult obesity trends using NHANES I (1971-1975), II (1976-1980) and III (1988-1994) showed that BMI increased everywhere in adults with and without asthma; however, the prevalence of obesity was higher in the group of patients with asthma (21.3-32.8%) compared with that without asthma (14.6-22.8%).[3] A retrospective study involving 143 adult patients revealed a similar relationship between the prevalence of EBM or obesity and BA.[4] It has been shown that patients with asthma who had a comorbidity of EBM or obesity have a lower response to baseline therapy and have a more uncontrolled course of the disease and, therefore, this cohort of patients has a nearly fivefold risk of hospitalization due to exacerbations of the disease.[5]

Therefore, based on the data above, it is necessary to search for new diagnostic and therapeutic measures in patients with asthma against the background of EBM or obesity.

Among immunological parameters, IgE is the most studied in patients with asthma. However, our attention in this study was given to another immunoglobulin, namely serum and secretion IgA, which is less investigated, but in our opinion, has a significant effect on the course of asthma.

It is common knowledge that IgA is a gamma globulin fraction, synthesized mainly in the plasma mucosal cells in response to local antigen effects. The main function of the serum IgA is the protection of the respiratory, urinary tract and gastrointestinal tract from infection.[6]

Secret IgA (sIgA) has a dimeric structure and is resistant to enzymes due to its structural features. This immunoglobulin lives only for 5 days. Therefore, for its constant replenishment in the body, differentiation of B-lymphocytes into plasma cells occurs daily and plasma cells synthesize sIgA. Recent studies have suggested the possible role of the epithelial cells in an antigenic presentation. Dendritic cells in the airways epithelium can directly provide anti-B cells and thus stimulate them to differentiate with cytokines synthesized by the epithelial cells, into plasma and prior to the synthesis of sIgA.[7]

Thus, sIgA is responsible for local defense, and its regulatory role in combination with local synthesis, transport and secretion is distinguished by the immunity of mucous membranes from systemic immunity.[8] This immunoglobulin is not able to bind the complement or cause its activation. However, it fulfils various protective functions by interacting with different receptors of the immune system, which protects the mucous surfaces of the body from the penetration of microorganisms into tissues. sIgA can bind toxins and, together with lysozyme, exhibits bactericidal and antiviral activity. It acts as an agglutinator of microorganisms and a toxin neutralizer, inhibiting the binding of viruses and bacteria to the surface of the mucous membranes, thereby suppressing replication.[9,10]

In the domestic literature, at present, there are no researches on the correlation of IgA and BA, but in foreign sources of these studies are already available. Woo-Jin Kim and colleagues conducted a series of studies in adult asthma patients regarding IgA correlation and showed that IgA levels may have an association with age, gender, bronchial hyperresponsiveness and serum IgG levels.[11,12]

A characteristic feature of modern infectious pathology is the growth of chronic infectious and inflammatory diseases.[13,14]

Considerable experience has been accumulated in the use of drugs of bacterial origin with immunotonic properties for several decades, in chronic infectious pathology of various organs and systems, and in allergic diseases. A typical moment in the appointment of immune drugs is to include them in complex therapy along with anti-inflammatory drugs, which greatly increases the effectiveness of treatment and compliance of the patient with the doctor.[15]

Currently, the drug Broncho-munal from the firm Lek in Slovenia is popular among patients and doctors. The drug affects different parts of the immune response. [16,17]

Among immunomodulators with antiviral activity of interest is the drug Inosine pranobex, namely the drug Novirin, of Kiev vitamin plant, PAS, Kiev, Ukraine. Clinical studies have shown that it is well tolerated (practically non-immunogenic), which is probably due to the similarity ("affinity") of its compounds to substances found in the body [18,19]

Aim

To evaluate the level of IgA in patients with bronchial asthma against the background of excessive body weight or obesity and to evaluate the effects of bacterial lysate and inosine pranobex.

Material and methods

According to the design, the study was conducted in two stages. The first stage was examination of 105 patients with asthma based on the Department of Family Medicine and General Practice of Odessa National Medical University and the formation of the main cohort group according to inclusion/exclusion criteria "Patients with BA against the background of EBM or obesity", which included 75 patients, and a comparison group according to the inclusion/exclusion criteria "Patients with BA against the background of normal body mass index (NBMI)", which included 30 patients. At the 2nd stage of the study, a treatment-and-prophylactic complex (TPC) was administered to the main group of the main cohort group of the 1st stage (the main group - 30 patients and the comparison group - 30 patients, control group - 15 patients) to complete the clinical trial, namely the use of the preparations of bacterial lysate, inosine pranobex. The average course of treatment was 4 weeks.

Body mass was measured on the OMRON BF 51 scales impedance meter, measured in light clothing, on an empty stomach, measuring accuracy was 0.01 kg.

Body mass index was calculated by the formula: BMI = m/p^2, where BMI is the body mass index (kg/m^2); m - body weight (kg); p - height, elevated in square (m^2).

Studies of IgA levels were determined by flow cytometry, and the patient's blood was taken in the morning on an empty stomach. Reference values: IgA-0.70-4.00 g/l. The level of sIgA was determined by the method of immunoassay analysis. The material for the study was the patient's saliva. The reference values of sIgA are 40-170 μg/mL determined by flow cytometry on Cobas 6000, Roche Diagnostics (Switzerland).

The treatment and prophylaxis complex included in addition to the basic treatment includes bacterial lysate 7.5 mg daily for 28-30 days in combination with inosine pranobex at a dose of 1000 mg three times a day for 3-4 weeks.

A statistical analysis was carried out according to generally accepted methods of variation statistics. Validity was evaluated by Student's t test. Differences were recognized as essential at the significance level of $p \leq 0.05$. The correlation relationship was established using Spearman correlation criterion and Pearson correlation-regression analysis.

All patients signed a voluntary informative consent to participate in the investigation at the beginning of the study.

Results

105 patients with a basic diagnosis of asthma were examined, whose average age was 41.19 ± 1.05 years, of which there were 72 women and 33 men. According to the anthropometric study, 75 patients were found to have EBM or obesity (BMI 31.67 ± 0.53) who were included in the main group and 30 patients with NBMI (BMI 22.13 ± 0.32), which were the comparison group.

In the study, it was found that in the main group, patients with severe 25 (33.33%) and moderate 35 (46.67%) degrees of severity of asthma and mild 15 (20%), predominated, while patients in the comparison group had a milder degree of the course, and only 10 (33.33%) of patients within 30 had a moderate severity of asthma, and 20 (66.67%) patients had a mild BP ($p>0.05$). In the first and second stages of the study, all patients with severe asthma did not control the disease. The patients did not receive systemic steroids.

Patients with EBM or obesity had significantly lower control, namely on an average ACT-test with 12.73 ± 0.31 points. In the distribution of patients according to the severity of the course, we received the following data: with a mild degree of severity of the course 17.65 ± 0.23 points, with a moderate - 13.05 ± 0.17, with a severe - 10.9 ± 0.25 points. Patients with EBM had an average of 17.10 ± 0.34 points (15.33 ± 0.24 points in patients with moderate severity and 19.78 ± 0.37 in patients with mild) ($p \leq 0.001$). It is noted that the AST test correlates with the severity of the course in both groups according to Pierce r = 0.98 and r = 0.98, respectively.

In the study of serum IgA levels, it was noted that the values did not go beyond the reference values, more detailed data is presented in Table 1.

Table 1. Serum IgA level in the examined patients according to the severity of bronchial asthma

Group	Severity of the course	Level of serum IgA g/l
Main	Mild	2.54 ± 0.16**
	Moderate	2.36 ± 0.15
	Severe	1.87 ± 0.15*
Comparison	Mild	2.35 ± 0.16**
	Moderate	2.72 ± 0.48
Control	-	2.00 ± 0.20

Note:*$p_{m\text{-}comparison}<0.05$
**$p_{m\text{-}control}<0.05$
^$p_{comparison\text{-}control}<0.05$

The table above shows that the patients in the main group with a severe course had significantly lower serum IgA values than patients in the comparison group ($p < 0.05$), but the statistically significant difference between this index in the patients with a severe course in the main group and the control group was not revealed.

When calculating the mean values for sIgA in the examined patients, it was found that the mean value in the main group had a large standard deviation, so we conducted an analysis of sIgA for the patients in the main group separately according to the severity of BA (Fig. 1).

Figure 1 shows data indicating that patients with a mild course had a level of sIgA close to the control

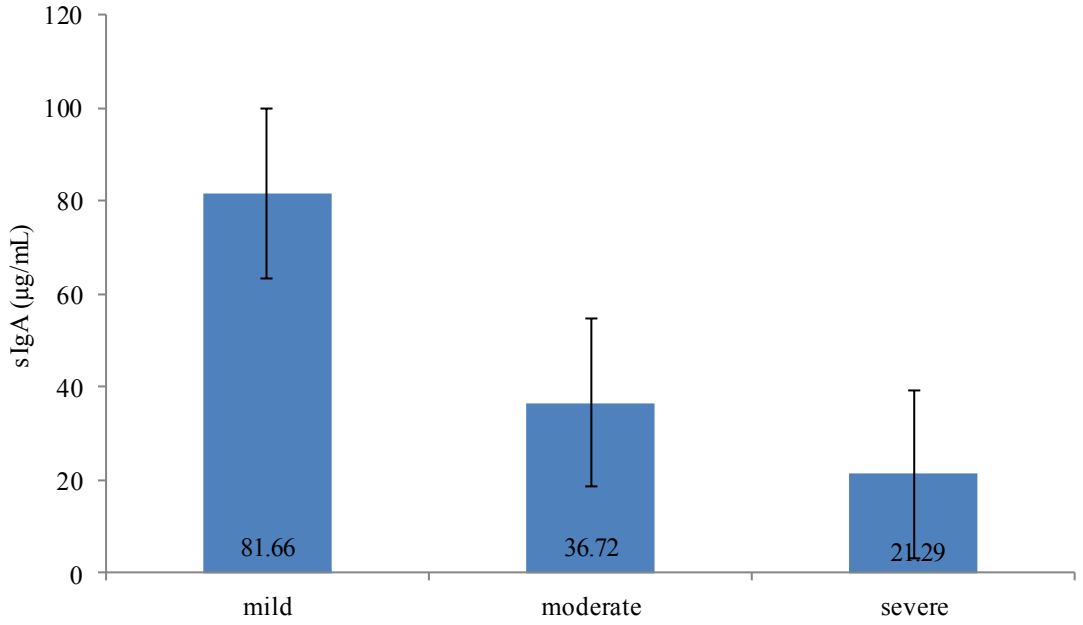

Fig. 1. The level of sIgA in the patients in the main group according to the severity of the course of bronchial asthma

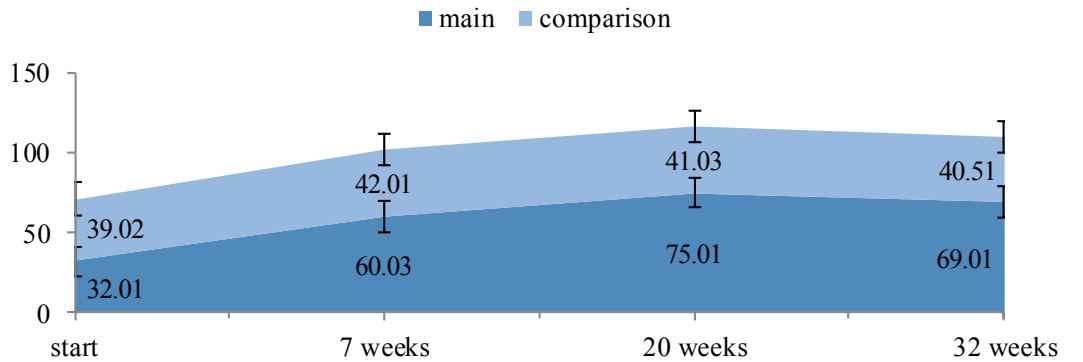

Fig. 2. The level of sIgA in the patients with bronchial asthma in the context of EBM or obesity in the use of the treatment and prevention complex

group, namely 81.66 ± 0.06 µg/mL, in the patients with an moderate severity – 36.72 ± 3.92 µg/mL, and in the patients with a severe course – 21.29 ± 0.01 µg/mL ($p<0.001$; $p<0.001$; $p < 0.001$, respectively). There was established a feedback close correlation relationship between the level of sIgA and the severity of the asthma course under by Spearman $\rho = -1.0$, according to Pearson $r = -0.96$. While analyzing sIgA in the control and comparison group, it was found that in the comparison group, this figure was lower, namely 52.66 ± 1.68 µg/mL versus 83.68 ± 3.68 µg/mL in the control group ($p<0.001$).

Therefore, it is established that the greater BMI, the lower is the level of sIgA, which can be explained by more frequent bacterial and viral infections in this cohort of patients, which further aggravates the course of asthma.

After examination, the second stage included patients from the main group of the first stage, that is, the patients with asthma against the background of EBM or obesity, which were given the proposed treatment.

The level of sIgA for the catamnesis period varied as follows (Fig. 2)

In Figure 2 it is evident that the patients in the main group had a significant increase in the level of sIgA against the background of the use of TPC with the inclusion of a preparation of bacterial lysate in combination with inosine pranobex against the background of training in Asthma school, receiving the basic treatment ($p < 0.05$).

In order to confirm the improvement of asthma control, all patients passed the ACT test before the start of the study and during the follow-up. Average figures in the groups are shown in Fig. 3.

Figure 3 shows that patients in the main group had a significant increase in asthma control, namely 17.98 ± 0.44 versus 12.77 ± 0.46 prior to the use of TPC

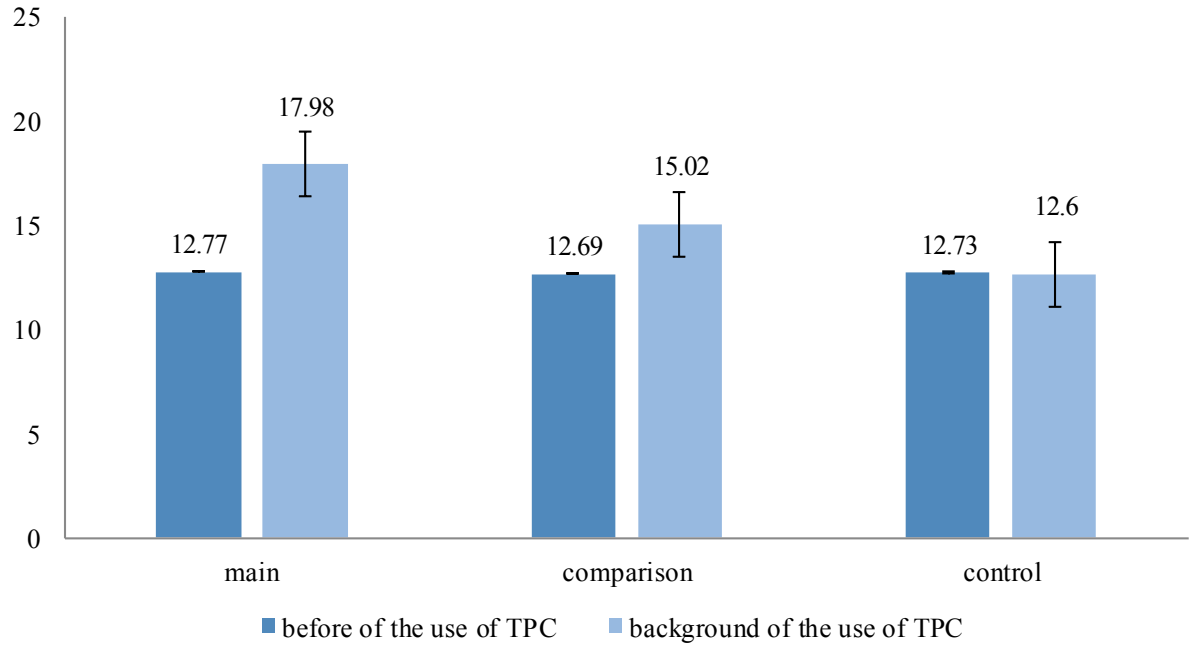

Fig. 3. Average values of the AST test in the patients with bronchial asthma in the presence of excessive body weight or obesity in the process of application of the treatment-and-prophylactic complex

($p < 0.001$). In the comparison group the patients who had undergone training at Asthma School also had improvements in asthma control, namely 15.02 ± 0.39 points versus 12.69 ± 0.56 points prior to training ($p < 0.01$). The patients in the control group who refused TPC and education at Asthma School did not have a statistically significant difference in the follow-up period, namely the pretest study 12.73 ± 0.74 versus 12.60 ± 0.79 points in 32 weeks ($p > 0.05$).

It should be noted that the data in the table corresponds to 20 weeks of the follow-up. During a re-examination on the 32nd weeks of the follow-up, the regression of spirographic parameters was detected in 17% of patients in the main group and in the comparison group.

As a result of the evaluation of the effectiveness of the developed TPC with the inclusion of pharmacological correction against the background of training at Asthma school the patients with asthma with EBM or obesity, it was found that the main group that used the above-mentioned TPC had the best results in improving Asthma control. The positive dynamics of increasing asthma control (RR = 0.38; RRR = 0.62; NNT = 2.12) is more reliable ($p < 0.05$) than in comparison groups (RR = 0.89, RRR = 0.11; NNT = 4.05) in patients with asthma against the background of EBM or obesity, which in turn affected the course of asthma by improving the compliance between the physician and the patient.

Discussion

The patients in the main group with severe asthma have a decreased level of sIgA ($p < 0.001$), which may be a criterion for the more severe course of bronchial asthma in patients with excessive body weight or obesity in the event that asthma control has not been achieved. Together with this, patients in the main group have a direct close correlation between the severity of the course and the level of sIgA ($r = 0.96$).

In analyzing similar studies, we did not find similar works. Woo-Jin Kim et al. in his study of the relationship between serum IgA level and allergy/asthma found a reduced sIgA level in only 12.2% of patients. However, it was not indicated how much body weight this patient population had.[11]

In another study, A. Gonzalez-Quintela et al. showed that obese patients had higher serum IgA levels than normal-weight people ($p = 0.006$) or overweight ($p = 0.005$).[20]

In 2018, Susanna Esposito demonstrated an increase in the production of IgA secretions of the nasopharynx and saliva when prescribing bacterial lysate to children with recurrent respiratory infections, wheezing and asthma.[21]

Bulgakova and co-workers also found that when inosine pranobex was used in children with asthma, humoral immunity returned to normal; while there was an increase in the concentration of IgG, IgA and IgM.[20]

In our study, the patients in the main group had a significant increase in the level of sIgA against the background of the use of TPC with the inclusion of a preparation of bacterial lysate in combination with inosine pranobex against the background of training in Asthma school, receiving the basic treatment ($p < 0.05$).

The data presented in this paper are intermediate and need further investigation.

Conclusion

Bronchial asthma is a widespread disease that often has confounding comorbidity as excessive body weight or obesity. Moreover, patients with asthma who have large BMI have a more severe course of bronchial asthma.

Correlation relationship was established in the group of patients with bronchial asthma and excessive body weight or obesity between the level of sIgA and the severity of the BA course; there is a direct strong Spearmen $\rho = 1.0$, and Pierce $r = 0.96$. Furthermore, patients in the main group had a significant increase in the level of sIgA against the background of the use of TPC with the addition of a basic treatment by the preparation of bacterial lysate together with inosine pranobex. The main group that used the above-mentioned TPC had the best results in improving asthma control.

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ORIGINAL PAPER

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Association of fat patterning, type 2 diabetes mellitus and MTHFR gene polymorphism: a study among the two ethnic groups of Tripura, North-East India

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ABSTRACT

Introduction. Type 2 Diabetes Mellitus (T2DM) is a group of metabolic disorders resulting from insufficient action of insulin. The etiology of T2DM is multi-factorial that includes genetic factors, obesity and lifestyles. Recent reviews of overall and stratified meta-analyses demonstrated the association between *MTHFR* polymorphism (C677T) including fat distribution and risk of T2DM. Publications of Indian context regarding fat patterning and MTFHR genetic polymorphism of the North East Indian population are insufficient and scant among the ethnic population of Tripura.

Aim. In this backdrop, the present study is the first attempt to understand the relationship of fat patterning, *MTHFR* gene polymorphism and T2DM among two Tibeto-Burman speaker endogamous ethnic populations (Chakmas-the migrant group and Tripuris – the aboriginal group) of Tripura, North East India.

Material and methods. The present study consists of age matched 280 males (Chakmas 147 and the Tripuris 133) from Tripura. Anthropometric and metabolic (Fasting Blood Glucose) variables and to discern obesity, blood glucose level and genotyping of MTHFR was performed following standard techniques.

Results. The result revealed significant ($p<0.05$) association of obesity, TT genotypes and fasting blood glucose among the Chakmas with in comparison to the Tripuris.

Conclusion. In this first attempt from North East India on the aspects of association of fat Patterning, Type 2 Diabetes Mellitus and MTHFR gene polymorphism suggests that the Chakmas are more diabetic, and this might be due to the concomitant effects of T alleles and higher central obesity and Percent Body Fat (PBF). More population screening from other under-represented indigenous populations of North East India is needed for prevention of metabolic disorders.

Keywords. fat patterning, MTHFR, obesity, T2DM

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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

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Introduction

Type 2 Diabetes Mellitus (T2DM) is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from insufficient action of insulin. In contemporary time, the prevalence of this disease is increasing steadily all over the world. T2DM is known to be a complex and heterogeneous disease resulting from a set of interacting factors that can be genetic or environmental and also behavioral. Patients living with T2DM are often at risk of facing both short-term as well as long-term complications and can often lead to premature death because nowadays this disorder is the ninth major cause of death. Factors like high blood glucose level and obesity affects T2DM patients more than a non-diabetic person.

The etiology of diabetes in India is multi-factorial and includes genetic factors coupled with environmental influences such as obesity associated with rising living standards, steady urban migration, and lifestyle changes.[1] The majority of genetic variations associated with Type 2 Diabetes Mellitus are thought to act by subtly changing the amount, timing, and location of gene activity. Genetic variations likely act together with health and lifestyle factors to influence an individual's overall risk of type 2 diabetes.[2] Recent research such as candidate gene approach and Genome Wide Association Studies (GWAS) in the field of type 2 diabetes genetics had until recently succeeded in identifying few genuine disease-susceptibility loci.[3,4] Ongoing studies focusing on the role of copy number variation and targeting low frequency polymorphisms should identify additional T2DM-susceptibility loci. Increased genetic activity in genes like MethyleneteTraHydroFolateReductase (MTHFR) can result in problems like high glucose level or insulin secreting pathway which leads to the physical condition known as T2DM.[5]

Furthermore, epidemiological studies found that general adiposity assessed by Body Mass Index (BMI) is a powerful predictor of type 2 diabetes, which has a strong relationship to diabetes and insulin resistance.[6] Subsequently, regional obesity (abdominal obesity) as measured by high waist circumference has been proposed as a better predictor of risk of T2DM development.[7,8] However, fat patterning associated with genetic polymorphism and T2DM seems to be a contemporary approach for this disease condition were reported in some works.[9-11] In obese or overweight people, the level of pro-inflammatory markers, and other substances that are involved in the development of insulin resistance, is increased. Fat distribution in terms of abdominal obesity may cause fat cells to release pro-inflammatory chemicals and these chemicals can make the body less sensitive to the insulin it produces by disrupting the function of insulin responsive cells and their ability to respond to insulin.[12]

Recent review of overall and stratified meta-analyses of the association between MTHFR polymorphism C677T and risk of type 2 diabetes mellitus, delineates a significant effect of C allele (CC genotype) compared to CT and TT genotypes.[13] More than 15 different genes were investigated for their possible influence on plasma homocysteine levels of which MethyleneteTraHydroFolateReductase (MTHFR) was one of the most studied.[14] MTHFR (677TT mutation) is associated with type 2 diabetes in and rs1801133 Single Nucleotide Polymorphism(s) (SNPs) has been found among the Asian population.[15] A study also reported that the TT genotype of MTHFR C677T contributes to susceptibility to T2DM and supports the hypothesis that elevated Homocysteine is causally related to increased risk of T2DM.[16] Indian studies reported variable result as no association with MTHFR C677T gene polymorphism and T2DM in South Indian Population and North East India has found the MTHFR gene polymorphic in both case and control for hyperhomocysteinemia.[17,18]

India, occupying the center-stage of Palaeolithic and Neolithic migrations, found to be somewhat under-represented in genome-wide studies of variation.[19] Being at the cross-roads of migration, Indian populations have undergone complex and ancient admixture events over a long period of time and have been the melting-pot of disparate ancestries originating from different parts of Eurasia and South-East Asia.[20-23] Although the date of entry of modern humans into India remains uncertain but it is reasonable to consider that by the middle Paleolithic period (50,000–20,000 years before present [ybp]), humans appear to have spread onto many parts of India.[23] Contemporary ethnic India is a land of enormous genetic, cultural, and linguistic diversity.[24,25] A more recent study exploring Indian genomic diversity demonstrated four major ancestral genetic components in mainland India that included four dominant ancestries in populations from mainland India: Ancestral North-Indian (ANI), Ancestral South-Indian (ASI), Ancestral Tibeto–Burman (ATB) and Ancestral Austro-Asiatic (AAA).[26]

Aim

The literature reviews on fat distribution and genetic polymorphisms from North East India reveals scant studies from Tripura.[18] In this context, the present study to best of the knowledge, is the first attempt to discern the relationship of fat patterning, Type 2 Diabetes Mellitus and MTHFR gene polymorphism among two Tibeto-Burman speaker endogamous ethnic populations (Chakmas-the migrant group and Tripuris – the aboriginal group) of Tripura, North East India.

Material and methods

The present study consisted of one hundred forty seven (147) male participants from the migrant Chakma

population from Manu, Longthorai Valley of Tripura and one hundred thirty three (133) male participants from the aboriginal Tripuri population from Agartala, Tripura. Prior to the study verbal and/or written consent from the each participant was obtained. Mouthwash was collected from all the participants in 15 mL centrifuge tubes. Genomic DNA was isolated from the mouthwash following a standard technique.[27] The quantity and quality of DNA was checked by Spectrophotometry and gel electrophoresis. DNA was stored at −20 °C. Genotyping of *MTHFR* (C677T) (rs1801133) was performed using PCR-RFLP with the locus specific primers (Forward Primer was 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ and reverse primer was 5′-AGG ACG GTG CGG TGA GAG TG-3′). PCR product was digested with HinfI enzyme (Biolab) following the manufacturer's protocol. Restriction fragment size analysis was performed by visualization of digested PCR product after separation by 3% Agarose gel electrophoresis. Height, weight, waist circumference, and hip circumference were measured using standard techniques.[28] BMI (Body Mass Index), WHR (Waist-Hip Ratio) and WSR (Waist to Stature Ratio) were calculated using standard formulas.[28] Fasting blood glucose was measured using Accu-Chek blood glucose monitoring following the machine manuals. The classification was done by the standard classification.[28]

Data checked and analyzed by SPSS (windows version 18.0). Allele frequencies for *MTHFR* allele frequency were estimated by MLH (Maximum Likelihood) estimation.[29] Descriptive and Inferential statistics in terms of paired 't' test was done to understand the mean difference between the two ethnic groups. On the other hand, genotype modeling has been done to find out the effects of the *MTHFR* genotypes along with odds ratio. Cut off was set as p = 0.05.

Results

Distribution of anthropometric and metabolic variables (Table 1) of the age matched participants revealed statistically significance (P<0.05) between the Chakmas and Tripuris. Except for the general obesity measure through BMI, Chakmas demonstrated significantly (p<0.05) higher stature, PBF and as well central obesity measured by WC and WHR in comparison to the Tripuris. Furthermore, significantly (P<0.05) higher mean fasting blood glucose level was found among the Chakmas compared to the Tripuris.

Distribution of *MTHFR* gene polymorphism (Table 2) demonstrated significant (p<0.05) difference in the genotypes between the Chakmas and Tripuris due to differential distribution (polymorphism) C and T homozygote and thus reflected on significantly (p<0.05) higher T alleles among the Chakmas in comparison to the Tripuris. The result of fasting blood glucose level (mg/dL) evinced higher prevalence of T2DM among the Chakmas in accordance to ADA (American Diabetic Association, 2016) and showed significantly (p<0.05) higher mean value (131.42±53.65 mg/dL) of fasting blood glucose level compared to that of the Tripuris (108.96±21.83 mg/dL). Analysis of genotype modeling, however, demonstrated TT genotype (TT vs CT + CC) had higher OR (OR=9.57, 95% CI, p<0.05) in comparison to CC genotype (CC vs TT +CT) (OR=0.1038, 95% CI, p<0.05).

Table 1. Anthropometric and metabolic characteristics of the studied population

Variables	Chakma (Mean±SD) N = 147	Tripuri (Mean±SD) N = 133
Age (years)	46.12±8.29	45.09±7.28
Height (cm)	154.05±9.01*	151.32±7.47
Weight (kg)	55.40±11.28	54.31±10.11
BMI	23.02±4.55	23.70±4.34
WC (cm)	86.85±10.32*	78.73±7.98
HC (cm)	94.17±8.38*	89.55±6.26
WHR	0.92±.06*	0.87±.03
PBF	28.82±7.52*	20.71±6.84
Blood glucose (mg/dl)	131.42±53.65*	108.96±21.83

*p<0.05, BMI - Body Mass Index; WC - Waist Circumference; HC - Hip Circumference; WHR - Waist to Hip Ratio; PBF - Percent Body Fat

Table 2. Distribution of *MTHFR* gene polymorphism among the Chakmas and Tripuris

		Genotypes			***Allele frequencies***	
Population	**N**	**CC**	**CT**	**TT**	**C**	**T**
Chakma	147	128 (87.07) 119	9 (6.12) 23.9	10 (6.81) 1.19	0.9013	0.0987
Tripuri	133	121 (90.98)	11 (8.26)	1 (0.752)	0.9511	0.0489

Figures in (parenthesis) denotes the percentage

Discussion

The overall cardinal result of the maiden study on these ethnic groups (Chakmas and Tripuris), one being migrant (The Chakmas) and another being aboriginal (The Tripuris) revealed that the Chakmas were significantly (p<0.05) heavier, obese and had significantly (p<0.05) higher fasting glucose level in comparison to the Tripuis. In accordance to WHO cut off Chakmas males revealed much higher WC (86.85±10.32 cm) and on the Tripuris demonstrated significantly (p<0.05) lesser WC (78.73±7.98 cm), which is within the normal range and

the present study found in corroboration with earlier studies from abroad and India.[7,8,30] The present study also found the association of significantly ($p<0.05$) higher central obesity marked by WC and PBF along with significantly ($p<0.05$) higher TT genotypes and higher fasting blood glucose level (131.42±53.65 mg/dl), which is significantly higher than (IEC 2009, ADA 2010, WHO 2011) among the Chakmas. In contrast the Tripuris had lower fasting blood glucose level (108.96±21.83 mg/dL) associated with lower abdominal obesity, PBF and lower TT genotypes.[31]

Conclusion

The first attempt from North East India with regard to the association of fat distribution, genetic polymorphism of MTHFR gene and T2DM gene polymorphism envisaged that the Chakmas are more diabetic than the Tripuris might be due to the concomitant effects of T alleles and higher central obesity and PBF. More population screening from much under-represented indigenous populations of North East India is needed for understanding and preventing of metabolic disorders.

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ORIGINAL PAPER

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Health behaviors in professionally active nurses – preliminary research

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ABSTRACT

Introduction. Health behaviors are all behaviors related to health. The study was aimed at recognizing the health behaviors of professionally active nurses.

Aim. The aim of the study was to evaluate health behaviors presented by professionally active nurses.

Material and methods. 103 nurses were included in the study. The method of diagnostic survey was used in the paper, and the research tool was the author's questionnaire for socio-demographic data and the Inventory of Health Behaviors.

Results. Nearly 60% of the respondents declared participation in preventive examinations. 70% of nurses put the family first in the hierarchy of life values. In the group of professionally active nurses, the value of health behavior index was 80.52 points.

Conclusion. The greatest value in the life of nurses was the family right after health and work. Nurses declared regular participation in preventive screenings. They presented the average level of health behaviors. The nurses' educational level positively affected the level of their health behavior. Nurses living in the countryside showed a higher level of health behavior. Nurses with chronic condition presented a lower level of health behaviors than their healthy colleagues.

Keywords. health, health behaviors, lifestyle, nurse

Introduction

According to the World Health Organization (WHO), "health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity".[1] The WHO definition points to three dimensions of health, i.e. physical, mental and social drawing attention to its multi-dimensional character.

The concept of health behavior refers to any health-related behavior. In other words, health (health-promoting) behaviors are actions taken by an individual for health reasons or such that have a documented impact on health.[2]

Numerous definitions of health behaviors may be found in the literature. According to A. Titkow, health behaviors are human activities and actions expressed by means of behavioral variables - related to the sphere of health and disease.[3] However, according to Puchalski, health behaviors are actions, behaviors or types of be-

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havior selected by an observer and / or a subject, which under a certain system of knowledge (colloquial beliefs, a given scientific theory or social ideology) remain significant, defined in the adopted option of a relationship with health in the sense assumed in this system.[3]

Health behaviors are those behaviors which, in the light of modern medical knowledge, cause specific - positive or negative - health effects in people who implement them, they are both reactions to all situations related to health, as well as habits and deliberate actions.[3]

The World Health Organization emphasizes the division of health into the following dimensions: physical, mental and social, therefore, health behaviors are to ensure maintenance, improvement and restoration of health in these areas:[4]

- physical health, which consists in ensuring personal hygiene of the body and surroundings, proper nutrition, adequate physical activity - motor and rehabilitation exercises as well as body immunity.
- mental health related to effective coping with stress, building self-confidence and strength, as well as trust and high self-esteem.
- social health means activities related to building relationships between social units, skillful communication, and solving social problems and disputes.[4]

Health-related behaviors are defined as e.g. health behaviors, health-promoting behaviors, anti-health behaviors, healthy lifestyle.

Everyone is responsible for one's health, therefore, they make own conscious choices of behaviors that are beneficial or negative for one's health.[5] The choice of behaviors by a person depends i.e. on gender, age, life goals, and the life situation in which a person finds oneself, it is precisely conditioned by origin, social roles, education, material conditions, and place of residence. These choices also depend on cultural views, family or national traditions.[6]

Aim

The aim of the study was to evaluate health behaviors presented by professionally active nurses.

Material and methods

The research material was obtained by means of diagnostic survey method, survey technique based on the author's questionnaire to collect socio-demographic data and the Health Behavior Inventory according to Juczyński. The questionnaire items concerned the socio-demographic situation, health situation and life values of the respondents. The level of health behaviors was determined based on the Health Behavior Inventory. It consisted of 24 statements about health-related behaviors. The respondents' task was to indicate how often during a year they observe the behaviors listed by choosing a value on the scale, where: 1 meant almost never, 2 rarely, 3 from time to time, 4 often, 5 almost always.[7]

On the basis of the frequencies of behaviors indicated by the respondents, the overall intensity of health-promoting behaviors was determined, as well as the intensity levels of four domains of health behaviors, i.e. proper nutrition habits, preventive behaviors, positive mental attitude, and health practices.

The score of general index of health behaviors was within 24-120 points. The higher the value, the higher the level of health behavior declared.[7] The research was conducted in May 2018 among nurses employed at John Paul II Podkarpackie Provincial Hospital in Krosno. The study was anonymous and voluntary, the respondents were informed about the purpose of the study in the header of the questionnaire. The inclusion criteria for nurses were following: minimum one-year seniority and voluntary participation in study. Exclusion criteria, nurses who did not meet the above requirements.

The subject of the analysis was to present the health behavior of nurses. The surveys were considered to be completed correctly, when answers were provided to all questions asked. All subjects were informed about the purpose of the study and agreed to it. Before starting the research, the respondents were instructed how to complete the questionnaires correctly. 120 questionnaires were distributed, of which 103 correctly completed questionnaires were included in the final analysis.

Results

The survey covered 103 people working as nurses. In the group of nurses included in the study, rural residents dominated (68%), every third respondent (32%) lived in the city. Over ¾ of the respondents were people over 40 years of age.

Considering the professional education of the nurses participating in the study, the largest group had a bachelor's degree (41.8%). Post-secondary education (Registered Nurse - 38.8%) occurred with a slightly lower frequency, and nurses with master's degree were definitely the least numerous group, which in the study group accounted for less than 20%.

40% of nurses had specialization in nursing (various fields), and 60% of the respondents did not have such professional qualifications.

The largest group of respondents declared specialization in conservative nursing (16.5%), followed by emergency nursing (6.8%) and anesthesia and intensive care (6.8%). Such specializations also appeared as surgical nursing (3 persons, i.e. 2.9%) and oncological nursing (2 persons, i.e. 1.9%).

Every tenth person had seniority up to 10 years. Half of the respondents (49.5%) worked from 21 to 30 years, and every fifth worked as a nurse for over 30 years.

Table 1. Characteristics of the study group

Variable	N	%
Place of residence		
city	33	32.0
village	70	68.0
Age in years		
< 30	6	5.8
30-40	17	16,5
41-50	44	42,7
51-60	31	30.1
>60	5	4.9
Education		
Secondary	40	38.8
BSc	43	41.8
MSc	20	19.4
Specialization		
yes	41	40.0
no	62	60.0
Seniority		
> 10 years	11	10.7
10-20	18	17.5
21-30	51	49.5
>30	23	22.3
Participation in preventive screenings		
yes	61	59.6
no	42	40.4
Chronic diseases		
yes	29	28.3%
no	74	71.7%

The surveyed nurses were also asked about participation in preventive screenings. In the light of the results obtained, it is worth noting that over 40% of nurses, i.e. people dealing with health care professionally, did not participate in any preventive screening. Among persons declaring participation in preventive screening, the largest group (30.1%) had mammography. Secondly, cytological tests were indicated (16.5%), and thirdly - colonoscopy (8.7%). Other preventive screenings included gynecological examinations (2 persons, i.e. 1.9%), and in individual cases: tumor markers, glucose control and blood tests.

Every fourth respondent (28.3%) was treated for chronic disease, 71.7% of nurses did not suffer from such diseases. Thyroid diseases (hyperthyroidism or hypothyroidism - 9.7%) were mentioned most often among chronic diseases, followed by hypertension (5.8%). Other chronic diseases mentioned by nurses were diabetes (2 people), and in individual cases: microscopic vasculitis, hyperhomocysteinemia, osteoarthritis and degenerative spinal disc disease.

The respondents were asked to rank their three life values to determine which ones were most important to them. Family was the most frequently mentioned as the most important value (rank I) (68%). Health was mentioned as the second one (29%). In the third place, nurses placed the value of work (3%) (Table 2).

Table 2. The most important values in life

No.	Study	Rank I	Rank II	Rank III
1.	Family	68.0%	29.1%	2.9%
2.	Health	31.0%	58.3%	10.7%
3.	Work	1.0%	12.6%	86.4%

By converting the obtained distribution of responses into point values, where 3 points were awarded for rank I, 2 points for rank II, 1 points for rank III, a hierarchy of three values was created in the lives of the nurses surveyed.

The final score shows the key role of family in the respondents' lives. Health came second and work third (figure 1).

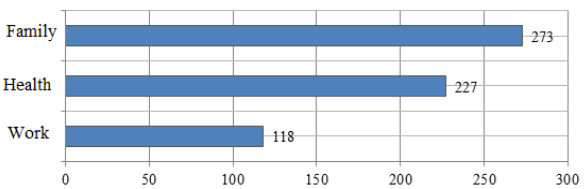

Fig. 1. Life value hierarchy, based on the point score

To gain knowledge about the health behaviors of nurses, study participants were asked to determine how often they followed specific behaviors during a year. A list of twenty-four behaviors was used to which respondents referred using a five-point scale, where 1 meant almost never and 5 meant almost always. The distribution of answers related to all twenty-four issues is presented in the figures below, followed by the calculation of the mean frequency level, to create a hierarchy of the most commonly implemented health behaviors in this professional group.

Based on the frequency of individual health behaviors indicated by the respondents, a general health behavior index was determined, as well as an intensity index in four categories, resulting from the grouping of all twenty-four analyzed health behaviors. Behaviors were assigned to the following broader domains:

a. proper eating habits: questions 1, 5, 9, 13, 17, 21,
b. preventive behavior: questions 2, 6, 10, 14, 18, 22,
c. positive mental attitude: questions 3, 7, 11, 15, 19, 23,
d. health practices: questions 4, 8, 12, 16, 20, 24.

Considering the use of a five-point scale to describe twenty-four behaviors, the value of the general index of health behaviors ranges from 24 to 120 points. In the analyzed group of nurses, the value of this index was 80.52 points. The minimum value of the index obtained

in the research sample amounted to 48 points, while the maximum - 108 points.

Taking into account the division into four domains of health behaviors presented above, it can be seen that the highest intensity of behaviors in the group of nurses is visible in the case of preventive behaviors (21.20 points), while the lowest in the case of health practices (18.08 points). The average intensity of behaviors that make up proper eating habits and a positive mental attitude is similar and approximates 20.60 points (figure 2).

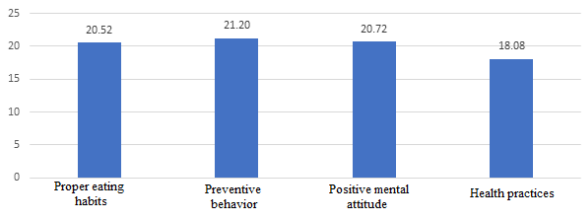

Fig. 2. Severity of nurses' health behaviors broken down into four domains

When analyzing the frequency of health behaviors of nurses, it is worth taking into account their characteristics, such as age, education, place of residence and occurrence of chronic diseases. For each of the variables listed, a calculation and comparison of the value of the general index of health behaviors was made, as well as the value of the index broken down into four domains of health behaviors.

Considering the value of the index of health behaviors of nurses, taking into account their age, it can be stated that it changes stepwise and not linearly. The highest value of the index was recorded for the youngest age category (up to 30 years), and then among people between 41 and 50 years of age and after 60 years of age. Definitely the lowest rate of health behaviors was found in nurses from the 31-40 age group (Figure 3).

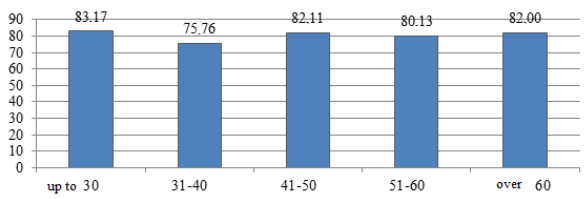

Fig. 3. General index of health behaviors and the age of respondents

The general index of health behaviors was higher in nurses with higher education than in registered nurses, however, in the case of persons with higher education, the value of the index was similar, with a slight advantage in the case of persons with BSc over persons with MSc (Figure 4).

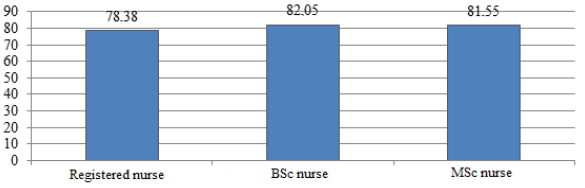

Fig. 4. General index of health behaviors and education of the respondents

When comparing the value of the general index of health behaviors with the place of residence of the respondents, it was found that nurses living in rural areas had a much higher index of health behaviors than nurses living in cities (difference of 4.57 points) (Figure 5).

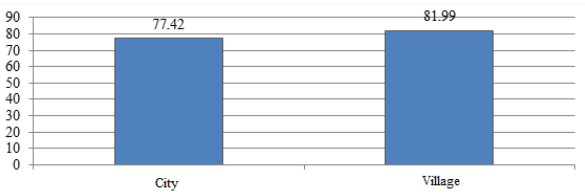

Fig. 5. General index of health behavior and the place of residence of the respondents

Interestingly, it seems that people treated for chronic diseases had a lower rate of health behaviors than those who did not suffer from any chronic disease (Figure 6).

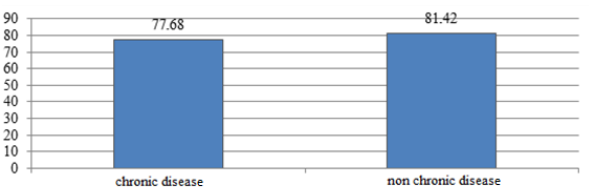

Fig. 6. General index of health behavior and the incidence of chronic disease in the respondents

Summarizing the discussed information on the frequency of health behaviors of the surveyed nurses, a summary of the mean was prepared - from the most common to the least frequently implemented. The nurses most often reduced smoking, had friends and regular family, avoided colds, ate a lot of fruit and vegetables and thought positively. The last positions in the created hierarchy, with the lowest declared frequency, were the avoidance of overwork and excessive physical exertion as well as sufficient rest (Figure 7).

Discussion

Numerous studies on healthy lifestyle are available in the literature of the subject. A survey by Kantar Public conducted in 2017 among 1,000 Polish residents aged 15-69 on physical activity of the population in line with

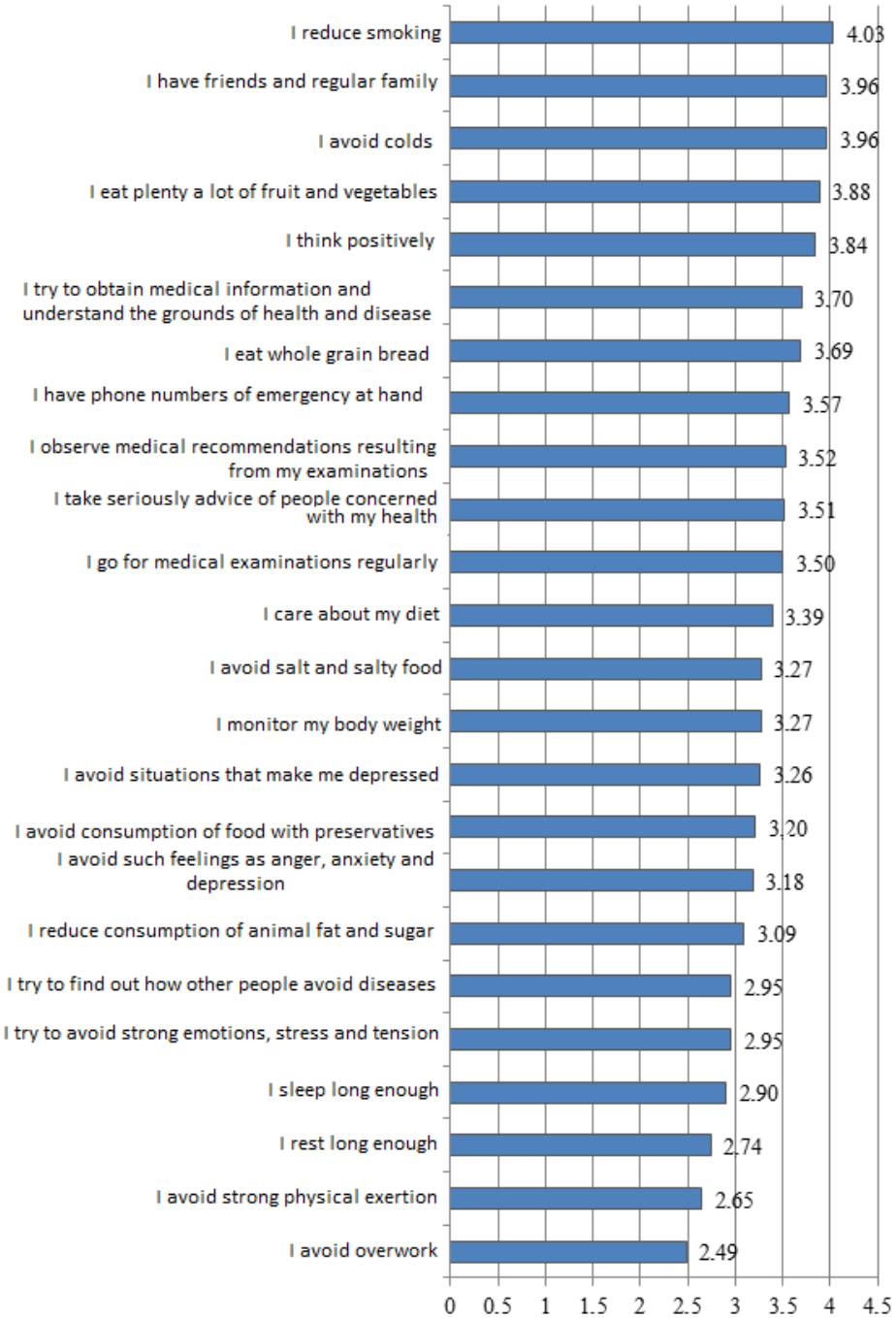

Fig. 7. Mean frequency of selected health-related behaviors

the requirements of the World Health Organization indicated the areas where the subjects are most often active physically.[8] The largest group, i.e. 30%, indicated work, 27% associated it with mobility, 22% undertake physical activity in their free time, and 21% at home and in the garden.

Nurses, as a group professionally prepared to work in health care, are expected not only to care for the health of their patients, but to serve an example for the entire society on how to care for their own health. The studies on nursing staff conducted in many countries confirm that nurses have professional knowledge of health behaviors, but do not apply this knowledge to themselves.[9-11] This is particularly visible in the area of diet.[11-13]

In our study, nurses were asked about the most important value in life for them. Family, health and work came first. In the paper by Barbara Ślusarska et al., nurses pointed to health as the first, family as the second and work appeared in the third place.[14]

The study by Muszalik et al. conducted on a group of 255 nurses indicates that majority of them (71.6%) assess their health at a good level, and every fifth nurse assesses their health condition as poor. In our study, 30% of nurses surveyed indicated that they were struggling with a chronic disease, most often thyroid conditions

- 10 people, hypertension - 6 people, diabetes - 2 people and 6 nurses indicated other diseases.[15] The study by Zagroba et al. on a sample of 100 professionally active nurses indicated that nurses' health condition is as follows: 45 people have back problems, 35 are overweight and 29 have frequent headaches, every fifth nurse declares no health problems - 23.[16] In the same study, the authors came to the conclusion that nurses present average level of physical activity, which undermines their credibility as health promoters.

Over 60% of the nurses surveyed admitted that they participate in screenings, which is a surprisingly low result considering that the respondents are representatives of the medical profession and are required to serve as an example in the field of health. In the study by Mrozowicz and Guta conducted on a group of 100 women, 49% of women surveyed (n = 100) take part in preventive screenings at least once a year, and 7% do not participate in them at all.[17]

On the other hand, in the Andruszkiewicz and Nowik study among nurses and teachers by means of the Positive Health Behaviors Scale for Women (n = 83), where five domains were assessed: health care, nutrition, body care, preventive behaviours, psychosocial health and physical activity indicated that the profession does not differentiate the level of health behavior. The highest results were obtained in the area of body care, i.e. the respondents underwent periodic examinations (cytological examination, breast self-examination).[18]

It is also worth paying attention to the study by Remigrońska and Włoszczak-Szubzda, where the results indicate the reproduction of disturbing anti-health behaviors such as: bad eating habits, passive lifestyle, exposure to stress and insufficient rest in working nurses (n = 110). [19]Lack of physical activity is also a problem for nurses in the USA, Turkey and Korea. [11, 13, 20-21]

In the analysis, the value of the health behavior index in the group of nurses amounts to 80.52 points. The minimum value of the index obtained in the study sample was 48 points, while the maximum was 108 points (the range of values from 24 to 120 points). Based on our research, it can be concluded that the highest intensity of behavior in the group of nurses is visible in the case of preventive behavior (21.20 points), while the lowest in the case of health practices (18.08 points). The average intensity of behaviors that make up proper eating habits and a positive mental attitude is similar with approximately 20.60 points.

In the study by Justyna Palacz on a sample of 664 students of Physical Education, Physiotherapy, Tourism and Recreation as well as Pedagogy, the general index of health behaviors in women amounted to 78.02, while in men 73.28 points.[22]

In turn, in the paper by Anna Walentukiewicz et al., where 77 nursing students were examined, the result was 73.19 points.[23] In another study by this author, where medical students were studied (n = 195), the lifestyle index evaluation showed that only every 10 student represents a pro-health attitude.[24]

In the paper by Zadworna-Cieślak and Ogińska-Bulik the studies of open population (n = 285) using the Inventory of Health Behaviors showed that women (74.87) had a higher level of the health behavior index than men (70.60).[25]

Taking into account our research and quoted studies of various authors, it can be stated that both in the open population and among medical professionals there are many deficits related to health-promoting behaviors.

Conclusion

The greatest value in the life of nurses was the family right after health and work. Nurses declared regular participation in preventive screenings. They presented the average level of health behaviors. The nurses' educational level positively affected the level of their health behavior. Nurses living in the countryside showed a higher level of health behavior. Nurses with chronic condition presented a lower level of health behaviors than their healthy colleagues.

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ORIGINAL PAPER

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The factors discriminating the results of screening test aimed at detection of scoliosis and detection of flexibility disorders in group of preterm children at the beginning of school age

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ABSTRACT

Introduction. The threshold of compulsory schooling for prematurely born children is of particular importance. It's a period of intense physical development which may increase the risk of scoliosis.

Aim. The aim of this research is to determine whether age, gender, BMI value and selected elements of perinatal interview discriminate the results of screening test aimed at detection of scoliosis and flexibility disorders in group of preterm children at the beginning of school age.

Material and methods. The study population consisted of 61 preterm children aged 5-8 years. The study included perinatal interview, BMI assessment, screening tests to detect scoliosis and flexibility.

Results. Statistically significant dependence was obtained between age and normal and abnormal results of the screening test aimed at detection of scoliosis and between the result of the screening test for detecting flexibility disorders and: age, number of foetuses, assessment on the Apgar scale.

Conclusion. The results of screening test aimed at occurrence of scoliosis in group of preterm children are significantly correlated with the age and screening test aimed at occurrence of the flexibility disorders are discriminated by age, origin from mono- or multi-foetus pregnancy and assessment on the Apgar scale.

Keywords. development of premature babies, health balance, prematurity, school readiness

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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

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Introduction

Children who are born between 22nd and 37th week of pregnancy are considered prematurely born.[1] In Poland, as well as other developed countries, the rate of premature labours is 6,3%.[2-4] Because of the amount and diversity of medical problems polish premature children are under a coordinated multi-specialized care during the first 36 months of their lives.[5] Own experience has shown this period is too short and the authors call for extension until the premature children reach school readiness.

School readiness is a functional term and is assessed during a year-long compulsory preschool preparation (class 0) or the first term of 1st class of primary school, right at the start of fulfilling schooling obligation. Health schooling maturity is defined as a balance achieved between schooling requirements and physical, intellectual and socio-emotional development. A group of children being assessed for school readiness might present a diversified age composition. According to a schooling reform carried out between 2015 and 2016 in Poland 5 and 6 year olds attended class 0 and 6 and 7 year olds attended 1st class. The tool which is used to assess school maturity is a specifically designed check-up protocol. One of the elements of the examination is a screening testing for scoliosis and suppleness.

Scoliosis is a three-dimensional spine deformation of a various etiology. Untreated leads to reduced suppleness, deformation and decreased chest movement, has a negative impact on circulatory and pulmonary systems thereby affecting the entire body.[7] Idiopathic scoliosis (of unknown origin) is the kind being diagnosed the most (80% of cases). Its diagnosed in 2-3% of children and adolescent populations. Idiopathic scoliosis occurs in the periods of accelerated length growth of axial skeleton: between 6th and 24th months old, 5 and 8 years old (early school growth spurt), 10 and 14 years old (pubertal growth spurt).[8] The early school growth spurt overlaps with the period of achieving the school maturity.

Performing a screening test for scoliosis and suppleness disorders during ordinary check-ups is necessary as most scolioses' onset is undefinable. Despite being criticised as fragmentary and for its inaccurate record keeping which prevents comparisons between consecutive check-ups, it has its use in medical practice, paediatrics and merely serves determining the symptoms indicative of scoliosis (1st and 2nd stage). It provides an introduction to detailed diagnosis based on instrumental method.[9-11] Radiological review is performed with an x-ray picture of the spine in posteroanterior projection. A change in Cobb's angle (the angle of lateral spinal curvature) during a year is used as a measurement of scoliosis' progression. The risk of further progression decreases as the skeleton develops. The assessment of skeleton's development is made with Risser test.[12] It's worth remembering that idiopathic scoliosis can present itself in patients of all ages.

Researchers investigated the connection between the age, gender, perinatal factors and the results of screening tests for scoliosis and suppleness disorders in prematurely born children who are starting school.

Aim

The aim of the paper is twofold. Firstly, to determine if age, gender, BMI, perinatal factors differentiate the results of screening tests for scoliosis in prematurely born children at the start of school. Secondly, to determine if age, gender, BMI, perinatal factors differentiate the results of screening tests for suppleness disorders in prematurely born children at the start of school.

Material and methods

The studied group consisted of 61 children aged between 5 and 8 born prematurely (x=6,38 years, Me=6 years, s=0,73). The group was functionally uniform - all children were starting school. High age spread derives from some children starting school a year earlier as well as children whose schooling obligation was postponed by a year. Girls consisted 52% (32 children) and boys 48% (29 children) of the group.

The study has been approved by the Bioethical Committee of Medical Faculty of University of Rzeszów (first act: 7th Dec. 2012, last 6th of Feb. 2017). The study was conducted between 2015 and 2016 at the Physiotherapy Institute and the Innovative Anthropometric Methods Laboratory of the Center of Innovative Nature-Medical Studies at the University of Rzeszów.

The study was conducted according to the check-up protocol and performed as per generally accepted rules and standard of conduct as described by well-child care covered by the compulsory pre-school preparation.[6] Medical history of perinatal period was gathered (subsequent pregnancy, labour date, foetus count, delivery method, birthweight, Apgar score) (table 1 A-F). An aggregated number (sum) of detrimental events in the perinatal period was established i.e. occurrence of one of the listed events equals 1 point: respiratory failure, respiratory distress syndrome, bronchopulmonary dysplasia, congenital pneumonia, acquired pneumonia, pulmonary emphysema, ventilation therapy (cmv, imv), infant flow, passive oxygen therapy, hyperbilirubinaemia, anemia, thrombocytopenia, leucopenia, bleeding from digestive/respiratory system/tamponade, serological conflict in major groups, blood or hemocompatible agent transfusion, exchange transfusion, hypoxic ischaemic encephalopathy, evidence of periventricular leukomalacia, I-IV grade stroke, epilepsy, seizures different than epilepsy, apnea, retrolental fibroplasia, patent ductus arteriosus/circulatory failure, TORCH

infections, other intrauterine infections, sepsis, purulent meningitis, encephalitis, bacterial gastroenteritis, urinary infections, perinatal necrotizing enterocolitis, gastroesophageal reflux, hypoglycemia, hypocalcemia, osteopenia of prematurity, intravenously administered drugs, parenteral nutrition, tube feeding, procedures under general anesthesia.

Table 1. Baseline characteristics of the group of premature children entering school readiness age

Perinatal history		
A. Birth order	**N**	**%**
First pregnancy	31	51
Second pregnancy	15	25
Third pregnancy	5	8
Fourth pregnancy	5	8
Fifth pregnancy	2	3
Sixth pregnancy	3	5
B. Foetus count	**N**	**%**
Single pregnancy	39	64
Twin pregnancy	13	21
Triple pregnancy	9	15
C. Delivery week	**N**	**%**
24	2	3
25	0	0
26	4	7
27	6	10
28	8	13
29	1	2
30	10	16
31	5	8
32	23	38
33	0	0
34	1	2
35	1	2
D. Type of delivery	**N**	**%**
Natural	10	16
C-section	51	84
E. Apgar evaluation [points]	**N**	**%**
0-3	9	15
4-7	39	64
8-10	13	21
F. Birth weight [g]	**N**	**%**
below 750	3	5
750 - 1000	10	16
1000 – 1500	21	34
1500 - 2500	26	43
above 2500	1	2

Basic anthropometric measurements have been taken. The measuring technique was based on methods utilized in international anthropological research. The following anthropometric characteristics were taken into account: weight (w) and height (h). Instruments used: medical scale (kg), anthropometer (cm). Quetelet II weight-height ratio was calculated (kg/m^2 WQ2, Body Mass Index, BMI). A screening test for scoliosis was conducted with the following criteria: child standing upright, examined from behind: long spinal axis is straight, a plumb hung from the centre of occipital protuberance aims at gluteal cleft, shoulders and shoulder blades on the same height, waist triangles symmetrical, lower limbs the same length, the child during Adams test examined from behind: rib hump absent, muscular prominence in the lumbar area absent. When this criteria were not met the result was considered positive. Next a screening test for suppleness disorder was conducted. A negative result was achieved when a child performing a forward bend test was able to reach the floor with fingertips. When this criteria were not met the result was considered positive

Statistical analysis

The data was analysed statistically. A relation between age, gender, BMI, perinatal factors and correct/incorrect screening test results for scoliosis and suppleness disorders was analysed based on statistical methods. A nonparametric Mann-Whitney U test was used to assess the differences in measurable characteristics of independent variables among two populations. Variables presenting qualitative characteristics were analysed with Pearson's chi-squared test. $p<0,05$ was assumed as statistically significant level.

Results

Based on perinatal history it was established that the children were born from pregnancies of different order (table 1A), foetus count (table 1B), delivered prematurely (table 1C), naturally or by C-section (table 1D), in various general condition and birth weight (table 1F). In the perinatal period premature children were encumbered with numerous adverse perinatal events (table 2A). A screening test for scoliosis presented negative results in 54% of children (table 2B) whereas suppleness disorder screening test presented negative results in 64% examined children (table 2C). BMI was analysed with statistical method (table 2D).

Table 2. Baseline characteristics of the group of premature children entering school readiness age based on the gathered data

A. Combined amount (sum) of adverse perinatal events				
$\bar{x}$	Me	Min	Max	s
11,93	12,00	0	26	5,75
B. Screening test for scoliosis				
Result		N		%
Negative		28		46
Positive		33		54
C. Screening test for suppleness disorders				
Result		N		%
Negative		22		36
Positive		39		64
D. Body Mass Index BMI [kg/m^2]				
$\bar{x}$	Me	Min	Max	s
15.36	15.26	10.46	27.80	2.43

Table 3. Differentiation of scoliosis screening test results in the group of premature children entering school readiness age

A. Scoliosis screening test results according to gender					
Variables	Female		Male		Chi^2/p
	N	%	N	%	
Negative result	18	30	10	16	Chi^2 =2,90
Positive result	14	20	19	32	p=0,88
Pearson's Chi-squared test					

B. Scoliosis screening test results according to age				
Variables	Age[years]			Z/p
	$\bar{x}$	Me	s	
Negative result	6,14	6,00	0,71	Z=-2,44
Positive result	6,58	7,00	0,71	p=0,015
Mann-Whitney U test (Z)				

C. Scoliosis screening test results according to birth from first/subsequent pregnancies				
Variables	Birth from first, second… sixth pregnancy			Z/p
	$\bar{x}$	Me	s	
Negative result	2,11	1,50	1,52	Z=0,26
Positive result	1,97	1,00	1,36	p=0,78
Mann-Whitney U test (Z)				

D. Scoliosis screening test results according to foetus count							
Variables	Single pregnancy		Twin pregnancy		Triple pregnancy		Z/p
	N	%	N	%	N	%	
Negative result	??????	26	6	10	6	10	Chi^2 =1,93
Positive result	23	38	7	11	3	5	p=0,38
Pearson's Chi-squared test							

E. Scoliosis screening test results according to delivery week				
Variables	Delivery week: from 24 to 35			Z/p
	$\bar{x}$	Me	s	
Negative result	30,25	30,50	2,40	Z=0,85
Positive result	29,70	30,00	2,51	p=0,39
Mann-Whitney U test (Z)				

F. Scoliosis screening test results according to type of delivery					
Variables	Natural		C-section		Chi^2 / p
	N	%	N	%	
Negative result	24	40	4	6	Chi^2 =0,16
Positive result	27	44	6	10	p=0,68
Pearson's Chi-squared test					

G. Scoliosis screening test results according to Apgar score							
Variables	0-3 p.		4-7 p.		8-10 p.		Chi^2 /p
	N	%	N	%	N	%	
Negative result	4	7	16	26	8	13	Chi^2 =1,66
Positive result	5	8	23	38	5	8	p=0,43
Pearson's Chi-squared test							

H. Scoliosis screening test results according to birth weight									
Variables	<750 g		750-1000 g		1000-1500 g		>1500 g		Chi^2/p
	N	%	N	%	N	%	N	%	
Negative result	2	3	4	6	11	18	11	18	Chi^2 =1,30
Positive result	1	2	6	10	10	16	16	27	p=0,72
Pearson's Chi-squared test									

I. Scoliosis screening test results according to combined amount of adverse perinatal events				
Variables	sum of the events			Z/p
	$\bar{x}$	Me	s	
Negative result	11,00	12,00	6,41	Z=-0,8
Positive result	12,73	12,00	5,09	p=0,40
Mann-Whitney U test (Z)				

J. Scoliosis screening test results according to BMI				
Variables	BMI [kg/m^2]			Z/p
	$\bar{x}$	Me	s	
Negative result	14,97	15,11	1,62	Z=-0,85
Positive result	15,68	15,43	2,94	p=0,36
Mann-Whitney U test (Z)				

A statistically significant relation between age and positive/negative result of scoliosis screening test (p=0,0015, Mann-Whitney U test). Older age correlates to a negative test result (table 3B).

Additionally a statistically significant relation was observed between negative/positive screening test result for suppleness disorder and:

– age (p=0,007, Mann-Whitney U test) - younger age correlates with positive test result (table 4B),
– number of foetuses (p=0,030, Pearson's Chi-squared test) - being born from pregnancies having higher foetus count correlates with positive test result (table 4D),
– Apgar score (p=0,008, Pearson's Chi-squared test) - higher Apgar score correlates with positive test result (table 4G).

No statistically significant relation was observed between negative/positive results of a screening test for scoliosis and gender (table 3A), birth from first/subsequent pregnancy (table 3C), fetus count (table 3D), delivery week (table 3E), type of delivery (table 3F), Apgar score (table 3G), birth weight (table 3H), combined amount of adverse perinatal events (table 3I), BMI (table 3J).

No statistically significant relation was observed between negative/positive results of a screening test for suppleness disorders and gender (table 4A), birth from first/subsequent pregnancies (table 4C), delivery week (table 4E), type of delivery (table 4F), birth weight (table 4H), combined amount of adverse perinatal events (table 4I), BMI (table 4J)

Discussion

During growth span the risk of scoliosis progression is at its highest.[13] The time gap between regular check-ups is widely criticised.[9] In paediatrics the check-ups are conducted every several years.[14-20] In the studied group of prematurely born children who are starting school the age correlated significantly with the screening tests for both scoliosis and suppleness disorders. Older age corresponds to the positive test result. It is worth noting that the children are between 5 and 8 years old which coincides with – widely described in literature – first critical postural development period as well as early school growth spurt.[21,22] Research carried out in Spain have shown that pupils 8,5 years old, among the studied 6 to 12 year olds, are susceptible to increased risk of developing scoliosis.[23] That confirms the necessity of conducting annual check-ups in that age group as well as in prepubertal period.

The development of the abilities needed to maintain correct posture and fulfilling complex motoric standards is a reflection of a maturing central nervous system.[24] Children with low birth weight present decreased motor skills including suppleness.[25] It is believed that in conjunction with the progression of gestational age at the delivery time the survival rate increases, the prognosis of motoric and cognitive development improves.[26] In prematurely born children the posture control is disturbed. It may result from the immaturity of the cortex processes related to motoric control and proprioception.[27] Motoric disorders starting at the first year of age are often connected to decreased neurodevelopment results at 6-7.[28] Nevertheless, no significant differences in the occurrence frequency of postural disorders in torso area between children born prematurely and their peers were noted. Before entering 12 years of age the posture control systems are not fully developed hence the deficits might not be spotted as a child develops. The entire scope of postural disorders can be fully observed during puberty and adulthood.[24,30]

The examined group of prematurely born children shown the following statistically significant dependencies: older age corresponds to negative suppleness disorder test, which is also confirmed by Touwslader et. al. research.[31] The same dependency was observed in screening test performed on 6-7 year old children entering schooling obligation age.[32] In contrast to the work of other authors who performed screening tests in schools no relationship between female gender and suppleness disorders was found.[33] Increased body weight in relation to body height did not affect the test results however obesity in later life may significantly increase a chance a positive suppleness disorder test.[34] Moreover, being born from a pregnancy with higher foetus count and higher Apgar score correlates to a positive suppleness disorder test.

Earlier a study was published on diversification of results of orientating questionnaire of motoric and psycho-social development in regard to the level of educational maturity (school readiness) in the same group of prematurely born children. The study established that post-natal Apgar score differentiates the results of the questionnaire on the indicative study of motor and psychosocial development in terms of school readiness in the in the areas of child's functioning – high motor skills, visual-motor coordination, memory and total score.[35]

In recent years an increase in the percentage of prematurely born children has been observed.[36] This is why the issue of preterm children medical care is important and requires further and deeper research.

Conclusion

In conclusion, age differentiates the result of scoliosis screening test in prematurely born children entering schooling obligation age which shows the necessity of performing the check-ups more frequently. The studied characteristics (age, being born from single- of multiple pregnancy and Apgar score) differentiate the result of the screening test for suppleness disorders in premature born children.

Table 4. Differentiation of suppleness disorder screening test results in the group of premature children entering school readiness age

A. Suppleness disorder screening test results according to gender					
Variables	Female		Male		Chi^2 /p
	N	%	N	%	
Negative result	10	16	12	20	Chi^2 =0,67
Positive result	22	36	17	28	p=0,41
Pearson's Chi-squared test					

B. Suppleness disorder screening test results according to age				
Variables	Age[years]			Z/p
	$\bar{x}$	Me	s	
Negative result	6,73	7,00	0,77	Z=2,71
Positive result	6,18	6,00	0,64	p=0,007
Mann-Whitney U test (Z)				

C. Suppleness disorder screening test results according to birth from first/subsequent pregnancies				
Variables	Birth from first, second… sixth pregnancy			Z/p
	$\bar{x}$	Me	s	
Negative result	1,68	1,50	0,95	Z=-0,78
Positive result	2,23	1,00	1,61	p=0,43
Mann-Whitney U test (Z)				

D. Suppleness screening test results according to pregnancy foetus count							
Variables	Single pregnancy		Twin pregnancy		Triple pregnancy		Chi^2/p
	N	%	N	%	N	%	
Negative result	18	30	4	6	0	0	Chi^2 =6,95
Positive result	21	34	9	15	9	15	p=0,03
Pearson's Chi-squared test							

E. Suppleness screening test results according to delivery week					
Variables	Delivery week: from 24 to 35			Z/p	
	$\bar{x}$	Me	s		
Negative result	29,50	30,00	2,60	Z=-1,20	p=0,22
Positive result	30,21	31,00	2,36		
Mann-Whitney U test (Z)					

F. Suppleness disorder screening test results according to type of delivery					
Variables	Natural		C-section		Chi^2/p
	N	%	N	%	
Negative result	20	33	2	3	Chi^2 =1,34
Positive result	31	51	8	13	p=0,25
Pearson's Chi-squared test					

G. Suppleness disorder screening test results according to Apgar score							
Variables	0-3 p.		4-7 p.		8-10 p.		Chi^2/p
	N	%	N	%	N	%	
Negative result	5	8	17	28	0	0	Chi^2 =9,77
Positive result	4	7	22	36	13	21	p=0,008
Pearson's Chi-squared test							

H. Suppleness disorder screening test results according to birth weight [g]									
Variables	<750		750-1000		1000-1500		>1500		Chi^2/p
	N	%	N	%	N	%	N	%	
Negative result	1	2	4	6	9	15	8	13	Chi^2 =0,98
Positive result	2	3	6	10	12	20	19	31	p=0,80
Pearson's Chi-squared test									

I. Suppleness disorder screening test results according to combined amount of adverse perinatal events					
Variables	Sum of the events			Z/p	
	$\bar{x}$	Me	s		
Negative result	12,50	12,00	5,52	Z=0,25	p=0,80
Positive result	11,62	12,00	5,93		
Mann-Whitney U test (Z)					

J. Suppleness disorder screening test results according to BMI					
Variables	BMI [kg/m^2]			Z/p	
	$\bar{x}$	Me	s		
Negative result	16,18	15,49	2,87	Z=1,61	p=0,11
Positive result	14,89	15,07	2,04		
Mann-Whitney U test (Z)					

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ORIGINAL PAPER

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Evaluation of neutrophil phagocytic, complement functions, and cytokines expression among diabetic patients in Abuja, Nigeria

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ABSTRACT

Introduction. Inflammatory response in Diabetes Mellitus (DM) begins with chronic sub-clinical inflammations as a result of insulin resistance and activation of both innate and adaptive immune system as the disease progresses to complicated diabetes. Hence, the present study investigated the neutrophil phagocytic, complement function (CH50), and some cytokine profiles among diabetic and non-diabetic patients attending the National Hospital in Abuja, Nigeria.

Aim. To evaluate the neutrophil phagocytic, complement function (CH50), and some cytokine profiles among post-operative septic diabetic and post-operative septic non-diabetic patients at the National Hospital in Abuja, Nigeria.

Material and methods. Subjects were recruited by convenient sampling technique through interviewer-administered questionnaires. Subsequently, blood samples were collected. Fasting blood sugar (FBS) (mmol/L) was determined using glucose oxidase method. Neutrophil function test (Fmol/phag) was assayed using nitroblue tetrazolium reduction test (NBT). Hemolytic complement function (CH 50) test was conducted using serum harvested from sheep sensitized with human group (O^{Rh} D +ve) red blood cells. While serum Interleukin-4, -6, -10 and TNF- α were determined using Enzyme Linked Immunosorbent Assay (ELISA).

Results. Mean ± Standard deviation (SD) of FBS concentration of 10.5 ± 1.3 (mmol/|L) among diabetic and 4.7 ± 0.9 (mmol/L) among non-diabetics was recorded. There is a decrease in neutrophil phagocytic function with a mean ± SD of 5.4 ± 2.1 (Fmol/phag) in diabetics compared to 9.2 ± 2.1 (Fmol/phag) in non-diabetics. Similarly, complement (CH 50) function and C-reactive protein were significantly lower in diabetics when compared to non-diabetics ($p<0.001$). There was a significant difference in

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IL-6 concentration between diabetics and non-diabetics groups, but no significant difference was observed in TNF-α, IL-4 and IL-10 concentrations between study groups (p>0.05). TNF-α and IL-6 was significantly higher in diabetics with cardiovascular disorders compared to non-diabetics subjects with cardiovascular disorders (p<0.001).

Conclusion. Findings from this study revealed the association of complement, neutrophil phagocytic function, CRP and IL-6 among septic diabetic patients,. In addition TNF-α and IL-6 expression was higher in DM patients with cardiovascular disorders.

Keywords. diabetes mellitus, effector molecules, pro-inflammatory markers

Introduction

Diabetes mellitus (DM) causes immune-suppression with an increased risk of either partial or permanent complications of certain tissues or organs such as the eyes, kidneys and heart.[1] Inflammatory response in DM begins with chronic sub-clinical inflammations as a result of insulin resistance and activation of both innate and adaptive immune system as the disease progresses to complicated diabetes over a period of 10–20 years.[2]

Cytokines regulate inflammatory and immune response through their activity on cells and they provide important signals in the pathogenesis of a range of diseases, including type 2 diabetes mellitus.[3] It has been reported by many studies that DM compromises the immune system by impairing leukocyte migration, phagocytosis and poor signal transduction that leads to increased risk of oxidative stress and increased cellular loss of function of which pro and anti-inflammatory cytokines play key role in the development and progression of diabetic complications over time.[4]

Cytokine secretions occur either in autocrine or paracrine manner in cells and trigger several cellular responses depending on diverse factors, such as cell type, timing, acting synergistically in many contexts to markedly amplify their effects.[4] They also have the capacity to induce expression of cytokine receptors on other cytokines.[5] In addition to being cells of acute inflammation and primary phagocytes of innate immune systems, neutrophils secrete cytokines and chemokines that further mobilize monocytes and macrophages to the site of inflammation.[5]

Metabolic and hemodynamic alteration associated with inflammatory response were hypothesized to be the major causes of cardiovascular and renal injuries.[6] Complications such as diabetic cardiomyopathy occur in 80% of all diagnosed type 2 diabetic patients, while 15% develop diabetic nephropathy and other complications account for 5%.[7]

Preventing diabetic complication is one of the major challenges in diabetes management, though previous diagnostic and treatment strategies in Nigeria and most developing countries do not include targeting immunological markers. The recent use of inflammatory cytokines is pivotal and very useful in monitoring diabetic patients during sub-clinical transformation to a life threatening diabetic complication.[8] Inflammatory cytokines, mainly IL-4 and TNF- α are involved in the development and progression of type 2 diabetes.[9]

IL-6 was initially thought to be a proinflammatory cytokine mainly with effects within the immune system, but this understanding of IL-6 was soon found to be too simplistic.[10] In the adaptive and innate immune systems, IL-6 is involved in both amplification of and protection against inflammation.[10] Thus, inappropriate regulation of IL-6 may play a direct protective or deleterious role in both antigen-specific immune-mediated diseases and in diseases where IL-6 or other inflammatory factors cause a low-grade inflammation (as seen in obesity and type 2 diabetes), which is likely to be involved in the pathogeneses of these diseases.[10] IL-6 has been suggested to be involved in the development of obesity-related and T2DM-related insulin resistance.[11]

TNF-α are pro-inflammatory cytokines whose expression increases chemotactic factors and adhesion molecules on vascular endothelium to effect the process of extravasation of leucocytes (especially neutrophils) in response to specific inflammatory stimulus such as bacterial infection.[12] IL-6, in more recent studies in type 2 diabetic patients in India, was demonstrated to be a strong predictor of progression to diabetic complications which has been related to alterations in endothelial permeability, induction of messengial cell proliferation and increased fibronectin expression.[12] Among various pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) has attracted the most attention, since it amplifies the inflammatory network of cytokines leading to a worsening progression of diabetes.[13]

In particular, it has been suggested that TNF-α is associated with insulin resistance and type 2 diabetes, given that TNF-α down-regulates the tyrosine kinase activity of the insulin receptor. Interleukin-10 (IL-10) is a centrally operating anti-inflammatory cytokine that plays a crucial role in the regulation of the innate immune system. It has strong deactivating properties on the inflammatory host response mediated by macrophages and lymphocytes, and potently inhibits the production of pro-inflammatory cytokines such as IL-6 and TNF-α.[14] IL-10 is produced by T-cells, B-cells, monocytes, and macrophages, under tight genetic control. The key roles of IL-10 as an inhibitory cytokine of autoimmunity and inflammation raise questions concerning the impacts of this cytokine on the pathogenesis of other diseases including type 2 DM and its nephropathic complications.[15]

Understanding the roles of inflammatory cytokines in the development and progression of diabetic cardiomyopathy and nephropathy is of critical importance. Early quantification of these markers may constitute a pivotal target in DM management and might open the possibility of new potential therapeutic targets.

There is paucity of studies on the roles of pro-inflammatory markers in predicting the risks for diabetes complications in subpopulations of interest, especially in Nigeria. Hence the need to carry out this study to evaluate the neutrophil phagocytic, complement function (CH50) and some cytokine profiles among post-operative septic diabetic and post-operative septic non-diabetic patients at the National Hospital in Abuja, Nigeria.

Material and methods

Study area

The study was carried out in the National Hospital Abuja, a referral tertiary health care facility with a 500 bed capacity.

Ethical approval

The ethical approval for this research was obtained from the Ethics and Research Committee of National Hospital Abuja (NHA) and the study was conducted in accordance with the declaration of Helsinki.

Study sample size

The sample size for the study was determined using the standard formula for calculation of minimum sample size by Rodrigues et al.[15] As calculated, the sample size was n = 38 case and n= 38 controls. In order to increase the precision, an attrition rate of 10% was added to each group. Hence, the sample size was increased to 45 case subjects and 45 controls. All eligible type 2 diabetic patients were within the age range of 25-60 years in both case and control groups.

For the purpose of this study, subjects were classified (inclusion criteria) as follows:

Groups (n = 45)	Case group (n = 45)	Control Group (n = 45)
Group 1: (n = 15)	Diabetic patients ≤ 5 years on treatment	Apparently healthy subjects
Group 2: (n = 15)	Diabetic patients with cardiovascular disease	Non-diabetic patients with cardiovascular disease
Group 3: (n = 15)	Diabetic patients with nephropathy	Non-diabetic patients with nephropathy

Exclusion criteria

a. Patients > 5 years on diabetic treatment without diabetic complication were excluded.
b. Patients with diabetic complication that were not clinically confirmed were excluded.
c. Diabetic patients with terminal complications (stroke, cardiomyopathy, myocardial infarction and renal dialysis) were excluded in this study.
d. Diabetic patients on anti-TB drugs, anti-retroviral drugs and cancer chemotherapy.
e. Patients with type 1 diabetes and gestational diabetes were excluded from the work.

Blood sample collection

8 mL of blood was collected from each patient, and 4 mL was collected into a sterile plain vacutainer tube. The blood was allowed to clot and was later centrifuged at 12000×g for 5 minutes and the clear sera was harvested and rapidly stored at -20°C until used for the determination of serum cytokines. 2 mL of blood was collected into EDTA vacutainer tube (BD® New Jessy, USA) and samples were immediately used for NBT assay. The remaining 2 mL of blood was collected into fluoride oxalate tube and later centrifuged at 12000×g for 5 minutes and the plasma was used for glucose determination and complement studies within 1 hour.

Table 1. Age and Sex Characteristics of Diabetic and non-diabetics subjects

Characteristic	Case (n = 45)			Controls (n = 45)			Total (n=90)
	Group 1 β (%)	Group 2 β (%)	Group 3 β (%)	Control 1 β (%)	Control 2 β (%)	Control 3 β (%)	
Age (years)							p<0.001
27 – 36	1 (2.2)	2(2.2)	0 (0.00)	0 (0.00)	0 (0.00)	9 (20.0)	12 (13.3)
37 – 46	4 (8.8)	3 (6.6)	3 (6.6)	4 (8.8)	2 (4.4)	2 (2.2)	18 (20.0)
47 – 56	8 (17.7)	6 (13.3)	6 (13.3)	7 (15.5)	4 (8.8)	4 (8.8)	35 (38.8)
≥ 57	2 (4.4)	4 (8.8)	6 (13.3)	4 (8.8)	9(20.0)	0 (0.00)	25 (27.7)
Mean ± SD	42 ± 1.3	29 ± 2.2	44 ± 2.4	42 ± 1.9	45 ±2.3	43 ± 1.5	p<0.001
Sex							P=8834
Male	9 (20.0)	8 (17.7)	8(17.7)	7 (15.5)	10(22.2)	9 (20.0)	51 (56.6)
Female	6 (13.3)	7 (15.5)	7(15.5)	8 (17.7)	5 (11,1)	6 (13.3)	39 (43.3)

n = number of case and control, group 1 = diabetic patients on treatment, group 2 = diabetic patients with cardiovascular disease, group 3 = diabetic patients with nephropathy, β = number of occurrence, (%) = percentage in parenthesis

Analytical Laboratory procedures

Patients were prepared for Fasting blood glucose (mmol/L). Plasma glucose was determined using glucose oxidase kit procured from Randox Laboratories, England. Neutrophils phagocytic function test was performed using the modified method of Onyenekwe et al.[16]. Serum samples were used to estimate the level of IL-4, IL-6, and IL-10 using ELISA Kits from Enzo-life-sciences (UK) LTD according to manufacturer's instructions. Serum TNF-alpha was quantified using sandwich ELISA by kits supplied by WKEA Med Supplies Corp, China. Human C-reactive protein (CRP) was estimated based on ELISA kit from Cayman Chemical (UK) LTD.

Data analysis

The data obtained were computed using Microsoft office Excel 2013 and SPSS version 20. The results were expressed as percentage and mean ± SD. All data were checked for distribution of variables and feasibility of using a parametric test. If the data is normally distributed and there is no violation of assumptions (normal or Gaussian distribution) independent t-test or one way ANOVA (parametric) was used to find the association between the variables. A p value less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

The mean ± SD age (years) of 42 ± 1.3, 44 ± 0.5 and 45 ± 2.3 in diabetic patients with ≤ 5 years of management, diabetic patients with cardiovascular diseases and patients with diabetic nephropathy, respectively, was recorded. In addition, 29 ± 2.2, 42 ± 1.9 and 43 ± 1.5 among apparently healthy controls, non-diabetic patients with cardiovascular disease, and non-diabetic patients with nephropathy, respectively, was recorded (Table 1). The age range between 47–56 years had the highest number of participants, 35 (38.8 %), while age range 27–36 years had the least participants, 12 (13.3%). From this study, 51 (56.6 %) of the participant were male and 39 (43.3%) were female (Table 1).

Findings from this study showed that neutrophil function consistently decreases among diabetic patients with ≤ 5 years treatment, diabetic patients with cardiovascular disease, and diabetic nephropathy, respectively. However, higher values were observed among the control subjects with 9.2 ± 2.1, 7.3 ± 1.9 and 8.5 ± 3.5 (fmol/phag) with no significant difference of neutrophil function among the groups ($p>0.05$) (Table 2). The findings also indicated that FBG of 10.5 ± 1.3, 10.2 ± 1.6 (mmol/L) and 8.6 ± 1.7 (mmol/L) in diabetic patients with ≤ 5 years on treatment, patients with cardiovascular disease, and patients with diabetic nephropathy, respectively.

Consistent normal FBG concentrations were observed among the control group. There was significant differences in FBG among the study groups ($p<0.05$) (Table 2). The serum CRP concentrations of 8.5 ± 1.8 (ng/L) and 3.5±2.8 (ng/L) was recorded in diabetic and non-diabetic subjects with cardiovascular disease while serum CRP concentrations of 5.2 ± 0.3 (ng/L) and 3.7 ± 0.3 (ng/L) was observed in diabetic and non-diabet-

Table 2. Neutrophil Phagocytic Activity, Fasting Blood Glucose, Serum C-Reactive Proteins and BMI (Mean ± SD) among Diabetic Patients and Controls

Analyte	Case (n=45)	Controls (n=45)	p value
NBT (fmol/phag)			
Group 1	5.4 ± 2.1	9.2 ± 2.1	0.0138
Group 2	6.7 ± 2.0	7.3 ± 1.9	0.2860
Group 3	6.8 ± 3.9	8.5 ± 3.5	0.2308
FBG (mmol/l)			
Group 1	10.5 ± 1.3	4.7 ± 0.9	<0.0001
Group 2	10.2 ± 1.6	4.8 ± 1.2	<0.0001
Group 3	8.6 ± 1.7	4.8 ± 0.9	<0.0001
CRP (ng/l)			
Group 1	4.2 ± 0.3	3.5 ± 1.3	<0.0002
Group 2	8.5 ± 1.8	3.5 ± 2.8	0.0678
Group 3	5.2 ± 0.3	3.7 ± 0.3	<0.0001
BMI (kg/m^2)			
Group 1	32.7 ± 1.3	26.3 ± 2.4	<0.0001
Group 2	39.2 ± 3.5	32.3 ± 1.0	0.05
Group 3	34.7 ± 2.5	29.2 ± 1.5	0.0723

n – number of case and control, group 1 – diabetic patients on treatment, group 2 – diabetic patients with cardiovascular disease, group 3 – diabetic patients with nephropathy, NBT – Nitro blue tetrazolium reduction test, FBG – fasting blood glucose, CRP – C-reactive proteins and BMI – body mass index

ic patients with nephropathy. There was a significant difference between CRP level between the study group ($p<0.05$) and significant BMI among the group of diabetic patients with ≤ 5 years of treatment, and diabetic patients with cardiovascular disease group ($p < 0.05$). There was no significant BMI difference among diabetic and non-diabetic patients with nephropathy respectively 34.7 ± 2.5 (kg/m^2) and 28.2 ± 1.5 (kg/m^2) ($p>0.05$) (Table 2).

Serum IL-6 concentration was significant higher (30.2 ± 2.1ng/L) among diabetic patients with cardiovascular disease and non-diabetic patients with cardiovascular disease (21.4 ± 1.3 ng/L) ($p<0.05$) (Table 3). However, no significant difference in serum IL-6 concentration was observed among diabetic and non-diabetic patients with nephropathy ($p = 0.1040$) (Table 3). The serum concentration of TNF-α (20.2±2.4 ng/L) among diabetic patients with ≤ 5 years on treatment was significantly higher than apparently healthy controls (18.1 ± 1.4ng/L) ($p=0.012$) (Table 2). Serum TNF-α concentration of 35.5 ± 2.5ng/L was observed among diabetic patients with cardiovascular disease and 29.4 ± 2.5ng/L among non-diabetic patients with cardiovascular disease. There was significant difference in TNF- α between the two groups ($p<0.05$). No significant difference in TNF-alpha was observed among diabetic patients and non-diabetic patients with nephropathy ($p=0.421$) (Table 3).

The mean serum IL-4 concentrations in diabetic patients ≤ 5 years on treatment (5.8 ± 3.1 ng/L), diabetes with cardiovascular disease (5.9 ± 0.6 ng/L) was not significantly different from diabetic patients with nephropathy (5.1 ± 4.7 ng/L) and the control subjects ($p=0.2443$). Similarly, serum IL-10 concentration of 8.4 ± 3.1ng/L among diabetic patients with ≤ 5 years on treatment was not significantly different from 8.8 ± 0.9 ng/L and 8.1 ± 0.6ng/L among diabetic patients with cardiovascular disease and nephropathy, respectively ($p=0.9849$) (Table 4). Similarly, no significant difference between serum IL-10 concentration among diabetic patients (8.0 ± 7.4ng/L) and non-diabetic patients with nephropathy (14.5 ± 7.4 ng/L) ($p=0.2146$) (Table 4).

Discussion

Nigeria is one of the countries most affected by type 2 diabetes mellitus. Diabetic patients suffer from multiple immunological disorders with increased risk of complications. This study revealed that neutrophil phagocytic activity significantly decreased consistently in DM patients compared to non-Diabetic controls. A Previous study established that insulin insensitivity affects glucose uptake into cells and peripheral tissues, and thus affects the neutrophil functions of the innate immune system.[17] These findings also agree with Oni et al. and Chenxiao et al., who reported that high

Table 3. Serum Pro-inflammatory Cytokines IL-6 and TNF-α Concentration (Mean ± SD) among Diabetic Patients and Controls

Cytokines	Case (n = 45)	Controls (n = 45)	p value
IL-6 (ng/l)			
Group 1	21.9 ± 1.4	18.1 ± 1.5	0.001
Group 2	30.2 ± 2.1	21.4 ± 1.3	0.001
Group 3	22.8 ± 2.4	20.5 ± 2.1	0.104
TNF-α (ng/l)			
Group 1	20.2 ± 2.4	18.1 ± 1.4	0.061
Group 2	35.5 ± 2.5	29.4 ± 2.5	0.001
Group 3	23.7 ± 2.3	22.7 ± 1.9	0.421

n - number of case and control, group 1 - diabetic patients on treatment, group 2 - diabetic patients with cardiovascular disease, group 3 - diabetic patients with nephropathy, the figures in parenthesis (%) - percentage

Table 4. Serum Anti-inflammatory Cytokines IL-4 and IL-10 Concentrations among Diabetic Patients and Controls

Cytokines	Case (n = 45) Mean ± SD	Controls (n = 45) Mean ± SD	p value
IL-4 (ng/L)			
Group 1	5.8 ± 3.1	6.1 ± 0.3	0.1648
Group 2	5.9 ± 0.6	5.6 ± 0.5	0.4193
Group 3	5.1 ± 4.7	8.9 ± 4.7	0.2443
IL-10 (ng/L)			
Group 1	8.4 ± 0.9	8.5 ± 0.7	0.9849
Group 2	8.8 ± 0.6	8.1 ± 0.6	0.1598
Group 3	8.0 ± 7.4	14. 5 ± 7.4	0.2146

n - number of case and control, group 1 - diabetic patients on treatment, group 2 - diabetic patients with cardiovascular disease, group 3 - diabetic patients with nephropathy, the figures in parenthesis (%) - percentage

blood glucose concentrations impaired superoxide production from isolated blood neutrophils in diabetic patients.[18,19] These authors further reported that high glucose concentration rapidly affects membrane receptors responsible for the activation of NADPH oxidase in neutrophils. Anelise et al. and Harshad et al. also reported similar observations in an animal study.[20,21] The result from this study confirmed high glucose concentration among diabetic patients. However, insulin inaction in type 2 diabetes leads to insulin toxicity and production of reactive oxygen species which changes the hemodynamic. This causes damage to much tissue especially the vascular endothelium and glomerular basement membrane.[22] This is in consonance with the findings of Dwijo et al., Cathy et al., Juan et al., Joachim et al. and Marisa et al.[4,6,23-25]

Findings from this present study revealed that the serum level of C-RP is high in both diabetic and non-diabetic patients with cardiovascular diseases, while it is relatively low in diabetic and non-diabetic patients with nephritic syndromes (p=0.001). It has been confirmed that C-RP in diabetes plays a central role in pathogenesis of atherosclerosis and athero-thrombotic conditions associated with huge prevalence of cardiovascular disease. It does also predict the risk of cardiovascular event in both diabetic and non-diabetic patients.[26] This result is in consonance with findings of Cathy et al. and Zaid et al.[6,26] However, this finding disagrees with report from Youn-Hee et al. who reported that C-reactive protein production is independent of diabetic complication or occurrence of cardiovascular diseases or nephritic syndrome.[27] Joachim et al. reported that high serum level of CRP is an indicator for diabetic progression toward complication especially in DM associated cardiovascular disease.[24]

This study showed a consistent increase in serum level of IL-6 in DM and diabetic patients with cardiovascular disease. Many studies established that increase serum concentration of pro-inflammatory cytokines IL-6 is associated with insulin resistance diabetes and is related to progression of vascular thrombotic accident, endothelial damage, cardiovascular disease and renal injury.[28-30] This finding is in consonance with reports from Weijiang et al. and Ingrid et al. that there is high concentration of IL-6 in diabetic, cardiovascular disease and patients with nephritic syndrome.[31,32]

This study showed that serum concentration of TNF-α is lower among DM than non-diabetic subjects. However, high TNF-α levels were recorded in DM patients with cardiovascular disease as compared with other groups and controls. This could be because TNF-α is associated with progression of vascular thrombotic and endothelial damage as seen in many cardiovascular diseases.[31] Thus, an anti-TNF-α drug can provide a promising therapy for insulin resistance in pre-diabetic stage. This is in agreement with findings from Weijiang et al. and Ingrid et al.[31,32]

Findings from this study showed that the serum concentration of IL-4 was significantly higher in DM and non-diabetic patients with cardiovascular diseases, but no significant difference was observed in diabetic and non-diabetic patients with nephritic syndromes. This could be because IL-4 has been demonstrated to be involved in immune-modulation of Th1 and Th2 in DM.[32]

There was no significant difference in the IL-10 expression all group of subjects studied. However, it was relatively high in non-diabetics with nephropathy. Even though IL-10 has anti-inflammation role, findings from a study did not associate IL-10 with nephropathic complications.[14] Notwithstanding, a cohort prospective study might give better insight into the end effects of IL10 levels in these categories of subjects.

Conclusion

Findings from this study revealed the association of complement, neutrophil phagocytic function, CRP and IL-6 among septic diabetic patients. In addition TNF-α and IL-6 expression was higher in DM patients with cardiovascular disorders.

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ORIGINAL PAPER

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Factors determining the level of knowledge about parabens in cosmetics

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ABSTRACT

Introduction. Parabens are preservatives that can be found in all types of cosmetics.

Aim. Analysis and evaluation of sociodemographic and psychosocial factors related to the level of knowledge of young people about parabens in cosmetics.

Material and methods. Three hundred students from randomly selected colleges in the Subcarpathian Province took part in the study. The author's questionnaire was used, which consisted of a statement, a scale of attitudes, and a test of knowledge as well as the Multidimensional scale of Health Locus of Control.

Results. A high level of knowledge about preservatives in cosmetics was possessed by 14 % of respondents. The respondents who read cosmetic ingredients showed a higher level of knowledge about parabens more often than others. Young people were most often characterized by type MHLC strong-internal, accounting for 34% of the sample, and this group had a higher level of knowledge about parabens more often than the other respondents.

Conclusion. The respondents who thought that their health depends only on them had a high level of knowledge about parabens. Respondents with a low level of knowledge about cosmetic ingredients are the type that increases the influence of coincidence and the type that magnifies the influence of others.

Keywords. cosmetics, MHLC, parabens, preservatives, young people

Introduction

Parabens are preservatives that can be found in all types of cosmetics, many products for infants and children and in food and pharmaceutical products.[1,2] Parabens are homologous esters of p-hydroxybenzoic acid and represent an aromatic carboxylic acid containing a carboxyl group bonded directly to a benzene ring. Parabens are white, odorless, and crystalline and exhibit adequate water solubility to achieve preservative activity. Parabens are absorbed via the gastrointestinal tract and, to a degree, percutaneously.[3] They are biodegradable by a number of nonspecific enzymes in nature, a fact that would suggest a potential environmental benefit in their use.[4] Methylparaben, ethylparaben, propylparaben, 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

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butylparaben are the most commonly used members, independently and in combination with each other or other biocides (Fig.1).[5] The paraben family has excellent coverage against fungi and gram-positive bacteria. They are more effective against fungi than bacteria, and antibacterial activity is most effective against gram-positive organisms. Evidence of antimicrobial activity for commonly used parabens shows broad inhibition of *E. coli, Pseudomonas aeruginosa, Aspergillus niger*, and *Candida albicans*, with higher inhibition of staphylococcal species, particularly *S. aureus*.[6] Their use has steadily increased. They are now among the most common biocides present in cosmetics. Cosmetic chemists use parabens in their formulations because they have no perceptible odor or taste, are effectively pH neutral, and do not discolor or harden.[7]

Many studies have shown that parabens are the safest preservatives in cosmetics. This status has been achieved because of documented minimal toxicity, low cost, chemical inertness, and near worldwide acceptance.[8] Parabens have been classified as generally regarded safe by the US Food and Drug Administration (FDA).[9] According to the report parabens exhibit a harmful effect on the body only after exceeding the acceptable daily dose. According to the report of the U.S. Food and Drug Administration, the average daily dose of parabens that can be absorbed by a man weighing 60 kg is about 75 mg, of which 1 mg comes from food, 25 mg from medicines and the remaining 50 mg from cosmetics.[10] There is no solid evidence of accumulation within body tissues or organs, although detectable tissue and organ levels of indeterminate duration have been reported. Few studies suggest that parabens penetrate into the body, accumulate in the tissue of the mammary gland. Using deodorants and antiperspirants is particularly dangerous because the parabens from the axillary region are absorbed the most rapidly into the mammary gland. After penetration into the human body, parabens mimic a female hormone – estrogen, thus being able to interfere with the hormonal balance of the body.[11,12]

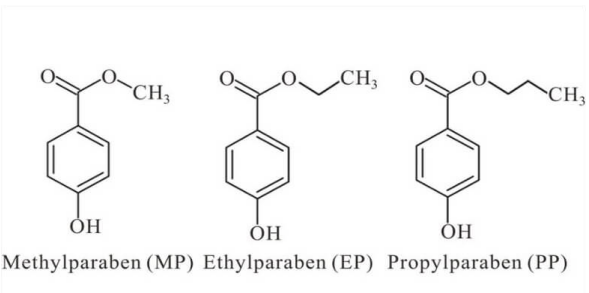

Fig. 1. Structural formulas of parabens

Other studies say that long-term and frequent use of parabens results in their accumulation in the stratum corneum of epidermis, causing disturbances in the morphology of skin cells. Such skin is exposed to UV rays, which react with the Methylparaben accumulated in the skin. This leads to discoloration, skin damage and neoplastic changes.[13] The Scientific Committee on Consumer Safety has reiterated its previous conclusion that the continued use of 4-methylparaben and ethylparaben as preservatives in cosmetics at the maximum authorized concentrations is considered safe for human health at 0.4% for 1 ester or 0.8% when used in combination.[14]

Aim

Analysis and evaluation of sociodemographic and psychosocial factors related to the level of knowledge of young people about parabens in cosmetics.

Material and methods

Organization and the study group

Three hundred students from randomly selected colleges in Subcarpathian Province took part in the study, i.e. University of Rzeszów, University of Information Technology and Management and the State Higher School of Technology and Economics. From among all the students, 100 members of medical, non-medical and cosmetology majors were drawn at random. The division of the students was important for showing the level of the knowledge about parabens. The students from nursing, public health and nutrition majors were randomly drawn to the medical group. The group of non-medical majors included students from the following majors: material engineering, mathematics and computer science. The third group formed students with a cosmetology major. The average age of the respondents was 23.3. The youngest student was 19 years old and the oldest one was 35 years old. The largest group of respondents were the people at the age of 21-25 (57%, N = 171), living in the countryside (59%, N = 177). In this group there were more women – (60.3%, N = 181) than men (39.6%, N = 119).

Research procedure

The research method used in this research was the diagnostic poll method. The author's questionnaire was used, which consisted of a statement, a scale of attitudes and a test of knowledge as well as a standardized the Multidimensional scale of Health Locus of Control tool (MHLC).The first part was respondent particulars, for collecting information about respondents such as: age, education, field of study, place of residence, and interests. The second part was a knowledge test, which was aimed at checking the general level of knowledge about parabens. The test consisted of 20 questions, which concerned: knowledge of the concepts related to parabens, products that most often have parabens in their compo-

sitions, their impact on the human body and the purpose of adding these substances to cosmetics. The third part was the scale of attitudes towards parabens, developed using the 5-point Likert scale. The Multidimensional Health Locus of Control (MHLC) - version B by Wallston and De Vellis in Juczyński's adaptation consists of 18 statements evaluated on a six-point Likert scale. MHLC has three dimensions. The internal dimension determines the extent to which the body is responsible for its own health. The other two dimensions represent man as a person whose aspirations are dictated by the influence of other people or accidental events. In each of the three dimensions, eight combinations of possible connection types can be distinguished: strong-internal, strong-external, diminishing and magnifying the influence of others, diminishing and magnifying the impact of coincidence, undifferentiated strong and weak.[15-16] The study was approved by the Ethics Committee of the University of Rzeszów.

Statistical analysis

For the purpose of this work, for the verification of the hypotheses, the following tests were used for questions on nominal scales: Kramer's V, Phi and in the situation when the dependent variable was on the quantitative/order scale and the independent variable on the qualitative scale, the nonparametric Kruskal-Wallis test (for more than 2 trials) was used. The statistical analysis was carried out using the SPSS program and all relationships are statistically significant when $p<0.05$.

Results

Statistical analysis showed that 2.3% (N=7) of respondents are characterized by a very high level of knowledge about preservatives in cosmetics. A high level of knowledge was presented by 14% of respondents (N=42). A low level of knowledge was shown by 36% (N=108), and very low by 17.3% (N=52) of people. It can be noticed that the age of respondents diversified their level of knowledge about preservatives added to cosmetics (p=0.05, Kendall's Tau-c = 0.08). In the percentage approach, the respondents at the age of 31-35 were characterized by a slightly higher level of knowledge about parabens in cosmetics than younger respondents (5.9%). Comparing percentage figures, it can be seen that students of cosmetology had a higher level of knowledge than students of other majors. The respondents from medical faculties were characterized by a lower level of knowledge about parabens than students of non-medical majors (45% vs. 32%). The respondents who were interested in cosmetology were more likely to have a high and very high level of knowledge about parabens (18%, 10%) than those with other interests (table 1).

The motifs that young people were governed by when choosing cosmetics also diversified their level of knowledge about preservatives added to care products.

Table 1. The general level of knowledge of the subjects on parabens and selected sociodemographic data

		The level of knowledge about parabens											
		Very low		Low		Moderate		High		Very high		Altogether	
		N	%	N	%	N	%	N	%	N	%	N	%
Age	18-20 years	15	20.0	32	42.7	21	28.0	5	6.7	2	2.7	75	100
	21-25 years	29	17.0	56	32.7	58	33.9	24	14.0	4	2.3	171	100
	26-30 years	5	13.5	13	35.1	10	27.0	9	24.3	0	0	37	100
	31-35 years	3	17.6	7	41.2	2	11.8	4	23.5	1	5.9	17	100
	Altogether	52	17.3	108	36.0	91	30.3	42	14.0	7	2.3	300	100
	p=0.05, Tau-c Kednalla=0.08												
Field of study	Medical	19	19.0	45	45.0	28	28.0	7	7.0	1	1.0	100	100
	Cosmetology	17	17.0	31	31.0	28	28.0	18	18.0	6	6.0	100	100
	Non-medical	16	16.0	32	32.0	35	35.0	17	17.0	0	0	100	100
	Altogether	52	17.3	108	36.0	91	30.3	42	14.0	7	2.3	300	100
	p=0.02, V Kramer=0.18, Chi-kwadrat=18.88												
Interests	Cosmetology	8	16.0	18	36.0	10	20.0	9	18.0	5	10.0	50	100
	Medicine	20	15.7	45	35.4	46	36.2	15	11.8	1	0.8	127	100
	Culinary	6	14.3	20	47.6	8	19.0	8	19.0	0	0	42	100
	Hairdressing	4	33.3	2	16.7	3	25.0	3	25.0	0	0	12	100
	Fashion	9	22.0	13	31.7	16	39.0	3	7.3	0	0	41	100
	Other	5	17.9	10	35.7	8	28.6	4	14.3	1	3.6	28	100
	Altogether	52	17.3	108	36.0	91	30.3	42	14.0	7	2.3	300	100
	p=0.04, V Kramer =0.16												

Table 2. The general level of knowledge about parabens and the motives of cosmetics selection

		The level of knowledge about parabens											
		Very low		Low		Moderate		High		Very high		Altogether	
		N	%	N	%	N	%	N	%	N	%	N	%
Reading the composition of cosmetics	Yes, always	3	6.3	8	16.7	17	35.4	15	31.3	5	10.4	48	100
	Yes, sometimes	34	17.0	74	37.0	65	32.5	25	12.5	2	1	20	100
	No, never	12	29.3	22	53.7	6	14.6	1	2.4	0	0	41	100
	I have no opinion	3	27.3	4	36.4	3	27.3	1	9.1	0	0	11	100
	Altogether	52	17.3	108	36.0	91	30.3	42	14.0	7	2.3	300	100
p<0.001, V Kramer =0.24													
The price of products proving the presence of parabens in cosmetics	Yes, the high price guarantees no parabens	11	57.9	5	26.3	0	0	3	15.8	0	0.0	19	100
	The price does not guarantee this	17	9.8	55	31.6	60	34.5	35	20.1	7	4.0	174	100
	I did not think about it	6	9.7	28	45.2	25	40.3	3	4.8	0	0	62	100
	I do not know	18	40.0	20	44.4	6	13.3	1	2.2	0	0	45	100
	Altogether	52	17.3	108	36.0	91	30.3	42	14.0	7	2.3	300	100
p<0.001, V Kramer =0.29													
Emotions related to labeling cosmetics "product contains parabens"	Yes	3	4.3	16	23.2	27	39.1	16	23.2	7	10.1	69	100
	No	26	20.3	45	35.2	38	29.7	19	14.8	0	0	128	100
	I do not read packages	7	17.5	18	45.0	12	30.0	3	7.5	0	0	40	100
	I do not stress because I do not know what parabens are	16	25.4	29	46.0	14	22.2	4	6.3	0	0	63	100
	Altogether	52	17.3	108	36.0	91	30.3	42	14.0	7	2.3	300	100
p<0.001, V Kramer =0.24, Chi-kwadrat=50.21 (df=12)													

Table 3. General knowledge about parabens and MHLC

			The level of knowledge about parabens					
			Very low	Low	Moderate	High	Very high	Altogether
MHLC type	strong-internal	N	8	30	37	22	5	102
		%	15.4	27.8	40.7	52.4	71.4	34.0
	strong-external	N	1	2	1	0	0	4
		%	1.9	1.9	1.1	0	0	1.3
	diminishing the influence of others	N	5	13	14	5	1	38
		%	9.6	12.0	15.4	11.9	14.3	12.7
	magnifying the influence of others	N	3	7	2	0	0	12
		%	5.8	6.5	2.2	0	0	4.0
	diminishing the impact of coincidence	N	5	15	12	9	1	42
		%	9.6	13.9	13.2	21.4	14.3	14.0
	magnifying the impact of coincidence	N	6	7	3	0	0	16
		%	11.5	6.5	3.3	0	0	5.3
	undifferentiated strong	N	8	12	13	4	0	37
		%	15.4	11.1	14.3	9.5	0	12.3
	undifferentiated weak	N	16	22	9	2	0	49
		%	30.8	20.4	9.9	4.8	0	16.3
	Altogether	N	52	108	91	42	7	300
		%	100	100	100	100	100	100
p=0.01, V Kramer =0.20								

The respondents who read the composition of cosmetics more often than others have high and very high knowledge about parabens contained in cosmetics (31.3%, 10.4%).The respondents who thought that the high price would ensure the lack of preservatives in cosmetics were characterized by a very low level of knowledge (57.9%). However, in people who are of the opinion that the price of the product does not guarantee the absence of parabens in cosmetics, there was a higher level of knowledge about them (4.0%). The respondents with a very high level of knowledge about preservatives are stressed to a greater extent than people with a very low level of knowledge when they reach for cosmetics with paraben content (10.1% vs 4.3%) (table 2).

Analyzing the results of the Health Locus of Control, it can be seen that young people were most often characterized by an intrinsically strong type (34%, N=102). Slightly fewer people exhibited the undifferentiated weak type (16.3%, N=49). The type magnifying the influence of others was found in 4% of respondents (N=12), and the type magnifying coincidence in 5.3% (N=16). The subjects with a very high level of knowledge about parabens contained in the cosmetics were much more likely than the others to have an intrinsic type (71.4%). Respondents with a very low level of knowledge more often exhibited the undifferentiated weak type (30.8%) (table 3).

Discussion

The level of knowledge of young people in Poland about preservatives added to cosmetics is very low. Woźniak-Holecka in her study reports that 38.58% of respondents have heard about parabens, but they do not have detailed information about them and cannot describe the impact of these substances on the body.[17] The author's own research shows that only 2.3% of respondents have a very high level of knowledge about preservatives in cosmetics. 45.3% of the respondents have heard about these preservatives, but not everyone knows how these substances work. A survey conducted among young women in South Asia shows that only 10% of the group under study exhibits a high level of knowledge about substances in cosmetics.[18] The fact that people do not read the chemical compositions of cosmetics is also a big problem. Kleszczewska E. and Jaszczuk A. in their study showed that women read labels of products only occasionally (39%), and 22% of respondents do not read them at all.[4] In the author's own study only 16.0% of the respondents admitted that they always read the composition of cosmetics. Research showing the lack of interest in cosmetics labels can be readily found worldwide. Soyun Cho et al. showed that in Korea, 79.2% of residents admitted to buying cosmetics without reading their composition.[19] Noiesen et al., after carrying out a questionnaire survey, concluded that for 46% of people reading the ingredients of cosmetics is too difficult and presented the fact that it is closely related to the low level of education of these people. This stems not only from the low level of knowledge about preservatives in cosmetics, but also from poor knowledge in the area of medical sciences.[20] Azam A. et al. showed that according to customers, the brand and high price will ensure high-class products.[21] The author's own study shows that people with a very low level of knowledge about preservatives added to cosmetics believe that a high price will guarantee them a cosmetic without paraben content. People who know what parabens are and how they impact the human body are aware that in most cosmetics, regardless of their price, one can find preservatives. Our own study showed that there is a considerable correlation between the Health Locus of Control and the level of knowledge about preservatives added to cosmetics. Statistical analysis shows that the majority of respondents are characterized as an internally strong type. These people believe that their health depends only on themselves. These individuals are characterized by a high level of knowledge about parabens. They realize that their level of health depends only on them, which is why they do not opt for cosmetics with preservatives. The subjects of the type magnifying the impact of coincidence and the type that magnifies the influence of other people have a very low and low level of knowledge about parabens to a greater extent. People with these types of MHLC believe that their health depends on other people or is coincidental. They are not interested in the composition of cosmetics until someone makes them interested in this topic or instruct them in the drugstore which cosmetics they should choose. Drugstore and pharmacy assistants play a big role in disseminating information about cosmetic ingredients. Educational materials may be helpful. A study published by Holt. et al. shows that people of the type that magnifies the impact of coincidence and the type that magnifies the influence of others are more sensitive to educational materials than those of the type with inner strength.[22] In the author's own study, 34.3% of the respondents admitted that during the next visit to a drugstore they would ask the assistant about parabens. This indicates that the employees of cosmetics stores enjoy high public confidence and should reliably broaden their knowledge and support clients in choosing the right cosmetics.[21]

Conclusions

The level of knowledge about parabens is low among young people. The low level of knowledge is caused by the lack of interest in cosmetic ingredients and selecting them according to their price. The age of the respondents influences their level of knowledge about parabens. The older the person has more knowledge about

parabens. Respondents interested in cosmetology had a greater level of knowledge about parabens than people with other interests. People who read the composition of cosmetics have a high level of knowledge about parabens. Respondents who think that the high price of cosmetics will ensure the lack of preservatives have a low level of knowledge about parabens. The respondents with a low level of knowledge about cosmetic ingredients are of the MHLC type magnifying the impact of coincidence and the type magnifying the influence of others. The respondents who thought that their health depends only on them had a high level of knowledge about parabens. Teach the public about substances added to cosmetics. Educational materials and social campaigns may be helpful.

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REVIEW PAPER

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Epidemiology of breast cancer in Podkapackie voivodship

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ABSTRACT

Introduction. Breast cancer is the second most prevalent reason for cancer deaths after lung cancer. Incidence rates have increased worldwide.

Aim. To present epidemiology of breast cancer in Podkapackie voivodship.

Material and methods. This analysis was performed using a systematic literature search.

Results. In the Podkarpackie voivodship up until 2014, the incidence rate of female breast cancer was rising and in 2015 it dropped by 68 cases. Even though the survival rate is increasing, it is still one of the leading causes of cancer deaths placing second after lung cancer.

Conclusion. Mortality rate, unfortunately, rose gradually. This shows that even though there is a National Health Program aiming at breast cancer awareness and prevention, it is still not enough to reduce the number of breast cancer deaths.

Keywords. breast cancer, statistics, epidemiology, Podkarpackie voivodship

Introduction

Breast cancer is a very serious problem all over the world. Most frightening is the fact that many deaths could have been avoided had the women been regularly screened. Many factors can contribute to the development of this malignancy, both modifiable and unmodifiable. Genetic factors that are responsible for approximately 10% of cases.[1-21] In the Podkarpackie voivodship during 2011-2014, there was an increase in breast cancer incidence as well as mortality. In 2015, however, the number of new cases dropped by 68. Most patients diagnosed with breast cancer were between 50 and 69 years old but the highest mortality rate was among women over 70. Breast cancer is the most common malignancy diagnosed in

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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

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women.[1-41] In this paper, we decided to present the incidence rate and mortality rate of breast cancer in the Podkarpackie voivodship between 2011 and 2015. Data that we present was collected by Podkarpackie Cancer Register and published in 2018.

Risk factors

There are many studies concentrating on risk factors of breast cancer due to the fact that it is so common. The scientists want to discover as much as possible about this neoplasm following the rule "know thy enemy" in order to prevent future cases or minimize the probability of development of this malignancy. Less than 10% of breast cancers can be attributed to an inherited genetic mutation. Breast cancer is more commonly associated with environmental, reproductive, and lifestyle factors, some of which are potentially modifiable.[21] Among the risk factors are increasing age, race, menarche history, breast characteristics, reproductive patterns, hormone use, alcohol use, tobacco use, diet, physical activity, and body habitus.[1] The incidence rate rises significantly with age, especially among women who are 50 and older. Long-term estrogen use is associated with a substantially increased risk of breast cancer.[10] A similar situation of increased risk occurs with alcohol and tobacco use. Low physical activity and diet poor in nutrients and rich in saturated fats can also lead to development of breast cancer. Obesity is associated with higher risk of breast cancer as well. [27]

Prevention methods

Prophylaxis is crucial in reducing the incidence rate of breast cancer. Regular check-ups and a healthy lifestyle can help save many lives. Early detection of breast cancer through different imaging methods increases chances of survival.

Breast cancer in Podkarpackie voivodship

According to data published in 2018 by the Podkarpackie Cancer Register, there were 665 new cases of breast cancer in 2011. Throughout the following years, the number of new cases rose to 859 in 2014. Surprisingly in 2015 there were 791 new cases. Figure 1 below shows crude rates of incidence and mortality rates between 2011 and 2015.

Among women with diagnosed breast cancer, the majority were between 50 and 69 years old. The highest mortality rate however, was among women aged 70 or more. This was probably because in those cases, it was more probable that the cancer was in a more advanced stage. Data published by the Podkarpackie Cancer Register shows that in 2011-2014, the number of breast cancer cases increased significantly. In 2015 it dropped by 68 cases. The reason for this is unknown. Maybe not all the incidents were reported by doctors to the Register or perhaps the cancer awareness of women was raised and they became more conscious of their lifestyle and health in general. Mortality rate, on the other hand, is gradually decreasing in some developed countries like the UK and US because of the developed technologies used in diagnosis and awareness. But in developing countries like India the situation is not good and some effective steps should be taken in this direction without any delay.[22]

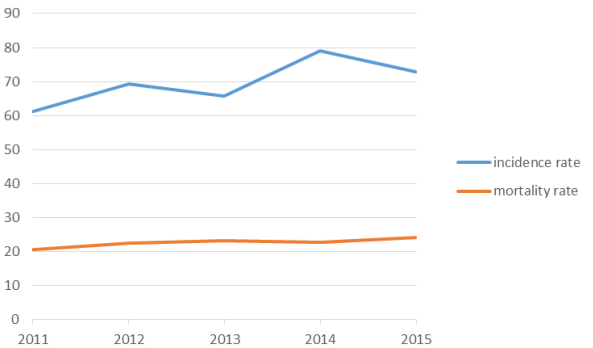

Fig. 1. Breast cancer incidence and mortality rates in Podkarpackie voivodship in 2011-2015, crude rates

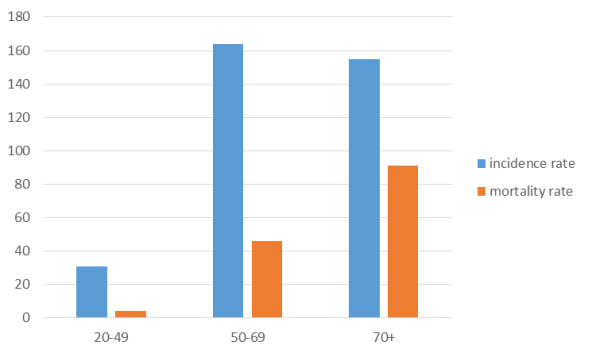

Fig. 2. Incidence and mortality rate of female breast cancer in 2015

Conclusion

In the Podkarpackie voivodship up until 2014, the incidence rate of female breast cancer was rising and in 2015 it dropped by 68 cases. Mortality rate, unfortunately, rose gradually. This shows that even though there is a National Health Program aiming at breast cancer awareness and prevention, it is still not enough to reduce the number of breast cancer deaths.

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REVIEW PAPER

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The use of imaging tests to obtain optimal margins in breast surgery

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ABSTRACT

Introduction. The proper negative margins (R0) breadth in the breast - conserving surgery for invasive breast cancer (IBC) and ductal carcinoma in situ (DCIS) is very important. The presence positive surgical margins (R1) is associated with the necessity of reoperation. It delays the adjuvant therapy and psychologically burdens the patient. The re-operation increases the costs of treatment. The introduction of mammography (MMG) increased detection of DCIS by 20%. With the increase in malignancy, cancer detection decreases in MMG, inversely in MRI groving. Effective preoperative and intraoperative diagnosis aims to reduce the number of R1 resections.

Aim. The size of the tumor next to its biology, determines the clinical course of the tumor. The accurate analysis of imaging tests is important.

Material and methods. This analysis was performed using a systematic literature search.

Results. Adequate surgical margins in breast cancer surgery for breast cancer have been reviewed. It is important to know if the cancer is multifocal and what the extent of the tumor is.

Conclusion. The adequacy of margins is important for adjusting the volume of excision. It is avoiding unnecessary resection of healthy breast tissue. It is essential for a good cosmetic result and the local recurrence rate. The combination of breast MRI with conventional breast imaging resulted in the lover rate of the R1 resectios and the lower rate of the re-operation.

Keywords. breast conserving surgery, ductal carcinoma in situ, extensive itraductal component, invasive breast cancer, lobular carcinoma in situ

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Introduction

Currently, in women with non-invasive breast cancer (DCIS - ductal carcinoma in situ) and in the majority with invasive breast cancer (IBC - invasive breast cancer), a breast-saving procedure is performed.[1-43] It is very important to obtain negative surgical margins (R0). The presence of positive surgical margins (R1) is associated with the necessity of reoperation. The re-operation delays the adjuvant treatment and psychologically burdens the patient. The re-operatin increases the costs of treatment. DCIS is a form of cancer cell proliferation in the breast ducts. DCIS cells do not exceed the basement membrane of the wires. The untreated DCIS almost always passes in the IBC. Until 1980, DCIS represented 1% of detected breast cancers. The introduction of mammography (MMG) increased detection by 20%. The MMG image for the detection of cancer includes microcalcifications, the presence of the nodule, the presence of nodules, asymmetry of the parenchyma, the presence of enlarged ducts, increased density of the parenchyma. The study demonstrated that patients with calcifications have an increased risk of local and distance recurence subsequent to BCS.[13] About 20% of DCIS cannot be detected in MMG. This is due to the small size of the cancer and the lack of microcalcifications. Preoperative breast MRI combined with conventional imaging results in a lower rate of surgical margin involvment and reoperations in patients.[31] Magnetic Resonance Imaging (MRI) detects from 77-96% DCIS and 90-100% IBC. With the increase in malignancy, cancer detection decreases in MMG, inversely in MRI-growing. MRI seems to be a better method for screening breast cancer. However, MRI is expensive and not always available. Currently used mainly to estimate the extent of the tumor before BCS.[1] Surgical margins in BCS are a strong prognostic factor for the recurrence of local cancer. The goal of BCS is to completely remove the tumor and get the best cosmetic effect.[1-20] During this year's conference in St. Gallen found that the width of the margins is not important. In the case of IBC, it is enough that the ink (the method for determining the boundaries of surgical cutting in the postoperative material) will not cover the tumor tissue. This is to remove the smallest amount of healthy tissue. This prevents breast asymmetry and improves the quality of life and patient satisfaction.[21-41] It has been proven that the presence of inc on the tumor surface increases the possibility of cancer recurrence in the same breast.[1-42] For women with DCIS, a margin of 2mm is adequate. The presence of R1 margins in DCIS and IBC greatly increases the possibility of cancer recurrence. This risk does not decrease after adjuvant radiotherapy, systemic chemotherapy or hormonotherapy.[1-30] The presence of R1 margins in LCIS does not increase the possibility of cancer recurrence. It is not certain whether this is similar with pleomorphic LCIS.[3] Young women under 40 have a higher risk of relapse after BCT. This risk is comparable to the risk after mastectomy. Cancers in young women are more aggressive than in older women. This is due to the biology of the tumor.[1-30] In the case of an IBC with a large intra-line component, there is a very high probability that there are numerous DCIS outbreaks. Removal of the tumor with the presence of EIC is associated with recurrences of cancer, especially in young women. Even if the operating margins are R0, MMG or MRI monitoring is recommended. In the case of microcalcification in MMG or changes in MRI, reoperation is recommended. The percentage of reoperations due to R1 resection is up to 38%. Reoperation is a psychological burden for the patient, the therapy is delayed, the cosmetic effect worsens and the costs of treatment are increased. Effective preoperative and intraoperative diagnosis aims to reduce the number of R1 resections. Information on the risk of local recurrence (lobular cancer type, histological malignancy, receptor status, young age) is also important.[41-42] Intraoperative methods for determining surgical margins play a key role. Intraoperative US (Ultrasonography) may cause reduction of positive margins. US provide useful information to the surgeon for incision site and extension of margin.[35,36,37] Endoscopy – assisted breast – conserving surgery (EBCS) was developed about ten years ago. Nowadays some studies have noticed the advantage of EBCS. The oncological outcomes and the aesthetic outcomes admittable. There is the less noticeable scar. A long-term follow – up studies are necessary to inquiry this method. The other findings shoved the poor prognosis of the HER-2 subtype. It is due to increased residua microscopic tumor rests after BCT. This information may help surgeons to choose the most usful surgical treatment.[18]Palpation examination, clip tagging, intraoperative histopathological examination, tissue X-ray, belong to the older methods and they have variable accuracy. Several studies describe the benefits of intraoperative ultrasound and shaving. Intraoperative ultrasound is more time-efficient but poorly detects DCIS. It has been proved that the use of intraoperative ultrasound reduces the amount of R1 resection compared to using clip or palpation. The use of shaving increases the detection of multifocal cancers. These cancers can be non-palpable or invisible in ultrasound.[1-41] A newer method is radio frequency spectroscopy (MarginProbe). An electromagnetic wave is used to identify the cancer tissue. Radifrequency spectroscopy is a new technique that analyzes the specimen. Several technologies such as Spectroscopic Optical, Fluorescence, X-ray, High-Freqency Ultrasound Techniques , Optical Coherence Tomography (OCT) with Interferometric Synthetic Aperture Microscopy (ISAM) can be used as an intraoperative method for analysis margins. OCT generates images that are the same as ultrasound but with

higher resolution. This method significantly reduce the number of reoperations.[43]

Conclusion

The adequacy of margins is important for adjusting the volume of excision. It is avoiding unnecessary resection of healthy breast tissue. It is essential for a good cosmetic result and the local recurrence rate. The combination of breast MRI with conventional breast imaging resulted in the lover rate of the R1 resections and the lower rate of the re-operation.

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REVIEW PAPER

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Nootropics: Phytochemicals with Neuroprotective and Neurocognitive Enhancing Properties

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ABSTRACT

Introduction. Neurological chronic conditions represent a healthcare concern worldwide. They hinder a person's functionality affecting family, social interactions, as well as academic and work performance. In addition, the complexity of these illnesses and the variable response to treatments, as well as the side-effects, call for the research and implementation of phytochemicals known as Nootropics to form part of an integrative treatment.

Aim. To present the influence of nootropics on neuroprotection and neurocognition.

Material and methods. Analysis of literature data found in the PubMed database.

Results. Nootropics, which can be synthetic or natural, possess properties that translate in enhancement of mental or neurocognitive functions. Within the natural options, vitamins, plants and even fungi have been found to produce cognitive enhancement with less side-effects. Continuing research has shown promising therapeutic uses for B vitamins, *Hericium herinaceus* and *Ginkgo biloba* as coadjuvants in the treatment of neurologic chronic conditions to improve an individual's neurocognitive functions and quality of life.

Conclusion. Nootropics open the door for new research, clinical implementation and promotion of integrative health especially in cognitive neuroscience by implementing products of natural sources. This is particularly important in order to identify side-effects, drug-herb interactions, proper posology and synergic actions that may prove to improve neurocognitive functioning and health improvement.

Keywords. cognitive enhancers, phytochemicals, neurologic conditions, B vitamins, *Hericium erinaceus*, *Ginkgo biloba*

Introduction

Attempts to enhance human potential and performance trough "potions" dates back from the ancient Greeks and is still present today, especially in light of the high incidence and prevalence of neurological disorders.[1] Cognitive disorders are majorly characterized by significant memory loss and inability to perform day to day normal activities which affect the life of individuals as well as their caregivers.[2] These conditions have become an emerging challenge to healthcare systems globally owing to the high burden of disease and lack of health-care infrastructure and resources, particularly in

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low- and middle-income countries.[3] The treatment and management of memory loss is highly challenging as no potential remedy is available at present for the complete cure.[2] At present, there is no potential therapy to cure dementia and although the approved therapies improve the symptoms they cannot modify the course of disease.[4] In the absence of effective pharmaceutical options for dementia, complementary medicines have been exhaustively explored.[5]

Neurocognitive Disorders

A wide array of acquired neurologic conditions exist that can produce both temporary and chronic cognitive impairment.[6] In this matter some recovery is expected in conditions like anoxia, stroke or head trauma, whereas Dementia and Multiple Sclerosis are progressive conditions in which a continuous deterioration is expected.[6]

Dementia and Alzheimer's Disease

Dementia is a syndrome comprising over 100 diseases characterized by a decline in cognition that interferes with the various types.[5] Among these types Alzheimer's disease (AD) has been given great attention due to the level of disability it produces. AD is one of the major causes of dementia in an estimated 60–80% of cases.[7] According to the Alzheimer's Association 2017 report, about 5.5 million individuals have AD.[8]

Alzheimer's disease is a heterogeneous disorder of multifactorial etiopathogenic factors with divergent clinical symptomatology, various ages of onset, presence or absence of germline mutations, degree and spread of pathological changes, existence or non-existence of risk factors and manifestation or non-appearance of polymorphic susceptibility alleles.[9] It is characterized by neurodegeneration associated with neuroinflammation.[10] Western medicine has revealed many genetic, cellular, and molecular processes that characterize AD such as protein aggregation and inflammation.[11] During the progression of AD, neurons from different parts of the brain are destroyed, including those areas that enable basic bodily functions like walking and swallowing.[9] Several studies report on the phytochemicals that have been clinically proven with significant anti-AD potentials.[7]

Multiple Sclerosis

Multiple Sclerosis (MS) is a complex and heterogeneous condition from the immunologic, neuropathological and clinical point of view, as well as the way it responds to different therapies. [12,13] This chronic complex neurodegenerative condition that targets the central nervous system (CNS) and is generally believed to be autoimmune in nature.[14] It is characterized by demyelination in the CNS and leads to inability of individuals.[15] MS begins in young adulthood and is more common in females and affects approximately 2.5 million people worldwide.[16,17]

Is has been stated that the etiology of MS is still unknown.[18-20] However, this is not quite correct according to Dobson and Giovannoni.[21] Genetic predispositions combined with environmental influences play an important role its pathogenesis.[18] Also Epstein–Barr virus, sunshine (UVB), smoking and vitamin D, combined with an individual's genetic background, play important roles in the condition's development.[22] As consequence the patient develops physical, neurocognitive and psychological symptoms and deficits.[23] According to Cree, Hollenbach, Bove, et al., long-term worsening is common in patients with Recurrent Remittent MS and is largely independent of relapses or new lesion formation detected by brain MRI.[24] In addition, neurocognitive deterioration is related to emotional problems, in particular, depression.[25]

These conditions serve as concrete examples of neurologic and neuro-immune conditions that affect the nervous system. The consequences are translated in physical, psychological and neurocognitive deficits that significantly affect patients and their families. In facing the reality of their complexity, the limitations of current treatments and the emerging evidence on the use of Nootropics make of them serious factors to be researched and implemented in treatment.

Nootropics

Phytochemicals as well as nootropics are vital cofactors with powerful effects on the body helping it to regain functionality.[12] In the last decade, an increasing number of herbal extracts, poly-herbal and herbo-mineral preparations and phytochemicals obtained from herbs have been studied for their neuroprotective potential in AD.[26] Analysis of antioxidant, anti-inflammatory, and neuroprotective phytochemicals used in various traditional medicines around the world reveal potential to ameliorate and prevent the neurodegeneration observed in AD.[11] This is also true in the case of many neurological conditions, where nootropics have been used. Therefore, research of phytopharmaceuticals obtained from medicinal plants of traditional origin can be beneficial.[2] In consequence, the natural system of medicine is exploring the remarkable benefits from the herbs used in various aspects and one of the aspects include brain function such as improving memory, improving alertness, improving intelligence, improving mental performance etc.[27]

The term nootropic was coined by Dr. Corneliu E. Giurgea in lexical analogy to "psychotropic", and comes from the Greek words for "mind" (noos) and "towards" (tropein).[1,28]They are also referred to as neuroenhancers, smart drugs, memory enhancers, cognitive enhancers, and intelligence enhancers and are used in cognition deficits commonly found in patients suffering from Alzheimer's disease (AD), schizophrenia, stroke, attention

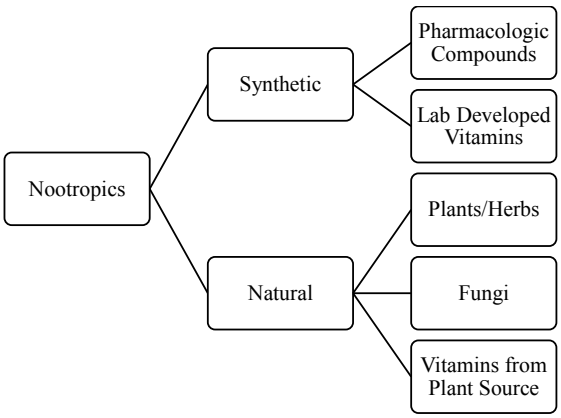

Fig. 1. Nootropic Classifications

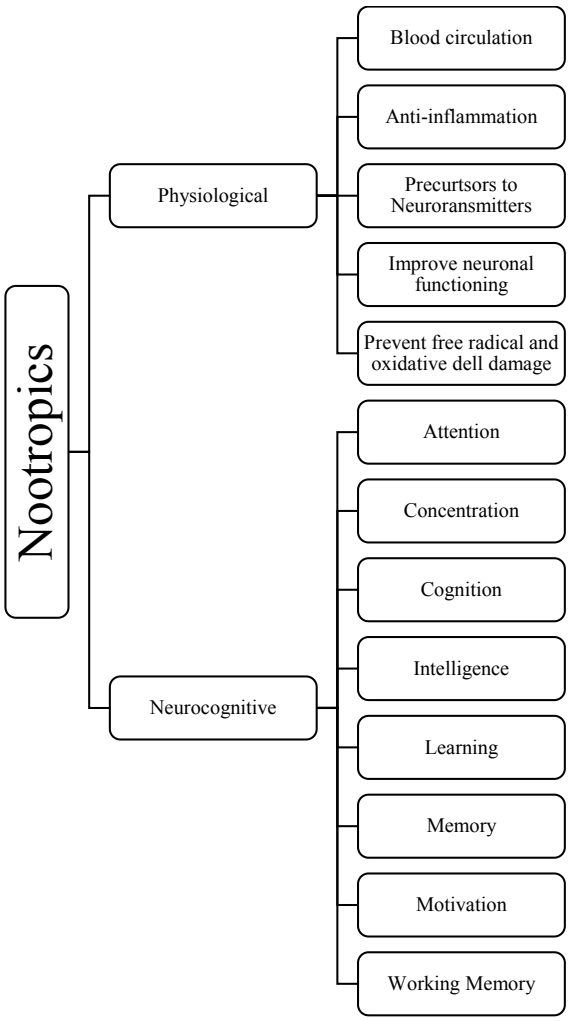

Fig. 2. Nootropic's Physiological and Neurocognitive Enhancing Attributes

deficit hyperactivity disorder (ADHD), or aging.[29-35] According to Margineanu, Dr. Giurgea detailed the definition of Nootropics, stating that they should: [28]

1. enhance learning and memory
2. increase the resistance of learned behaviors/memories to conditions that tend to disrupt them (e.g. hypoxia)
3. protect the brain against various physical or chemical injuries (e.g. barbiturates)
4. increase the efficacy of the tonic control mechanisms of the cortex on the subcortical levels of the brain
5. lack the usual pharmacology of other psychotropic drugs (e.g. sedation, motor stimulation) and possess very few side effects and extremely low toxicity.

There are two different nootropics: a) synthetic, which are compounds created in laboratories such as Piracetam, and b) natural and herbal nootropics, such as *Ginkgo biloba* and *Panax quinquefolius* known as American Ginseng.[36] More specifically, within the synthetic classification, we can include not only pharmacologic compounds, but lab developed vitamins also. Natural nootropics include plants/herb, fungi and vitamins from plant/food sources (Figure 1).

Nootropics are an extensive and structurally heterogeneous class of drugs, supplements, nutraceuticals, and functional foods that enhance one or more aspects of mental function.[27,29] Among the better known aspects (Figure 2) of mental and brain functions enhanced by nootropics reported in the literature are: a) attention, b) blood circulation, c) concentration, d) cognition, e) intelligence, f) learning, g) memory, h) motivation, and, i) working memory.[9,27,29,37]

Nootropics site of Action

The Nootropic site of action is the brain, and they must overcome all barriers to reach brain tissue, and the blood-brain barrier (BBB) is the last critical obstacle for the permeation of drugs or substances that require CNS action.[29] The BBB is a delicate membrane synthesized by the endothelial cells, which makes up the inside layer of cerebral microvessels and consists of brain capillaries that support endothelial cells and are surrounded by astrocytic end-foot processes. [7,29] It is a structure with complex cellular organization that separates the brain parenchyma from the systemic circulation.[29] The BBB controls the entrance of plasma components, red blood cells, and leukocytes into the CNS, while exporting the neurotoxic molecules from the brain to the blood.[7]

Purpose of Nootropics

At this point where natural compounds and neuroscience meet, the mandatory question is why use nootropics? The brain is the center of the nervous system which controls memory, thought, reason, judgment, consciousness and emotion, and supporting its health is vital to ensure successful regulation and coordination of body activities.[27,38] Proper brain functions are essential in the therapeutic approach and treatment of both neurocognitive and mental health conditions. Cognitive enhancers can help the brain work properly.[38] Therefore, the implementation of nootropics as coadjutants in pro-

moting the protection of the brain and nervous system should be taken into account.

Neurodegeneration is a process involved in both neuropathological conditions and brain ageing.[39] On the other hand, neuroprotection refers to the strategies and relative mechanisms able to struggle down the Central Nervous System (CNS) against neuronal damage caused by neuropsychiatric and neurodegenerative disorders such as Alzheimer's disease, anxiety, cerebrovascular impairment, seizures, and Parkinson's disease, among others.[40] However, treatment of these disorders with prolonged administration of synthetic drugs will lead to severe side effects.[40] Therefore, in terms of integrative healthcare, it is imperative to move toward an approach that not only treats, but nourishes the individual's organism in order to improve neurocognitive, emotional and physical functionality.

The therapeutic effect of nootropics is based on positive affection of metabolic pathways in brain tissue (improved utilization of nutrients and mediators) and their impact manifests after some time of administration.[29] They are neuroprotective or extremely nontoxic and act through: a) increasing circulation to the brain, b) providing precursors to neurotransmitters, c) improving neuron function, d) preventing free radical and oxidative damage to brain cells, and e) providing usable energy to the brain and among others.[27] Nootropics improve memory and learning by acting as Ca-channel blockers, acetylcholinesterase inhibitors (AChEI), glycine antagonists, antioxidants, serotonergic, dopaminergic, and glutamic acid receptors antagonists, at the time they exhibit neuroprotective potentials by decreasing the burden of Aβ accumulation, apoptosis, synaptic dysfunction, inflammation and oxidative stress.[9]

Evidence on the Usefulness of Nootropics

In an evidence-based healthcare system the different therapeutics require scrutinized evidence on its effectiveness. Therefore, we ask, is there evidence on the usefulness of natural or non-chemical Nootropics? Within the natural nootropics division there are plants/herb, fungi and vitamins from plant/food sources. Vitamins are organic compounds that are characterized by high levels of potency, so they are only required in very small amounts.[41] The B vitamins, also known as B Complex perform a wide array of important functions throughout the body, like helping to convert food into energy and maintain the immune system, healthy skin, blood cells, the brain, and the nervous system.[42] B Vitamins are vital in cognitive function, however, vitamins which have a significant influence in brain function include: a) Thiamine (B_1), b) Cobalamin (Cyanocobalamine/Methylcobalamine, B_{12}), c) Niacinamide (B_3), d) Folic acid, and e) Choline.[27] In addition several epidemiological studies have exposed that blood concentrations of vitamins B_6, B_{12}, and folic acid are linked to people's performance on tests of memory and abstract thinking.[43]

On the other hand, *Hericium Erinaceus* (HE) also known as Yamabushitake or Lion's Mane is an edible fungus abundant in bioactive compounds that include β-glucan polysaccharides, hericenones and erinacine terpenoids, isoindolinones, sterols, and myconutrients, which potentially have neuroprotective and neuroregenerative properties. [44,45]

According to Jiang, Wang, Sun, et al. the functions of HE on the nervous system are divided into two types: a) HE can regulate the growth and development of the neurons and accessory structures and b) it can coordinate the functions of neurons which are associated with the complex neurodegeneration diseases.[46] On a research conducted by Ratto, Corana, Mannucci, et al., two-month oral supplementation with HE was found to reverse the age-decline of recognition memory in mice.[47] Proliferating cell nuclear antigen (PCNA) and doublecortin (DCX) immunohistochemistry in the hippocampus and cerebellum in treated mice supported a positive effect of an HE on neurogenesis in frail mice. Vigna, Morelli, Agnell, et al., assessed whether a treatment with HE improved depression, anxiety, sleep, and binge eating disorders after 8 weeks of supplementation in subjects affected by overweight or obesity under a low calorie diet regimen.[44] They found out that HE promoted an improvement in mood disorders of a depressive-anxious nature and of the quality of nocturnal rest.

Finally, there is a plant widely used and researched for its uses in cognitive improvement, named *Ginkgo biloba* (GB). This plant contains bilobalide and ginkgolide and has demonstrated antioxidant and vasoactive properties as well as clinical benefits in several conditions such as epilepsy, ischemia, and peripheral nerve damage. [7,48] A meta-analysis of seven randomized controlled trials that consisted of 2,684 patients with Alzheimer- or vascular type dementia indicated that standard measures of overall cognition and activities of daily living improved in those who received *Ginkgo biloba* extract at 240 mg per day, whereas a daily dosage of 120 mg had no effect.[49]

As it can be appreciated, Nootropics from plant, vitamin and fungi sources represent a feasible coadjuvant supplementation in the treatment of neurocognitive conditions. They will serve as functional agents as well precursors that will promote functionality and neuroprotection.

Conclusion

Natural products in general and medicinal plants in particular, are considered an important source of new chemical substances with potential therapeutic efficacy.[37] A number of nootropics are synthetic analogues of physiological compounds like Acetylcholine (ACh), pyridoxine (B_6), GABA, or coenzyme Q_{10}), while others are natural compounds (e.g., vinpocetine), and finally there

are other cerebral-active compounds such as nimodipine, pentoxifylline.[29] These examples of nootropics open the door for new research, clinical implementation and promotion of integrative health especially in cognitive neuroscience by implementing products of natural sources. This is particularly important in order to identify side-effects, drug-herb interactions, proper posology and synergic actions that may prove to improve neurocognitive functioning and health improvement.

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REVIEW PAPER

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Therapeutic possibilities of botulinum toxin in neurological disorders – treatment of limb spasticity in the course of brain damage

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ABSTRACT

Introduction. Botulinum toxin is produced by the anaerobic bacterium *Clostridium botulinum*. The sporulation form of the *C. botulinum* is widely found in the environment (in soil) and may develop in inappropriately stored food. The symptoms of poisoning occur 18-36 hours after consumption of contaminated food.

Aim. The aim of this study is to present the benefits of using botulinum toxin in the treatment of spasticity of the upper and lower limbs in both adults and children.

Material and methods. A literature review of the following databases was carried out: PubMed, UpToDate.

Results. Botulinum toxin interferes with neural transmission by blocking the release of acetylcholine and causes muscle paralysis. The typical symptoms are diplopia, xerostomia, enteroparesis, speaking and swallowing disorders, as well as paralysis of respiratory muscles which leads to death. However, botulinum toxin is also a very potent medication. The biggest application is found in the field of neurology, *inter alia*, in the treatment of spasticity.

Conclusion. The study provides current evidence regarding the safety and efficacy of botulinum toxin injection for spasticity of the upper and lower limbs. Botulinum toxin injections are applicable in the treatment of many neurological disorders and the list of indications will certainly become wider.

Keywords. botulinum toxin, neurology, spasticity

Introduction

History of botulinum toxin

Botulinum toxin was discovered by a German doctor, Justinus Kerner, who carried out experiments with botulinum toxin. In 1820, Kerner published his first monograph on sausage poisoning entitled 'New observations on the lethal poisoning occurring so frequently in Württemberg through the consumption of smoked sausages'.[1] Later, he began animal experiments and experiments on himself to isolate the unknown toxin from sausages.[1] These results were published in a second monograph in 1822 entitled 'The fat poison or the fatty acid and its effects on the an-

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imal organism, a contribution to the examination of the substance which acts toxically in bad sausages'.[1] In 1946, Edward J. Schantz created the first clinical product using the crystallization techniques.[1] Allan Scott was the first to use the toxin to treat strabismus patients - from this episode, the first name of the toxin was Oculinum. Approval by FDA (US Food and Drug Administration), National Institutes of Health, and American Academy of Neurology, was obtained in 1989 to market Oculinum for clinical use in the United States for the treatment of adult (patients over the age of 11 years) strabismus, blepharospasm and hemifacial spasm.[1,2] Since 1989, the effectiveness of botulinum toxin A in reducing spasticity after stroke has been demonstrated, with reversibility and low prevalence of complications, obtaining the approval of the US Food and Drug Administration and European regulatory agencies for this indication.[3] Nowadays, there are eight types of botulinum toxin, labelled A–G, but only types A and B are used for treatment.[4] In Poland, botulinum toxin has been used since 1991. On the market, three 'A' types of preparations are available, although none of them is a generic form. They differ from one another according to the protein content, the number of units and potency.

Botulinum toxin in the field of neurology

In neurology, botulinum toxin is injected into affected muscles or glands using a needle. Before the injection, botulinum toxin must be dissolved in 0.9% saline solution.[4] Injection of botulinum toxin into affected muscles blocks the presynaptic release of acetylcholine from motor endplates of the lower motor neuron at the myoneural junction, and decreases tone by limiting muscle contraction.[5] Intraglandular administration of botulinum toxin acts at the neuroglandular junction to block the secretion of saliva and sweat by inhibiting the release of acetylcholine from presynaptic motor neurons.[4] Botulinum toxins A and B interfere with SNARE protein complex.[4] Botulinum toxin A cleaves the host protein SNAP-25, whereas botulinum toxin type B cleaves synaptobrevin.[4,6] The cleaved SNAP-25/synaptobrevin is unable to mediate fusion of vesicles with the host cell membrane, and it is impossible to release the neurotransmiter acetylcholine from axon endings.[4,6]

The biggest application of botulinum toxin has been found in neurology.[7-11]

The mechanism of the action of botulinum toxin can be described as chemical denervation. The beginning of the action is observed 2-5 days after the administration of botulinum toxin and lasts for 2-3 months.[12] The restoration of muscle function is related with reinnervation and formation of new synaptic contacts (sprouting of nerve terminals).[4]

Botulinum toxin is also used for the treatment of pain syndromes affecting secretion of pain mediators (substance P, glutamate and calcitonin gene related protein (CGRP)) from the nerve endings and dorsal root ganglions, reduces local inflammation around the nerve endings, deactivates the sodium channel, and exhibits axonal transport.[13]

There are few contraindications which refer to the administration of botulinum toxin. The most important are: conditions which show disorders connected with neuromuscular transmission, myasthenia gravis, Lambert–Eaton myasthenic syndrome, pregnancy and breast-feeding, allergy to medication, and infections at the injection site.[14]

Botulinum toxin should not be combined with aminoglycosides, penicillamine, quinine, chloroquine and hydroxychloroquine, calcium channel blockers and blood thinning agents e.g. warfarin or aspirin.[14] Anticoagulation only marginally increases the hematoma frequency, provided INR is controlled and appropriate injection techniques are used.[15] Caution should be exercised while administering botulinum toxin to patients with amyotrophic lateral sclerosis, neuropathy, myopathy, dysphagia, respiratory failure or low body weight. Although botulinum toxin is a strong neurotoxin it is a safe medication. The most common adverse effects are injection pain and local oedema, erythema, transient numbness, headache, malaise or mild nausea.[14] The most feared adverse effect is temporary weakness/paralysis of nearby musculature caused by the action of the toxin.[14] The weakness induced by injection with botulinum toxin A usually lasts about three months.[14] Patients receiving injections into the neck muscles for torticollis may therefore develop dysphagia.[14] This usually lasts a few days or weeks.[14] Other systemic side effects include an influenza-like illness and brachial plexopathy.[14]

An important problem arising from the administration of botulinum toxin seems to be systemic immunization with the formation of antibodies against the proteinaceous molecules of botulinum toxin, which leads to resistance to treatment.[16] Patients who receive higher individual doses or frequent booster injections seem to have a higher risk of developing antibodies.[14] It is important to distinguish between immunogenicity and the clinical classifications of secondary non-response (patient initially responds to therapy, but then loses clinical responsiveness over time with repeated injections) and primary non-response (patient fails to respond to the first and any subsequent administration of a therapy).[16]

During the last 30 years, since botulinum toxin started to be applied in the treatment of neurological disorders, the list of indications has become wider. It is undoubtedly a breakthrough in the treatment of many neurological symptoms and disorders where previously, before the 'botulinum toxin's era', medicine was found to be helpless.

The reason for the increased therapeutic application of botulinum toxin A is due to its marked prolonged clinical efficacy and proven safety record.[17]

A definite limitation in the treatment using botulinum toxin is the cost of therapy. The medication is expensive, and additionally requires frequent injections. In Poland, the National Health Fund (NFZ – Narodowy Fundusz Zdrowia) reimburses the cost of treatment of some disorders. There are four treatment programs:

1. Treatment of spasticity in cerebral palsy.
2. Treatment of focal dystonia and hemifacial spasm.
3. Treatment of upper limb spasticity after stroke – since 2014.
4. Treatment of lower limb spasticity after stroke – since 2017.

Application in neurology:
DYSTONIAS
- Blepharospasm
- Torticollis
- Mogigraphia
- Hemifacial spasm
- Laryngeal dystonia, dysphonia
- Bruxism
- Meige's syndrome
- Dystonia which is responsive to L-dopa
- Dystonia musculorum deformans
- Idiopatic and symptomatic dystonias

SPASTICITY
- After craniocerebral and spinal trauma, after ischemic and hemorrhagic stroke
- In multiple sclerosis
- In cerebral palsy

Spasticity is a velocity-dependent increase in muscle tone as a part of the upper motor neuron syndrome and is seen in a wide variety of neurologic diseases.[18] The most frequent causes of damage to the upper motor neuron in adults include: stroke, injury and multiple sclerosis. In children, the most frequent cause of spasticity is cerebral palsy (CP).[19] The Modified Ashworth Scale is commonly used to grade spasticity. It is useful for the assessment of specific muscle groups before and after botulinum toxin administration. The medication can be injected into the specified site under USG supervision.[20-23] It ensures safety and precision of the application. In some cases, EMG can also be used. A prospective, blinded study in Denmark showed that botulinum toxin treatment guided by EMG improves the outcome of the treatment of torticollis.[24]

HEADACHES
- Migraine
- Neuralgia
- Tension headache

CHRONIC PAIN SYNDROMS
- Pain of the small of the back which is connected with increased muscle tone
- Fasciomyalgia
- Fibromyalgia
- Syndromes with neuromuscular conflicts
- Local pain syndrome

AURICULOTEMPORAL SYNDROME (FREY'S SYNDROME)
FACIAL NERVE PARALYSIS
NYSTAGMUS
DRY EYE SYNDROME
SIALORRHEA
ESSENTIAL TREMOR
STIFF-PERSON SYNDROME
NEUROGENIC BLADDER
MYOCLONUS OF PALATE
EXCESSIVE SWEATING

Clinical application of botulinum toxin in other fields of medicine

Botulinum toxin is also used in other medical specialties, not only in neurology.

In ophthalmology: strabismus, nystagmus, oculomotor disorders.[25,26]

In gastroenterology: swallowing disorders, achalasia, pyloric stenosis, Hirschsprung's disease, dyskinesia of biliary tracts, anal fissure.[27-29]

Botulinum toxin treatment

Botulinum toxin type A is a first-line treatment for post-stroke spasticity.[30] Its injection into the muscles brings about satisfactory outcomes, which has been confirmed by many studies.

In one of the randomized, placebo-controlled, double-blind trials conducted by the International Abobotulinum toxin A Adult Lower Limb Spasticity Study Group, the effectiveness was compared between the effect of abobotulinum toxin A and placebo, with respect to the reduction in the lower limbs muscle tone in adult patients who ≥ 6 months ago had undergone stroke/brain damage.[31] It was confirmed that in the case of chronic hemiparesis, a single administration of abobotulinum toxin A decreased muscle tension.[31] The subsequent injections of abobotulinum toxin A during the year were well tolerated by patients, and improved the speed of walking on foot, and the probability of adjustment to the social conditions.[31]

In a systematic review and meta-analysis of studies concerning the use of abobotulinum toxin A in the treatment of lower limb spasticity of various etiology, published in Medicine*, from among 295 records, six randomized clinical trials were selected and evaluated which verified the action of abobotulinum toxin A.[32] The data collected from these studies provided a scientific basis for the use of abobotulinum toxin A in order to reduce spasticity in the muscles of the lower limbs.[32] A statistically significant reduction in the muscle tone

versus baseline values was achieved for the majority of evaluations performer using the MAS (Modified Ashworth Scale).[32]

At present, abobotulinum toxin A is the only preparation accepted by the US FDA for the treatment of lower limb spasticity in pediatric patients aged ≥ 2 years.[33] Intramuscular administration of abobotulinum toxin A has been approved, based on the results of phase 3 of clinical trials in children with lower limb spasticity caused by cerebral palsy.[33] In this sample, a single cycle of treatment was applied using abobotulinum toxin A at a dose of 10-15 U/kg/leg injected into the gastrocnemius muscle and the soleus, considerably improved the tone in the ankle plantar flexor muscle (the main end point).[33] In the recipients of abobotulinum toxin A, a significant response to treatment was observed, compared to placebo.[33]

A randomized double blind controlled trial carried out to determine the safety profile of Incobotulinum toxin A in children with cerebral palsy and gastrocnemius muscle spasticity, demonstrated that there were no significant differences in the frequency of occurrence of adverse effects, compared to the administration of abobotulinum toxin.[34] The study included 35 patients aged from 3-18 years, who were divided into two groups – the control group – 18 children, and the study group – 17.[34]

A systematic review of literature concerning the use of abobotulinum toxin A in the treatment of lower limb spasticity after stroke in adults showed, based on 9 of 12 randomized clinical trials, the high efficiency and safety of such a treatment.[35] The doses of the toxin were from 500 – 1,500 U.[35]

As mentioned above, botulinum toxin is also used in the treatment of spasticity in the course of cerebral palsy. Cerebral palsy is the most common musculoskeletal disability in childhood[36], and it has a worldwide incidence of approximately 2-2.5 cases per 1,000 live births.[37] It is classified physiologically on the basis of predominant tone into spastic, choreoathetoid and ataxic types, with spastic type being the most common, accounting for 80% of the cases.[37] In a Japanese study, sequential physical changes were examined after injection of botulinum toxin A.[38] Nine children with cerebral palsy participated in the study.[38] Measurements were taken of the maximum bending angle and maximum extension in the hip, knee, and ankle joints, step length, walking speed; the observed speed was determined using the Foot Contact Scale (FCS) and the Physician's Rating Scale (PRS).[38] The lower limb range of motion (ROM), Modified Tardieu Scale (MTS), knee joint extension torque, and Gross Motor Function Measure-66 (GMFM-66) were also measured.[38] The measurements were performed before treatment, after 4, 8 and subsequently 12 weeks.[38] A significant increase in the outcomes of treatment with respect to measurements in the ankle joint were observed after 8 weeks, and in the knee joint after 12 weeks. This demonstrates that the effects of action of botulinum toxin do not occur instantly at early stages of treatment.[38]

However, this is not the only study which proves the effectiveness of administration of botulinum toxin in the treatment of spasticity in the course of cerebral palsy. In the study presented below, injections of botulinum toxin A were performed under USG control in muscles of the lower limb (adductor longus, gracilis, medial hamstring muscles, gastrocnemius and soleus).[39] The dose of toxin did not exceed 12 IU/kg.[39] The study included 25 children aged from 3 - 16 years, suffering from unilateral (2) or bilateral cerebral palsy (23).[39] Spasticity in the knee and ankle joints decreased at week 4 and 12 of control, compared to the period prior to treatment.[39] After 12 weeks, spasticity in the hip joint was also reduced.[39] An improvement of the motor functions according to the Gross Motor Function Classification System was observed at weeks 4 and 12 after the administration of botulinum toxin A.[39] During the whole procedure, no adverse effects were noted.[39] In addition, 3 patients, when asked about the perception of pain accompanying spasticity, mentioned that this pain decreased after treatment.[39]

A study was also conducted in Poland to determine the effectivness of repeated administration of botulinum toxin to children suffering from cerebral palsy. In 2004–2010, 60 children with spastic cerebral palsy, aged 2–16, were treated with abobotulinumtoxin A injections (in the gastrocnemius and soleus muscles).[40] Thirty patients were diagnosed as tetraplegic, 20 diplegic, and 10 hemiplegic.[40] In each patient, muscle tone was rated by the Modified Ashworth Scale, passive range of motion in ankle joint, with extended, and flexed knee joint and gait using the Physician Rating Scale.[40] Assessment was performed before and after 8 injections (average dose of injection - 13.2 j/kg/mc).[40] The study showed that the abobotulinumtoxin A injections were effective in children with cerebral palsy, regardless of the number of sessions.[40] The best results were obtained in children under the age of 7 with hemiplegia, and greater impairment than level I on the Gross Motor Function Classification System scale.[40] It was proved that the treatment gain was highest up to 3 months after the injection, and for this reason abobotulinumtoxin A therapy can be safely and effectively repeated every 3–6 months.[40]

Also, a study concerning treatment of spastic equinovarus foot in 23 patients who had undergone stroke, confirmed that an improvement in mobility may be observed 4 and 12 weeks after the injection of botulinum toxin A under USG control.[41] The injections were performed into the following muscles: gastrocnemius, soleus and tibialis posterior.[41] The BoNT-A was injected at 2 sites, with 25 U each for the GC medial head, GC lateral head, S, and TP.[41] No adverse effects were observed in any of the patients.[41]

Botulinum toxin is recommended for use in many clinical cases, and although there is still no consensus about the moment when therapy with the toxin should be undertaken, and how long it should last; nevertheless, it is considered a first-line treatment in the case of focal spasticity.[42] In order to adjust the proper dose of the toxin, it is necessary to regularly monitor the degree of increased muscle tone.[42] The most commonly applied scale is the Modified Ashworth Scale (MAS), where the resistance while passive muscle stretching is assessed at 5 points according to an ordinal scale).[42] However, this scale is not sufficiently reliable, because it has no standardized speeds of elongation of the muscle fibers, does not provide the determination of an overall muscle resistance and, to a great extent, the result depends on the method of performing the examination by a physician. In addition, it concerns only the distal parts of the body, and is not sensitive to slight changes in the muscle tone. The instrument which enables, in a painless and non-invasive way, a quantitative and objective assessment of the properties of muscles is the MyotonPRO®.[42] An article published in Toxins® concerned a retrospective study conducted in order to confirm the safety and effectiveness of the use of Incobotulinumtoxin A at doses from 100 – 1,000 UI in the treatment of spasticity, according to the individual needs of patients.[42] The patients were divided into 3 groups, according to the dose of botulinum toxin A they received. During observation, some patients were assigned to another group due to an increase in the dose of the toxin administered.[42] It was confirmed that a long-term therapy with Incobotulinumtoxin A at a dose up to 1,000 UI is safe.[42] The reported adverse effects, such as transient generalized weakness or dysphagia, were rare.[42] Moreover, the study proved that a repeated long-term therapy (2 years) with botulinum toxin A does not lead to any reduction in the clinical efficiency caused by the formation of antibodies against botulinum toxin A, and/or auxiliary substances in a pharmacological preparation.[42]

Conclusions

This systematic review of studies concerning the use of botulinum toxin in the treatment of spasticity of various etiology, both in adults and children, confirms the great benefit from the introduction of botulinum toxin into the treatment.

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REVIEW PAPER

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Multi Drug Resistant Tuberculosis

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ABSTRACT

Introduction. Tuberculosis is one of the oldest infections known to mankind. Of all infectious diseases, tuberculosis causes the most fatalities of any infection. The incidence of tuberculosis on the rise due to the increased prevalence of HIV infection. The incidence of drug resistance strains of mycobacterium is also on the rise. When the mycobacterium is resistant to both INH and rifampicin it is called multi drug resistant tuberculosis. There is a primary and an acquired type of drug resistance. Multidrug resistant tuberculosis is a not only a problem for the patient but also for society at large. The treatment of multidrug resistant tuberculosis requires an entirely different approach.

Aim. In this review, we are going to describe the etiopathogenesis, diagnosis, investigations and treatment of multi drug resistant tuberculosis.

Material and methods. Analysis of the current literature.

Results. Genetic factors, previous treatment, and other factors predisposes the onset of drug resistance. By early detection and prevention of spread of drug resistant strains we can prevent the spread of resistant strains.

Conclusion. Drug resistance in tuberculosis is a very complex and dangerous problem. We have to prevent the development and spread of MDRTB. Good quality drugs should be used and made available to all sections of the population. Enhancing the National tuberculosis programs is the best way to attain an effective way to control this menace.

Keywords. BACTEC, MDRTB, multi drug resistant tuberculosis, *Mycobacterium,* tuberculosis

Introduction

Tuberculosis is believed to be as old as mankind's documented history. It is associated with poverty, malnourishment and poor hygiene and hence is more common in developing countries.[1] The incidence is gradually increasing all over the world. There is resurgence of tuberculosis associated with emergence of HIV infection worldwide. One third of cases are found to live in South East Asian countries.[2] It is the single largest cause of death due to infection. The incidence varies in different parts of the world from 10 per 100,000 (North America), 100 to 300 per 100,000 (Asia and Western Russia) and more than 300 per 100,000 people in central and Southern Africa.[3]

Tuberculosis is caused by *Mycobacterium tuberculosis*. There are two strains of *Mycobacterium tubercu-*

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losis, human and bovine type. Most human infection is caused by the human type. Bovine type produces gastrointestinal infection especially in persons consuming unpasteurized milk. Initially mycobacterium was thought to be a bacteria living in the soil which caused tuberculosis like disease in animals. After man started domesticating cows the disease got transmitted to man. However, sometime later this theory was disproved by studying the genome of bovine and human species.[4] It was thought that migration of man to different regions helped in the spread to various parts of the world. Indo-Europeans spread TB to Asia and Europe.[5] Robert Koch was first responsible for identifying tubercle bacilli. According to Rene Dubos, environmental factors also play major role in the causation of TB.[6]

Pathogenesis and clinical features

Tuberculosis usually occurs as an aerosol spread. Sometimes it can occur following drinking unpasteurized milk, but is very rare nowadays. Hence, the most common form of TB is pulmonary. Extra pulmonary TB usually occurs as a result of spread from the pulmonary site. According to Walleran, there are four stages in the pathogenesis of TB. During the first 2 to 8 weeks following inoculation the bacilli enters into the lymphatic circulation and reaches the regional lymph node – Gohn complex. In the next stage, there is hematogenous dissemination of the bacilli to different parts of lung and other parts of body. In this stage, fatal disease like meningitis or disseminated tuberculosis can occur and can last for about 3 months. During next 2 years there can be pleurisy. It may be due to hematogenous spread or spillover of bacteria into pleural space. In the fourth and last stage, the disease regresses in most cases.[4,7] In non-HIV infected patients about 3-5 % develop extra pulmonary disease within a year. 3-5% of patients will have a deactivation of the disease later. This stage lasts for about three years. In HIV infected patients, more than 50% will have either a reactivation or get new infection. In either case, the lung is the most common site and disease is severe and progressive as compared to non HIV positive patients.[8]

Common clinical symptoms of tuberculosis are loss of weight, loss of appetite, malaise, etc. In pulmonary TB there will be cough, chest pain and hemoptysis. Back pain, spinal deformities and neurological deficits can occur in Potts disease whereas severe muscle wasting with painful limitation of movements and ankyloses occur in osteo-articular TB. Meningitis is common in neurological involvement. Diarrhea, anemia, weight loss in gastrointestinal variety and matted lymph node enlargement in lymph node involvement.[9]

When the infecting mycobacterium is resistant to both INH and rifampicin it is called multi drug resistant tuberculosis (MDRTB or MDR/RR-TB). Now we will go into the details about the commonly used anti-tuberculous drugs both first and second line and their characteristic features. After that we will discuss about the drug resistance, the mechanisms, investigations, prevention and WHO consolidated guidelines for drug resistant tuberculosis (2018).

Anti-tuberculous Drugs

INH is one of the most common bactericidal drugs used in the treatment and prevention of TB. Food interferes with its absorption. It is metabolized in the liver. It acts by inhibiting cell-wall synthesis. Rifampicin is another bactericidal drug. It inhibits DNA-dependent RNA polymerase enzyme. It is also metabolized in liver. According to concentration, pyrazinamide can be either bactericidal or bacteriostatic. It can be used for the treatment of MDRTB.[10] Ethambutol resistance can occur when administered to previously given patients. This can be prevented by administering along with a second line drug. Ethambutol inhibits production of various metabolites in the bacilli. Because of ototoxicity and nephrotoxicity, streptomycin is rarely used for the treatment. It is used for short periods along with other drugs when other less toxic drugs are less effective.[11] Levofloxacin is one of the common oral drugs given in MDRTB. It is a safe fluoroquinolone, acts by inhibiting bacterial topoisomerase 4 and DNA gyrase enzymes which are essential for DNA replication, repair and recombination. In MDRTB where the organism is sensitive to fluoroquinolone and other first line drugs cannot be used moxifloxacin is an option. It also acts by inhibiting bacterial DNA gyrase.[12] Rifapentine was an agent used along with INH in DOTS regimen twice weekly in the intensive phase and once weekly in the continuation phase. When used along with other sensitive drugs ethionamide can be used to treat any form of active TB. Ethionamide is a bactericidal or bacteriostatic second line drug according to its concentration. Amikacin and cycloserine are other second line drugs which can be used in MDRTB. Capreomycin is a second line drug which is effective when first line drugs are ineffective or cannot be used because of toxicity. Rifabutin is particularly effective second line drug especially in people with HIV on treatment where rifampicin is contraindicated. Para-aminosalicilic acid can prevent the onset of resistance to INH and streptomycin. When used along with other drugs it is effective in MDRTB. Clofazimineis very rarely used for treating MDRTB.[13,14] Budaquiline is a diarilquinoline which acts by inhibiting mycobacterium adenosine 5-diphosphate synthetase by breaking the pathway for energy generation. It is reserved drug for treating MDRTB when other treatment regimens are ineffective for MDRTB of lung.[15]

In a tuberculosis granuloma, there will be rapidly dividing bacilli and non-rapidly dividing bacilli. The central

caseous area is relatively hypoxic and having low metabolic activity. Here the non-rapidly dividing bacilli become non-dividing. They are called persisters or dormant bacilli. They cannot be killed by usual drugs or body's immune mechanism. They get reactivated once immune system is weak. Prolonged treatment is necessary to eradicate the non-rapidly multiplying or dormant bacilli. Both these types are resistant to bactericidal drugs. But prolonged treatment with second line drugs is showings some promising results. INH is the most potent drug for Sputum conversion and preventing transmission. Rifampicin helps to sterilize the colony by preventing relapse.[16,17]

Drug resistance in tuberculosis

According to WHO, in 2016 there was a 4.1% increase in new occurrences of resistant tuberculosis and about 19% of the existing patients develop resistance to one or more drugs. There are 123 countries from which at least a case of extensive drug resistance is reported (XDRTB). XDRTB means resistance to at least four core anti tuberculosis drugs. It can involve MDRTB. 88% of MDRTB casea are seen in middle or high income countries. Out of these, 60% occur in China, India, Brazil, Russia and Africa. In some eastern European countries, more than one third of cases of tuberculosis is MDRTB. In 2012 more than 90% of notified cases of MDRTB are from 30 countries. There is an increased incidence of MDRTB due to increased incidence of HIV infection.[18]

A combined resistance to both INH and Rifampicin is called multi drug resistant tuberculosis (MDRTB). It can be associated with resistance to other drugs also. To establish the diagnosis of MDRTB the organism must be cultured to get a sensitivity test in-vitro. Most of the MDRTB patients are labelled as treatment failures, re-treatment failures and chronic cases. The problem of MDRTB is not only for the patient themselves but to the entire society due to spread of resistant bacilli in the community.[19]

Primary resistance is the resistance of the bacilli in a patient who has never been exposed to that drug. It can be resistance seen in wild strain or when the bacilli become resistant due to exposure to that drug in another patient earlier. Initial resistance include primary resistance, and the patient can conceal exposure to drugs and developed resistance often unknowingly. Acquired resistance is the resistance developing due to exposure to a particular drug. It is very difficult to establish this diagnosis because it may be bacilli which developed primary resistance in another patient. To diagnose acquired resistance we have to culture and verify the sensitivity of the drug before and after developing resistance which is not possible always. So drug resistance among previously treated patients will be a better term rather than acquired resistance. Resistant strains can grow in a high concentration of antibiotics.[20,21]

Chromosomal mutations occurring in *Mycobacterium tuberculosis* is the reason for drug resistance. They occur at a predictable rate. Resistance to one drug doesn't cause resistance to an unrelated drug because these mutations are unlinked. The number of bacilli in a tuberculosis lesion is about 10 million to 100 million and the chance of developing spontaneous resistance to both INH and Rifampicin is due to spontaneous mutation is very remote. Hence scientists now believe that perturbation in the individual drug target genes is responsible for primary resistance to multiple drugs in tuberculosis. It is said that if there is resistance to Rifampicin there is probability of resistance to other drugs also.[22]

Drug

- Isoniazid
- Rifampicin
- Pyrazinamide
- Streptomycin
- Ethambutol
- Fluoroquinolones
- Enoylacpreductase (inhA)
- Catalase-peroxidase (katG)
- Alkyl hydroperoxidereductase (ahpC)
- Oxidative stress regulator (oxyR)
- RNA polymerase subunit B (rpoB)
- Pyrazinamidase (pncA)
- Ribosomal protein subunit 12 (rpsL)
- 16s ribosomal RNA (rrs)
- Aminoglycoside phosphotransferase gene (strA)
- Arabinosyltransferase (emb A,B and C)
- DNA gyrase (gyr A and B)

Diagnosis of MDRTB

The conventional methods involve culturing the bacilli in Lowenstien-Jensen medium (LJ medium). But the disadvantage is that it takes 6 to 8 weeks for obtaining sensitivity results. There are three conventional methods (1) absolute concentration method (2) the resistance ratio method and (3) the proportions method. In absolute concentration method the minimum inhibitory concentration (MIC) is determined by inoculating the control media and drug containing media with controlled inoculated of Mycobacterium tuberculosis. Mediums containing Sequential two fold dilution of each drug is used. In resistance ratio method the chance of intra and inter observer error is less. Here the MIC of the testing sample is expressed as the multiple of the MIC of the standard strain tested simultaneously. In proportion method there is no need to strictly control the size of inoculums. In this method, the number of colonies grown in the medium with and without drug is compared. There is a critical proportion of number of colonies expected to form from which the proportion of drug resistance bacilli is determined.[23,24]

Other advanced methods

It has been shown that liquid based medium can detect paucibacillary tuberculosis in a shorter time. BACTEC-460 is a liquid based medium, radiometric method. 7H12 medium containing palmic acid labelled with radioactive carbon is used. The amount of carbon dioxide produced due to metabolism of the bacilli is quantified. In this method, the results will be available within two weeks.

The BACTEC – MGIT (Mycobacterium growth indicating tube) is the most common liquid based medium for detection of bacilli from tissue other than blood, urine, and bone marrow culture. The IGT contains Middlebrook medium 7H9 broth supplemented with antibiotics and other enrichment materials. The inoculated tube is incubated at 36 degrees and continuously monitored by BACTEC 960 or manually for 42 days. Mycobacterial growth is detected by fluorescent indicator embedded in silicone at the bottom of the tube. Studies have shown that there are comparable results with both proportion method and BACTEC.[25]

Restriction fragment length polymorphism (RFLP) is a technique used for genome mapping. Here the DNA fragment is divided into pieces by restriction enzymes and divided fragments are separated according to their length using gel electrophoresis. This technique is used to categorize and compare the DNA sequence of *M. tuberculosis*. It is found that DNA fingerprinting of M. Tuberculosis is not changed during development of drug resistance. Hence, RFLP can be used to track the drug resistant bacilli in the community. Recently Fluorescent amplified fragment length polymorphism (FAFLP) is also used for genomic mapping of *M. tuberculosis*.[26]

Luciferase reporter mycobacterophage: Here a suitable mycobacterophage is identified which can infect *M. tuberculosis*. Using genetic engineering techniques, luciferin is taken up by mycobacterophage. Later the cultured sputum sample is infected with phage. If viable bacteria are present, light is produced in presence of luciferin. If there is absence of diminishing light on treating with a particular drug it is indicative of resistance. This technique is a rapid and affordable method of detecting tuberculosis and MDRTB which give results in 2 days.[27] In many parts of the world, rifampicin resistance is considered as a good predictor of MDRTB. FAST plaque TB-RIF is a bacteriophage based test for detecting susceptibility to rifampicin.

Polymerase chain reaction (PCR) is used to identify the genetic basis for drug resistance. PCR can detect recognized mutations and new mutations. PCR is not routinely used for detection of drug resistance, but target mutations of rpo - B are useful for detecting rifampicin resistance and used commonly. Line probe assay is based on a reverse hybridization method. It is also useful for detecting rifampicin resistance. It consists of PCR amplification of rpo-B gene.[28]

Predisposing factors for MDRTB

Genetic factors

It is shown that there is an increase in IL-2 levels and decrease in IL 4 and IL10 in patients with MDRTB. It has been shown that interleukin gene polymorphism is associated with drug resistance in tuberculosis. There is an increased predisposition for MDRTB in patients with HLA DRB*13, HLA DRB*14 in the Indian population and HLA DRBI*08032and HLA DQB1*0601 in the Korean population. The mechanism of resistance is mainly due to a barrier mechanism where there is decreased permeability or efflux of drug and enzymatic degradation.[29,30]

Previous treatment with anti-tuberculosis drugs

Previous treatment failures can relapse after successful treatment, discontinuation of treatment, inadequate treatment, treatment with single drug or addition of drug to a failing regimen. It is very difficult to treat patients with recurrence and treatments remain infective for long time. There is a four-fold association of previous incomplete treatment with MDRTB. Incomplete treatment means discontinuation of treatment during any phase of treatment. The main reason for discontinuing treatment are feeling of well-being after sometime, they feel that the treatment is doing no good to them and when a smear-positive case become smear-negative. Patients with supervised treatment regimen showed less resistance. There is increased risk of toxicity to treatment of MDRTB if they were treated with second line drugs during their previous treatment. According to WHO the prevalence of MDRTB is 22% (newly detected cases) and 60% in previously treated patients. Inadequate compliance to treatment due to any reason can leads to drug resistance. If a person is infected with a strain resistant to a medicine is given additional medicines along with resistant drug chance that the organism developing resistance to other drugs also. Poor quality drugs and inadequate supply of drugs can cause acquired resistance.[31]

Lack of resources for diagnosis and treatment

Tuberculosis is a disease of the past in most developed countries. Most of the burden of tuberculosis is in the countries with poor resources. Out of 9.4 million new cases of tuberculosis in 2008, 60% of cases were in Asia and 33% in Africa. One of the major causes for tuberculosis in poor / developing countries is poverty. It can lead to malnutrition, over-crowding and lack of access to free or accessible treatment. In developing countries lack of laboratory facilities for the diagnosis and lack of medical experts for the treatment of such patients make the problem of MDRTB even worse. Even though chemotherapeutic agents are available, second line drugs are costlier and need to be taken for a lon-

ger time making such treatment inaccessible for most people. More than that empirical treatment with second line drugs without definitely diagnosing drug resistance and lack of compliance can lead to increased prevalence of MDRTB in developing countries.[31]

HIV infection

There is an increased incidence of tuberculosis with HIV infection. The major cause of death in HIV infected patients is tuberculosis. There is an increase incidence of tuberculosis due to prevalence of HIV infection and also an increase in MDRTB. But there is no conclusive evidence to suggest that HIV infection is a cause for MDRTB. There is higher mortality for MDRTB treatment in adults even though the success rate of treatment for MDRTB is same irrespective of HIV infection status.[30,31]

Prevention of MDRTB

The first step to prevent spread of MDRTB is early detection and treatment of drug sensitive tuberculosis and making sure that they adhere to proper treatment regimen. Screening of at risk patients like immunocompromised patients and close contacts of tuberculosis patients should be done for early diagnosis. The causes for non-adherence to treatment must be analyzed and rectified. Social and financial support should be provided at times.

In areas where drug resistance is prevalent, early and prompt detection and proper treatment is essential to curtail the spread of MDRTB. In many low and middle income countries, the absolute number of MDRTB cases is more than the retreatment cases. Unfortunately, there is no routine test for detection of MDRTB in all cases; it is done only in retreatment cases. So a large number of MDRTB cases remain undetected. Hence screening for resistance must be done for new cases also though it is not practical in developing countries.[32]

General health levels and work level conditions needs to be improved. Make the general population well aware of the means of spread and problems of MDRTB. We have to take measures to prevent poverty, overcrowding, malnutrition especially in homeless shelters, refugee camps, nursing homes and boarding schools.

We have to improve the quality of health care. It should be available to everyone and should ensure that high quality medicines are available and accessible. Many countries have regulations which control the availability of first line drugs only through the national tuberculosis program (NTP). It has been found that many first and Second line drugs distributed through the private sector is of inferior quality. Over the counter sale of tuberculosis drugs should be banned. Make tuberculosis a notifiable disease. Government should make diagnosis and treatment of tuberculosis free of cost. There must be social protection schemes for the patients. These can help the patient to adhere to treatment.[33]

Treatment of MDRTB

For the purposes of treatment, drug resistant tuberculosis is divided into rifampicin resistant TB (RRTB) and INH and rifampicin resistance (MDRTB) and MDRTB associated with resistance to other drugs also. The common drugs used for treatment of resistant tuberculosis are classified as follows:

Group A (Fluoroquinolones)

Gatifloxacin, levofloxacin and moxifloxacin are the fluoroquinolone which are very effective in the treatment of RRTB and MDRTB. They should be included in the treatment of resistant tuberculosis unless contraindicated. The use of ciprofloxacin and ofloxacin as second line drugs is no longer recommended. High doses of group A drugs have shown benefits in RRTB and MDRTB with a high safety profile. It can prolong QT intervals so used cautiously with drugs like budaquiline, clofazimine anddelamind which are having the same effect.[34]

Group B (Injectable second line drugs)

Aminoglycosides like amikacin, capriomycin, and kanamycin are commonly included in the long term treatment regimen for RRTB and MDRTB. Due to potential side effects it is not recommended in children unless there is resistance to fluoroquinolones. Unless contraindicated, it should be included in the treatment. Streptomycin is not used as a second line injectable drug routinely. Only when other three drugs cannot be used and if streptomycin is sensitive, it is included in the treatment. We have to monitor the development of ototoxicity and nephrotoxicity when these drugs are used.

Group C (Other core second line drugs)

Ethionamide/prothionamide ,cycloserine or terizidon, linazolid and clofazimine are used in this order of preference. Prothionamide can replace ethionamideandterizidon for clofazimine. The main use of these core drugs is to increase the number of effective drugs in the intensive phase to four. If pyrazinamide cannot be included then other agent is added. Gastrointestinal side effects can occur with ethionamide and prothionamide. When used in combination with PAS can produce reversible hypothyroidism. Cycloserine causes neuropsychiatric side effects. Lactic acidosis, thrombocytopenia, andanemia can occur with linizolid.

Group D

These are agents which are not included in the core group of drugs. These drugs are added to core group of drugs unless there is resistance, no interaction with other drugs or pill burden. They are sub classified as follows.

D1 group

This group includes pyrazinamide, ethambutol and high dose INH. Pyrazinamide resistance is seen associated with RRTB and hence avoided when rifampicin resistance is present. Otherwise pyrazinamide is shown to produce success when added to any regimen. High dose of INH is found to be effective in RRTB. In short course regimens for MDRTB, high dose INH are an integral part of treatment. High dose INH is also effective in children even in HIV positive cases. Due to ocular toxicity, the use of ethambutol must be weighed against other factors.

D2 group

These include two newer drugs. WHO recommends their limited use. Unless a large series of RCTs available, their wide spread use of them is not common. The drugs available are delamind in children and adolescents and budaquiline in adults.

D3 group

These include para-aminosalicylic acid (PAS), imipenam – cilastatin, merepenamclavulanate , amoxicillin clavulanate and thioacetazone. PAS is found to produce poor success in the treatment hence its use is not recommended routinely. Imipenam and amoxicillin are having same adverse effects but they should be always used together with clavulanate. Until 1990, thioacetazone was used as a first line drug but stopped using because of severe skin reactions especially in HIV positive individuals. Hence its use is contraindicated in HIV positive individuals.

The WHO consolidated the guidelines on drug resistant tuberculosis in 2018, by dividing the second line drugs into three main groups.

Group A: Fluoroquinalones (Levofloxacin and Moxifloxacin), bedaquiline and linezolid. These drugs are highly effective and recommended to include in all regimens unless contraindicated.

Group B: Clofazimine and cycloserine or terizidone are conditionally recommended as agents of second choice.

Group C: This includes all other drugs that can be used when a regimen cannot be composed with Group A and Group B agents. These drugs are ranked by the relative balance of benefit to harm usually expected of each. They include kanamycin and capriomycin (not recommended for use in MDRTB regimen), gatifloxacin and high dose isoniazid, thioacetazone (not recommended). Clavulanicacid is used only as companion agent along with carbapenems.

Longer MDR-TB regimen drug composition

To start with a four drug regimen, all three group A agents and at least one group B agent should be included in MDR/RR -TB. At least three agents are included after bedaquiline is stopped. If only one or two agents are included from group A, both group B agents are to be included. If agents from group A and B cannot be included group C agents are added to complete it.

Kanamycin and capriomycin are not included in longer MDR/RR-TB regimen where as we can include levofloxacin and moxifloxacin. There is a strong recommendation for inclusion of bedaquilinein patients older than 18 years. Linezolid should be included in longer regimen. In the case of drugs like clofazimine, cycloserine, ethambutol,pyrazinamide, imipenam-cilastatin, morepenam and amikacin, there is conditional recommendation with very low certainty in the estimates of effects. There is conditional recommendation against the use of ethionamide or prothionamide, para amino salicylic acid in long term regimen. Clavulanic acid should not be included in the treatment of MDR/RR-TB longer regimen. Delamind may be included in long term regimen in children above 3 years

The total duration of a longer regimen treatment is 18 months and can be modified according to patient's response to treatment. At least 15 to 17 months of treatment after culture conversation is recommended for most patients and can be modified according to response to therapy. In longer regimen that contain amikacin or streptomycin an intensive phase of 6 to 7 months is suggested for most patients.

Short term regimen

In MDR/RR –TB patients who have not been treated for more than one month with second line medications used in short term regimen or in patients in whom resistance to fluoroquinolone or second line injectable agents has been excluded, a shorter MDRTB regimen of 9 to 12 months may be used instead of longer regimen.

The monitoring of patients with long term regimen is done using sputum culture instead of sputum smear microscopy to assess the response to treatment. This has to be repeated every month.

For patients with combined HIV and MDRTB, the antiretroviral treatment need to be started as early as possible, say, within 8 weeks of starting second line treatment for MDRTB.

Lobectomy or wedge resection of lung may be used along with recommended MDRTB regimen in indicated patients.

Health education and counselling on the disease and treatment adherence should be provided to patients on TB treatment.

Tuberculosis produces weight loss and cachexia. The exact mechanism is not known. Since tuberculosis affects malnourished and immunocompromised patients. It is more common for MDRTB to occur in such patients. It is said that tumor necrosis factor alpha by its catabolic ac-

tivity can also lead to cachexia. So it is essential to screen the nutritional status of the patients regularly throughout the treatment. Also necessary actions should be taken to improve the nutritional status of the patients.[34,35]

Several other treatments like immunotherapy using *M. vaccae*, using interferons and IL-2 are there for the treatment of MDRTB. But further research is needed before they can be used for treatment.

Conclusion

Drug resistance in tuberculosis is a very complex and dangerous problem. We have to prevent the development and spread of MDRTB. Good quality drugs to be used and made available to all sections of population. Enhancing the National tuberculosis programs is the best way to attain an effective way to control this menace.

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CASUISTIC PAPER

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Open mesh repair of a voluminous recurrent inguinal hernia complicated by strangulation and intestinal obstruction

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ABSTRACT

Introduction. Inguinal hernia is a common surgical pathology in Nigeria but a giant (voluminous) recurrent strangulated inguino-scrotal hernia causing intestinal obstruction is very uncommon. Such a hernia, when it is recurrent and becomes complicated with strangulation and dynamic intestinal obstruction, presents many difficulties in management.

Aim. To present the successful management of a case of a strangulated and obstructed giant recurrent inguinal hernia.

Description of the case. Here we present the case of 47 year old man who had intestinal resection and anastomosis with prolene mesh repair of the posterior wall for a strangulated recurrent large inguinal hernia using the technique of tension free sutured prolene mesh popularized by Lichtenstein

Conclusion. The patient recovered, was satisfied with his care and has been symptom free at 18 months of follow up. Giant recurrent hernias complicated by strangulated and intestinal obstruction are uncommon in Nigeria today, despite our resource-poor status. When they occur, tension free repair with sutured onlay prolene mesh after Lichtenstein, can be a useful and the best option with satisfactory results, as in the case reported.

Keywords. Inguinal hernia, recurrent, voluminous, strangulated, mesh repair

Introduction

Globally, over one quarter of males will have inguinal hernia during their life time.[1] The prevalence of inguinal hernias in adults seems to be the same in both Western countries and countries in Africa at approximately 25% for males and 3% for females.[2,3] However, Africans tend to present late with large, or complicated hernias, and the repair rate is significantly lower at less than 42%.[4-6] The voluminous type, which is defined as the extension of hernia sac to the midpoint of the inner thigh or beyond in the standing position, is rare but occasionally seen in the developing world where factors such as poverty, ignorance of conventional treatment options and phobia for surgery significantly contribute to delay in surgical intervention.[2]

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Giant inguinal hernia starts gradually as a neglected new or recurrent hernia.[7,8] It grows massively and affects the quality of life by interfering with activities like intercourse, walking, urination etc. It is prone to skin excoriation and ulceration.[7] The content varies widely, with the omentum, small intestine, and colon commonly reported.[9] The pancreas, kidney etc. have also been seen by some surgeons.[10] The content may incarcerate, obstruct or strangulate with increased morbidity and mortality.[10]

Inguinal hernia repair remains the most common operation performed by general surgeons all over the world.[11] Surgical procedures for inguinal hernia repair generally fall into three categories: open repair without use of mesh (i.e. sutured, e.g. Bassini, Darning, McVay, Shouldice, Desarda), open repair with a mesh (Lichtenstein, Plug and Patch), and laparoscopic repair with a mesh. The non-sutured "tension -free" open mesh repair is the mostly widely used technique in the world today. However mesh based repairs have not been fully embraced in resource-poor countries because of poor socio-economic status, non-affordability of patients, non-availability of mesh and laparoscopes and lack of appropriately trained staff in most centers. Therefore the traditional tissue based sutured techniques are still widely practiced.[12,13.]

The repair of giant inguinal hernia poses an enormous challenge even among experienced surgeons, because there is loss of domain within the abdominal cavity which can lead to closure difficulty and subsequent increase in intra-abdominal pressure.[7,14] There is also a higher risk of recurrence owing to a large hernia defect.[7] The high risk of recurrence has made Lichtenstein technique of repair the most effective and preferred method of giant hernia repair.[15,16] This tension free method involves covering hernia defect with a mesh (foreign body) .[15] Many surgeons are, however, skeptical about using this method in repairing strangulated hernia for fear of infection.[17-20]

Here we present the successful management of a case of a strangulated and obstructed giant recurrent inguinal hernia using the technique of tension free sutured prolene mesh popularized by Lichtenstein.

Description of the case

A 47-year old man presented to our emergency department on account of a six day history of intermittent lower abdominal pain of increasing severity that became continuous in the last 8 hours. There was associated vomiting, abdominal distention and constipation. There was a huge right inguinoscrotal hernia which was first noticed 32 years ago. Initially, it was small in size at the groin, which was spontaneously reducible and gave no other symptom. It however progressively became massive and on presentation was very large, hanging down the groin and upper right thigh. About 6 months prior to presentation it became incompletely reducible and occasionally painful. He had a bilateral inguinal hernia in his childhood which was repaired when he was 12 years old but the right recurred soon after repair. There was no chronic cough and no straining in passing urine and stool. He is a mason and recreationally partakes in weight lifting. He drinks alcohol and smokes cigarette and cannabis. He is married with children.

On examination, he appeared obese, sick, febrile (39.5°C) and dehydrated. His vital signs were deranged (respiratory rate of 30/minute, pulse rate of 104/minute, and blood pressure of 98mm Hg/60mm Hg). The abdomen was uniformly distended with bilateral groin scars and a huge right inguinoscrotal swelling, which was tense and tender (Fig 1). His penis was buried in the swelling. The prostate was enlarged and rectum was empty on digital rectal examination. A diagnosis of strangulated giant recurrent inguinoscrotal hernia causing dynamic small intestinal obstruction was made.

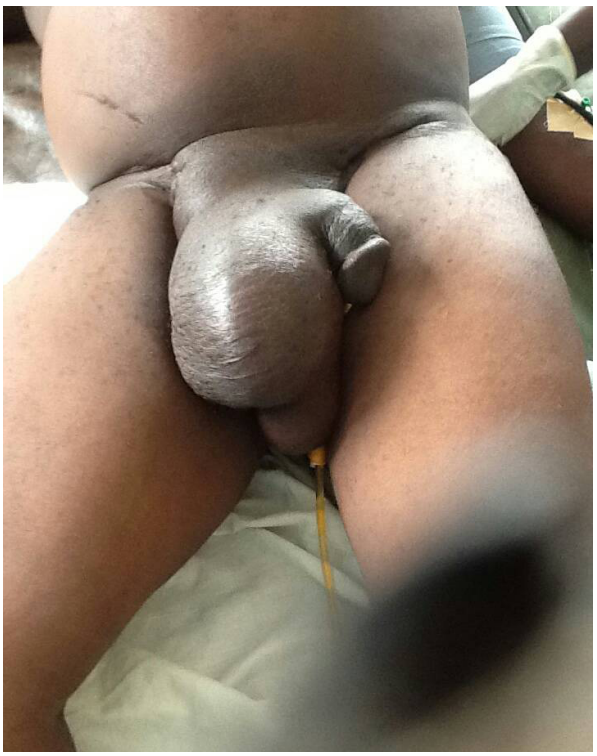

Fig. 1. Patient on the operating table

Immediate resuscitation with intravenous normal saline, intravenous antibiotics (ceftriaxone and metronidazole), nasogastric intubation and urethral catheterization, was commenced. Plain abdominal X-ray and abdominal ultrasound findings were suggestive of small intestinal obstruction. Laboratory investigations show hemoglobin concentration of 16.1g/dL and a white blood cell count of 13.3×10^9/L (neutrophilia). He was moved to the operating theatre for an emergency exploratory laparotomy after obtaining informed consent.

The surgery was done under general anesthesia and it commenced at exactly 6 hours of presentation. Imipenem and metronidazole were administered on induction of anesthesia. Right groin incision was used to minimize intra-peritoneal spillage.

Intraoperative findings include edematous but viable omentum, strangulated ileum; 30cm in length and 60cm from the ileocecal junction, and approximately 500 mL of serosanguinous fluid. Ileal resection and intestinal decompression then ilio-ilial anastomosis in two layers with 2/0 vicryl suture and partial omentectomy were done. Wound lavage with normal saline was also done.

The next task was to repair the hernia defect in the posterior wall of the inguinal canal which was very wide and it was repaired with onlay prolene mesh (Lichtenstein technique).[3] Incision was closed in 2 layers with nylon, continuous for external oblique and loose interrupted for skin.

Post operatively, the patient was nursed in the intensive care unit with intravenous imipenem (500mg 4 hours after surgery then 8 hourly for 72 hours), metronidazole (500mg 8 hourly for 72 hours), subcutaneous clexane (40mg daily for 7 days) and daily wound dressing with normal saline. On the third postoperative day, he began supportive mobilization. Bowel action returned on the fourth day, and was then commenced oral feeding on the following day. On the same 4th day post-operation, psychiatrist input was sought when the nurses reported he was unable to sleep during the night, for which he was placed on nitrazepam 5mg nocte for 2 weeks. His wound healed primarily and he was discharged home on the tenth day post-operation. Follow up visits at two weeks, six weeks, six months, one year and 18 months showed he was well and happy. He was referred to a urologist, on discharge, for evaluation of his enlarged prostate.

Discussion

An inguinal hernia can grow to reach a giant size with time if not repaired early. Our patient had recurrent hernia which he neglected. Recurrence is the most frequent complication of hernia repair and patient seeking repair of their hernia should be made aware of this so that if it does occur, they can return to the hospital for appropriate action.[21] Our patient couldn't seek repair of his hernia when it was still small; this is common in developing world where finance is often a constraint. In addition, he most probably had lost confidence in orthodox treatment after the first repair failed. Strangulation is not a common complication of giant inguinoscrotal hernia owing to the large defect.[22] Many studies observed that the risk of hernia complication increase with age because of comorbidities such as COPD and bladder outlet obstruction (BOO). The later was found in our patient although incidentally since he denied having any symptom suggestive of that (BOO).[23]

Recurrent hernias rarely grow large before causing symptoms due to fibrosis from previous surgery which makes the boundaries of the defect unyielding. In the case presented, we can assume that the initial repair at 12 years of life was a herniotomy with consequently minimal fibrosis to allow growth to a large size before strangulation.

The part of the intestine that frequently gets strangulated in inguinal hernia is the ileum as it has long mesentery, which is at risk of twisting.[3] In our patient, the ileum was involved. Partial omentectomy was added to the intestinal resection to reduce the volume of viscus to be returned to the abdomen. Patient was monitored closely after surgery in the ICU to detect early and vigorously manage any development of abdominal compartment syndrome which can led to mortality. In this syndrome increased abdominal contents can lead to increased intra-abdominal and intra-thoracic pressures which can compromise respiration.

There is continuing controversy concerning the use of mesh in strangulated giant hernias because there is expected increase in the risk of surgical site infection.[18-28] In our patient the possibility of increased risk of surgical site infection was considered hence the escalated use of antibiotics from induction of anesthesia to 72 hours after surgery. We chose open mesh repair for this patient as the only option likely to succeed because tissue repair method would have been difficult and definitely would have recurred. Again we have neither the equipment nor the expertise for laparoscopic mesh hernia repair.

Conclusion

Giant recurrent hernias complicated by strangulated and intestinal obstruction are uncommon in Nigeria today, despite our resource-poor status. When they occur, tension free repair with sutured onlay prolene mesh after Lichtenstein, can be a useful best option with satisfactory result, as in the case reported.

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Adrenomyeloneuropathy – a case report

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ABSTRACT

Introduction. Adrenoleukodystrophy is a genetic disorder linked to the X chromosome, in which the peroxisomal beta-oxidation process is disturbed. It is a metabolic disease that results in the accumulation of very long chain fatty acids (VLCFAs - very long chain fatty acids) responsible for the symptoms of damage to the adrenal cortex, gonads and the brain.

Aim. A clinical case is reported.

Description of case. This article describes the case of a 64-year-old woman who had neurological symptoms for many years, gradually increasing without significant improvement after the treatment (periodic steroid therapy).

Conclusion. Based on tests (including the determination of the ABCD1 gene and very long chain fatty acids - VLCFA), adrenoleukodystrophy was suspected.

Keywords. adrenoleukodystrophy, fatty acids, steroid therapy

Introduction

Adrenoleukodystrophy is caused by the mutation of the ABCD1 gene on the Xq28 chromosome.[1-4] This recessive mutation causes a defect of peroxisomal beta oxidation and the storage of saturated very long-chain fatty acids in all tissues of the body. It is most manifested in the adrenal cortex, myelin of the central nervous system and in Leydig cells in the testes.[4-7] The ABCD1 gene is responsible for proper functioning of the protein ALD, which belongs to transport proteins with an ATP binding cassette.[8-10] In 1997, Moser et al. distinguished seven phenotypes:[1]

Brain children's figure
1. Brain juvenile
2. Brain form of adults
3. Adrenomyeloneuropathy

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4. Adrenal insufficiency without neurological symptoms
5. Asymptomatic form
6. Heterozygotes

The most common phenotype is a childlike brain form that occurs in boys with normal early development. It was described by Siemerling and Creutzfeld in 1923.[3] There is a rapidly progressing demyelination of the white matter of the brain. The boys are asymptomatic, the mean duration of symptoms is 7 years. Initially, the disease is manifested by lack of concentration, hyperactivity, emotional lability.[8-12] Then, ophthalmologic symptoms (atrophy of the optic nerve), auditory (deafness), and coordination problems are added. The progress of symptoms is very fast, which results in a quick transition to the vegetative state - on average, 1-2 years. The basic diagnostic method is magnetic resonance imaging, which shows damage to the white matter of the posterior parietal and occipital areas as well as frontal areas.[12-18] Occasionally, there is also a juvenile figure, similar in its symptoms to the childish form, whose beginning falls on adolescence.

The adult brain form described in 1976 is mainly characterized by spastic paraplegia. There is no brain demyelination here either clinically or pathomorphologically. Additional ailments include cerebellar dysfunction or olive-bridge-cerebellar atrophy. There may be disturbances in the functioning of the adrenal cortex, progressive disturbances of hearing, vision, and headaches.[4]

In 1977, adrenomyeloneuropathy was described, in which hypogonadism, adrenal insufficiency - beginning in childhood, and paraparesis in the third decade of life were described. Additional symptoms associated with the above ailments include neuropathy, impotence, and sphincter disorders.[5]

The diagnosis of adrenoleukodystrophy includes:

1. Family history allows to determine the diagnosis in 95% of cases
2. Determination of serum VLCFA level - the level of hexacosic acid (C26: 0) and its ratio to docosan (C22: 0), rarely tetrakosan (C24: 0) is determined
3. 3) VLCFA determination in skin fibroblasts and red blood cell cultures
4. Genetic examination - analysis of the ABCD1 gene mutation
5. Prenatal tests - determination of VLCFA level or DNA test from cells obtained from chorionic tube or amniotic fluid
6. Screening test - determination of the level of lysophosphatidylcholine C26: 0 in a dry drop of blood
7. MR examination - abnormalities in the MR picture are usually better than clinical symptoms
8. Endocrine examination - may show a decrease in the level of some hormones (ACTH, testosterone, DHEA)
9. In cerebrospinal fluid an elevated level of protein with intratekinal IgG synthesis, pleocytosis
10. Extended visual evoked and auditory evoked potentials from the brainstem

Description of the case

A 64-year-old female patient was admitted to the Department of Neurology due to the worsening of the lower limbs, dizziness, and disturbances of the balance, which had been increasing for 2 months. In addition, she reported recurrent headaches in the temporo-parietal region of a stabbing nature, usually on the right side, short-term memory disturbances, difficulty in finding words, and incontinence. The above-mentioned complaints have been occurring for about 39 years and have been intensified periodically. The patient was repeatedly hospitalized and diagnosed in the direction of multiple sclerosis, despite the absence of demyelinating lesions in imaging studies. Family history showed similar symptoms in the patient's daughter - the family underwent genetic testing, which revealed a mutation in the ABCD1 gene. In the neurological examination on the day of admission to the ward: conscious patient, logical contact, auto and allopsychic, psychomotor slowing, negative meningeal symptoms, nystagmus absent, bilateral temporal temporal vision limitation with greater severity on the left side, in the range of the remaining cranial nerves pathology, discrete paresis of the pyramidal type of lower limbs, increased muscular tension of the lower limbs spastic type with greater severity on the left side, knee and step reflex increased with greater severity on the right side, Babinski's symptom is present on both sides, Openheim's symptom on the right, left side +/-, deformation of the lower limbs, feet hollowed with greater severity on the left side, dysmetria in the upper limbs with greater severity on the right side, Romberg's test lability without direction, skin reflexes absent, and symptoms of absent deliberation.

During the hospitalization, the department performed basic laboratory tests that showed hypercholesterolemia (statins were included in the treatment). The level of folic acid and vitamin B12 is correct. ACTH concentration and diurnal cortisol profile in the norm. The corticotropin stimulation test was not performed due to lack of preparation (Synacthen). In the magnetic resonance imaging of the head, single focal lesions of a vasogenic / demyelinating character were imaged in the white matter of both brain hemispheres, and in the deep left brain hemispheres, focal lesions of 15/13/8 mm with the presence of hemosiderin deposits - after hemorrhage into the cavernous haemangioma. The diagnosis was supplemented by an electroneurographic study that revealed sub-acute sensory-motor neuropathy of the axonal type. Based on the clinical picture, family history and additional tests (including the de-

termination of the ABCD1 gene and very long chain fatty acids - VLCFA), suspicion of adrenoleukodystrophy was suspected.

Discussion

In the patient for many years, it was suspected that these symptoms are indicative of multiple sclerosis despite the lack of previous demyelization typical for this disease entity. Therefore, multiple sclerosis should always be included in the differential diagnosis of adrenoleukodystrophy. In addition, in any person with Addison's disease, adrenoleukodystrophy should also be considered because of the typical symptoms resulting from damage to the adrenal cortex. It is noteworthy that in the family members of the patient, genetic tests for the mutation of the ABCD1 gene were positive. In adults, the course of the disease is significantly slower compared to children, hence the complaints accompanied the patient for many years. Although the disease due to the method of inheritance should affect only men, the patients may be in mild or moderate form, as in the presented patient. There is no specific treatment for the abovementioned unit. The very avoidance of VLCFA in the diet does not lead to biochemical changes due to their endogenous synthesis.

Although the prevalence of adrenoleukodystrophy is 1:16,800 live-born, it is the most common peroxisomal disease and the most common inherited disease involving the white matter of the central nervous system, its course may be varied.[12-18] Even within members of the same family, there may be different forms of the disease. Symptoms may appear at various ages and with varying severity and prevalence of neurological or endocrine-related disorders. In the case of the presented patient, symptoms appeared in the adult period, mainly manifesting as ailments of the nervous system.

Conclusion

Only the combination of a restrictive diet low in VLCFA and the use of glycerol oil or Lorenzo oil results in a reduction in the concentration of VLCFA, but biochemical changes are not accompanied by clinical improvement. The sense of using this type of treatment is effective only in boys with a pre-symptomatic period. Despite the lack of effective treatment, the most important in this case is diagnostics especially in people with a history of genetic diseases.

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CASUISTIC PAPER

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Bladder Mullerianosis – a case report

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ABSTRACT

Introduction. Bladder mullerianosis is a rare and proliferative lesion that contains at least two types of ectopic Mullerian tissue in its wall.

Aim. To present case of bladder mullerianosis.

Description of case. The text contains a description of a clinical case of a 50-year-old woman admitted to a gynecological ward due to diarrheal symptoms and abdominal pain. In a CT examination of the abdominal cavity with contrast, within the posterior left-sided wall of the bladder a 43x25mm proliferative lesion suggestive of neoplastic character was revealed. Transurethral resection of the lesion (TURB) was performed. Histopathology revealed endosalpingiosis with small endocervical foci. The picture of hyperplasia met the criterion of mullerianosis.

Conclusion. Bladder Mullerianosis is a very rare disease that occurs mainly in women of reproductive age. It has very good prognosis. It is important to differentiate the lesion with malignant tumor. The basis for the diagnosis is the histopathological examination of the lesion tissues taken during the surgery.

Keywords. endocericosis, endosalpingiasis, mullerianosis, urinary bladder

Introduction

Mullerianosis was first described by Young and Clement in 1996 as a rare unit consisting of the endometrium and mucous tissue of the fallopian tube or the cervical mucosa, occurring within the lamina propria mucosa or the muscularis proper to the bladder.[1-5] Mullerianosis is a mild growth occurring mainly in the posterior wall of the bladder, especially in women of childbearing age. Clinical symptoms include haematuria, pelvic pain and diarrheal symptoms.[10-16] From the clinical, cytological and histopathological point of view, this change is similar to tumor growth. A thorough clinical examination, imaging and histopathological examination allow for some differentiation of these hyperplasia.[17-25] Treatment consists of surgical removal of lesions, by transurethral resection (TURB) and, in some cases, homonaglama therapy.[26-33]

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Description of the case

A 50-year-old woman was admitted to the clinical urology department with diarrheal symptoms and abdominal pain, which initially suggested pyelonephritis. During the distant time, the patient underwent supra-hysterectomy. Urinalysis and urine sediment analysis showed inflammatory changes with the presence of bacteriuria and leucocyturia. In addition, the results of laboratory tests were normal. In computed tomography of the abdominal cavity and pelvis with contrast, a pathological size of 43x25mm was diagnosed within the posterior left-sided wall of the urinary bladder. In the left kidney cup, a 3-mm-thick deposit was revealed, and in the pancreas the focal widened Wirsung cable. In addition, abdominal and pelvic organs without pathological features.

Undergoing general anesthesia, transurethral resection of the bladder was performed. Post-secretion material in the form of numerous tissue fragments was fixed in buffered formalin and tissue and H+E staining was performed. In the microscopic examination, endosalpingiosis was diagnosed with the presence of small endocervikosis foci. The image corresponded to mullerianozie. The image showed fragments of the wall of the urinary bladder covered with mucosa with features of edema. Within it and in the musculature, small, partially cystic glands with lining of the serous type of the fallopian tube and glandular cervix were present, which did not show signs of tumor atypia (Fig. 1).

After about 6 weeks, the patient re-visited the doctor because of abdominal pain. In the ultrasound examination, changes of the infiltrating form in the same region of the bladder were again diagnosed. The TURB procedure was resumed. In the histopathological examination mullerianosis was again diagnosed. After about 8 weeks after the procedure in the performed magnetic resonance imaging (MRI), in the place after previous surgery, an image suggesting the recurrent nature of the lesions was visualized. In addition, no other pathological changes were observed in the urinary bladder and adjacent organs.

Discussion

Mullerianosis was first described by Young and Clement in 1996 as a rare unit consisting of the endometrium and mucous tissue of the fallopian tube or the cervical mucosa, occurring within the lamina propria mucosa or muscularis proper bladder.[1-5] Unlike endometriosis, mulleriasis occurs in the organ, not on the surface of the organ. Other mullerianosis sites are: inguinal lymphatic tubules, ureter, mesosalpinx.[1] There are many theories about the pathogenesis of this disease. Theory of implantation, when the changes precede the surgery and the metaplasia theory, when the changes occur in people without a previous operation. There is a suggestion that the Mullerian system, which during the development creates the mesothelial mesothelioma, has the ability to differentiate into the epithelium of the fallopian tube, cervix and endometrium.[6] Mullerianosis is a pseudocancer change. The differential diagnosis should include benign and malignant bladder cancer. Clinical symptoms, imaging results, and cytological examination of urine play a role in the diagnosis. The key to the diagnosis is the histopathological examination of change

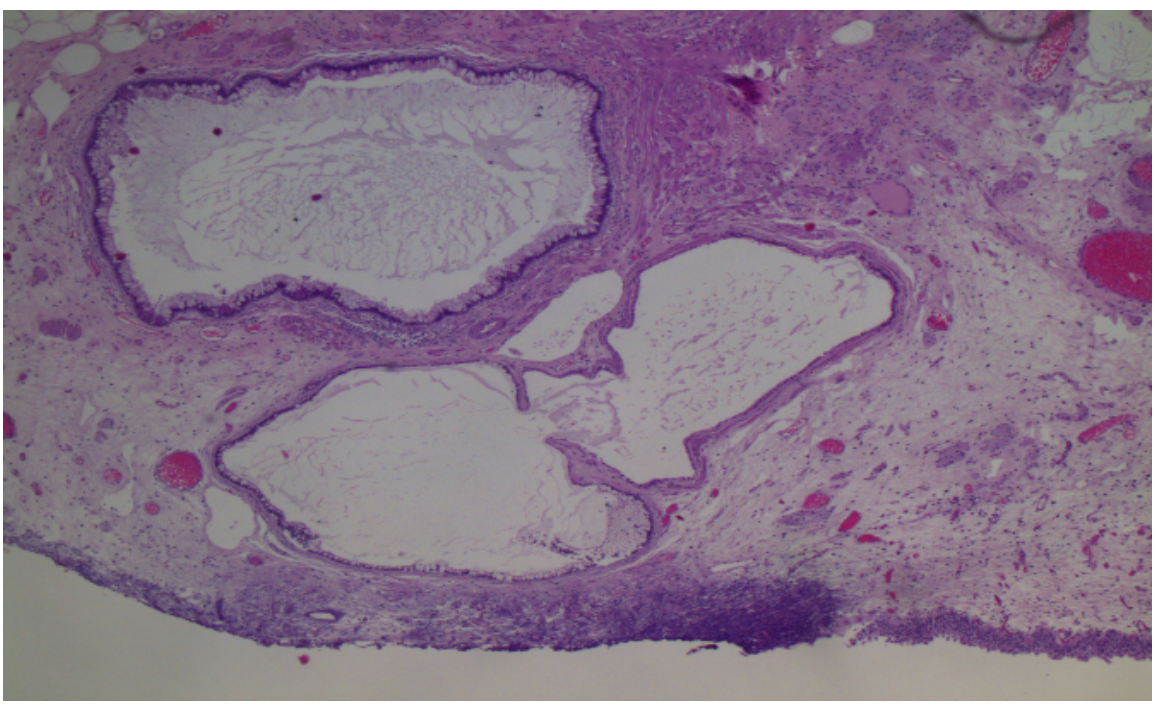

Fig. 1. Bladder Mullerianosis (Staining H+E, area 4X. Own material)

tissues taken during surgery (TURB), which is also an option for the treatment of change.[33-35] The second option is conservative treatment with hormone therapy using the LH-RH agonist.[8-16]

Conclusion

Bladder Mullerianosis is a very rare disease that occurs mainly in women of reproductive age. It has very good prognosis. It is important to differentiate the lesion with malignant tumor. The basis for the diagnosis is the histopathological examination of the lesion tissues taken during the surgery.

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