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Мак Мед Преглед

Списание на Македонското лекарско друштво

Journal of the Macedonian Medical Association

Наслов на Редакцијата и издавачот / Address of the Editorial Office and Administration:
1000 Скопје, Даме Груев 3, Градски ѕид блок 2
тел. 02/3162 577
www.mld.org.mk / mld@unet.com.mk
Жиро сметка / Bank Account
300000000211884 - Komercijalna banka Skopje

Печати: Бранко Гапо графичко производство - Skopje

Македонски медицински преглед се печати три пати годишно. Претплатата за списанието изнесува 10 евра за лекари, 50 евра за установа, странство 80 евра.

Основано 1946 Founded 1946

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*Мак Мед Прегл*ед 2023; 77(1): 1-7

ММР

Original article

EXPLORING THE EFFECTS OF BETAMETHASONE ON TOTAL FETAL LUNG VOLUME, AND ITS RELATIONSHIP IN THE PREDICTION OF NEONATAL RESPIRATORY DISTRESS SYNDROME

ИСТРАЖУВАЊЕ НА ЕФЕКТОТ НА БЕТАМЕТАЗОН ВРЗ ВКУПНИОТ ФЕТАЛЕН БЕЛОДРОБЕН ВОЛУМЕН И НЕГОВАТА УЛОГА ВО ПРЕДИКЦИЈА НА НЕОНАТАЛЕН РЕСПИРАТОРЕН ДИСТРЕС СИНДРОМ

Elena Dzikova, Goran Dimitrov, Irena Aleksioska Papestiev and Valentina Tofiloska

University Clinic for Gynecology and Obstetrics, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

Introduction. Every year, 15 million infants are born prematurely, and 1 million of them surrender to preterm birth complications, accounting for 1 in every 3 neonatal deaths. Globally, about 28.5 premature births occur per minute, resulting in approximately 4 fatalities, with 2 of these attributed to respiratory distress syndrome (RDS). This study aimed to investigate how betamethasone affects fetal lung development by assessing fetal lung volume and its association with neonatal RDS. The study received ethical approval by the Ethics Committee of the Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, North Macedonia.

Methods. This prospective clinical observational-interventional study involved 100 patients, including 50 with a history of preterm birth (study group) and 50 full-term cases (control group). The study was conducted at the University Clinic for Gynecology and Obstetrics, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, North Macedonia. Fetal lung volume was measured before and after betamethasone therapy, and its relation to neonatal RDS was evaluated. Patients were categorized into five groups based on gestational weeks.

Results. Significant differences in total fetal pulmonary volume were observed before and after betamethasone treatment. Additionally, the measurement of total fetal lung volume has proved to be effective in predicting neonatal respiratory distress syndrome.

Conclusion. Total fetal pulmonary volume measurement is a highly sensitive and specific method for predicting fetal lung maturity. This non-invasive, cost-effective, and easily accessible technique can be routinely employed in hospitals equipped with suitable ultrasound devices and adequately trained staff.

Keywords: fetal total pulmonary volume, fetal lung maturity, respiratory distress syndrome

Correspondence to: Elena Dzikova, Bul. Ilinden No.59, 1000 Skopje, R. N. Macedonia; E-mail: elena.dzikova@gmail.com

Апстракт

Вовед: Секоја година предвремено се раѓаат 15 милиони новороденчиња, а 1 милион од нив се соочуваат со компликации поради прематуритет, што претставува 1 од секои 3 смртни случаи кај новороденчињата. Глобално, секоја минута се раѓаат приближно 28,5 претерминиски новороденчиња, што е водечка причина за околу 4 смртни случаи, при што 2 од нив се поврзани со респираторен дистрес синдром (RDS). Ова истражување имаше за цел да го исследи влијанието на бетаметазонот врз феталната белодробна зрелост со мерење на феталниот белодробен волумен и неговата поврзаност со неонаталниот RDS. Истражувањето доби дозвола од Етичкиот комитет на Медицинскиот факултет при Универзитетот "Св. Кирил и Методиј" во Скопје, Северна Македонија.

Методи. Оваа проспективна клиничка опсервацијско-интервенциска студија вклучуваше 100 пациентки, 50 со историја на претерминирано породување (испитувана група) и 50 контролни случаи во термин за породување. Истражувањето се изведуваше во Универзитетската клиника за гинекологија и акушерство, Медицински факултет, при Универзитетот "Св. Кирил и Методиј" во Скопје, Северна Македонија. Анализирани беа: феталниот белодробен волумен пред и по терапијата со бетаметазон, и неговарта улога во предикција на неонаталниот RDS. Пациентките беа поделени во пет групи според гестациките недели.

Резултати. Се утврдија значителни разлики во феталниот белодробен волумен при третманот со бетаметазон. Исто така, методот се покажа ефикасен во предвидување на неонаталниот RDS.
Заклучок. Мерењето на феталниот белодробен волумен е метод со висока осетливост и специфичност за предвидување на зрелоста на феталното белодробие. Оваа неинвазивна, економична и пристапна техника може да се извршува рутински во здравствените установи обезбедени со соодветна ултразвучна опрема и обучен персонал.

Клучни зборови: вкупен фетален пулмонален волумен, фетална белодробна зрелост, респираторен дистрес синдром

Introduction

Preterm birth, defined as childbirth before 37 completed gestational weeks, is a global health concern with significant implications for neonatal mortality and morbidity. Each year, approximately 15 million infants are born prematurely [1], constituting around 11% of all live births worldwide. Tragically, 1 million of these preterm newborns succumb to the complex and often life-threatening complications associated with their premature birth, making preterm birth a leading cause of neonatal mortality. This grim reality highlights the pressing need for comprehensive research and interventions to address this public health issue.

One alarming aspect of preterm birth is its disproportionate impact on neonatal mortality. Astonishingly, one in every three neonatal deaths is attributed to complications related to prematurity. These fragile infants, born before full development, face signifycant challenges from their first moments of life. Respiratory distress syndrome (RDS), characterized by immature lung function, is a particularly devastating consequence of preterm birth, responsible for a substantial proportion of neonatal deaths, with about two out of every four fatalities among preterm infants linked to this respiratory condition.

Furthermore, the global prevalence of preterm birth is a pressing concern, with approximately 28.5 premature births occurring worldwide every minute. This phenomenon's prevalence varies across regions and countries, with data from 184 countries showing percentages ranging from 5% to 18% [1].

To address this issue comprehensively, it is imperative to intensify efforts in understanding the causes of preterm birth, developing effective preventive strategies, and improving the care and outcomes of preterm infants. This study aims to contribute to the understanding of factors surrounding preterm birth and its consequences, particularly focusing on fetal lung development and RDS. By doing so, this study seeks to pave the way for innovative approaches that reduce the global burden of preterm birth, mortality, and morbidity, offering hope to families worldwide.

Child mortality is a critical global health issue, with significant implications for public health and societal well-being. In 2015, 5.9 million children under the age of 5 lost their lives, with a mortality rate of 42.5 per 1,000 live births. Notably, 45% of child mortality occurs during the neonatal period, with a neonatal mortality rate of 19% [1].

Within child mortality, examining specific challenges faced by different regions and countries is crucial. In North Macedonia (NMK), the 2012 infant mortality rate was alarmingly high, at 9.8 per 1,000 live births - twice the European Union average. A significant proportion of these infant deaths (81%) occurred within the first 30 days of life, emphasizing the importance of early-life healthcare interventions to reduce mortality [2].

Prematurity emerges as the primary contributor to perinatal mortality in NMK, often leading to neonatal respiratory distress syndrome (RDS), a life-threatening condition. Understanding the dynamics of child mortality, particularly prematurity and RDS, is vital for improving healthcare practices and saving lives in NMK and worldwide.

Prematurity, a critical concept in neonatal and perinatal medicine, was defined by the World Health Organization (WHO) back in 1948 as childbirth with a neonatal birth weight below 2500 grams (g). However, it is essential to recognize that within this weight range, approximately one-third of newborns are born at full term, experiencing normal growth during gestation. In 1967, Battaglia and Lubchenco [3] introduced a practical classification system considering both birth weight and gestational week, offering a more comprehensive understanding of prematurity.

Preterm birth, defined as birth before 37 completed gestational weeks, can be categorized into three groups: extremely preterm birth (<28 gestational weeks), very preterm birth (28-32 gestational weeks), and moderately to late preterm birth (32-37 gestational weeks).

The leading cause of neonatal mortality worldwide and in North Macedonia is respiratory distress syndrome (RDS), resulting from atelectasis due to hyaline membrane disease. As such, predicting fetal maturity prenatally is crucial to ensure better postnatal adaptation and reduce morbidity and mortality among newborns. While various techniques exist for determining fetal maturity, many are invasive and carry

risks, particularly for infants already compromised by prematurity. This study examined the effects of betamethasone on fetal lung development, assessing fetal lung volume and its relationship with neonatal RDS. Ethical approval was obtained by the Ethics Committee of the Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, North Macedonia.

Material and methods

This prospective clinical observational-interventional study involved 100 patients, including 50 with a history of preterm birth (study group) and 50 full-term cases (control group). The study was conducted at the University Clinic for Gynecology and Obstetrics, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, North Macedonia. Fetal lung volume was measured before and after betamethasone therapy, and its relation in the prediction of neonatal RDS was evaluated. Patients were categorized into five groups based on gestational weeks.
Patients included in the study comprised those with premature rupture of membranes (PPROM), those experiencing preterm contractions and expected premature birth (PPI), or those with an anticipated preterm cesarean section (PSC). Patients excluded from the study were those with fetal anomalies, multiple pregnancies, and the presence of maternal illnesses.
A Voluson Expert E8 ultrasound machine equipped with a 3.5 MHz semi-convex ultrasound probe was used in the study. A Patients were examined transabdominally, in a semi-seated position. Fetal maturity was assessed both before and 72 hours after fetal lung maturation treatment using measurements of the total pulmonary volume (VolBD). These assessments were compared with the determination of postnatal respiratory distress syndrome (RDS) as the gold standard.
Patients were divided into 5 groups; the first four groups of study patients with a history of preterm birth and the fifth group comprised control cases at full term for childbirth:
- 28-30 gestational weeks
- 30 (+1 day) - 32 gestational weeks
- 32 (+1 day) - 34 gestational weeks
- 34 (+1 day) - 36 (+6 days)
≥37 gestational weeks (control cases).
The study was conducted in two phases, before and 72 hours after administering ampules Betamethasone at a dose of 14 mg/II every 24 hours, following a standard protocol for fetal lung maturation. The results obtained were monitored for up to 72 hours and then compared with the degree of postnatal RDS, which was used as the gold standard. If the patient had not given birth within 72 hours of the last measurement, she was excluded from the study.
MS Excel, SPSS, MedCalc, Social Science Statistics, and GraphPad were used for conducting standard statistical procedures, including mean value, standard deviation, Pearson's and Spearman's Rho correlations, analysis of variance, Student’s t-test for paired analysis, and p-value for statistical significance.
The specificity and sensitivity of the new method (total fetal pulmonary volume) were evaluated against the gold standard, postnatal RDS.
A linear regression analysis was performed to assess its distribution in relation to gestational weeks and the predictive value of RDS tests.
A diagnostic test was evaluated for sensitivity and specificity, including determining cut-off values using ROC (Receiver Operating Characteristic) curves and AUC (Area Under Curve) values.
The statistical significance was set at $p<0.05$.

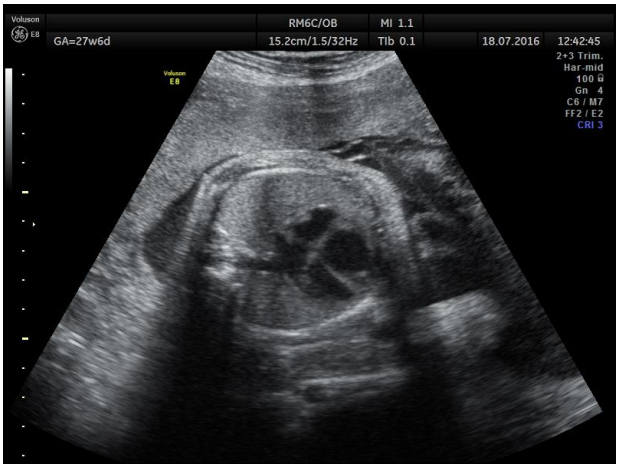

Fig. 1.

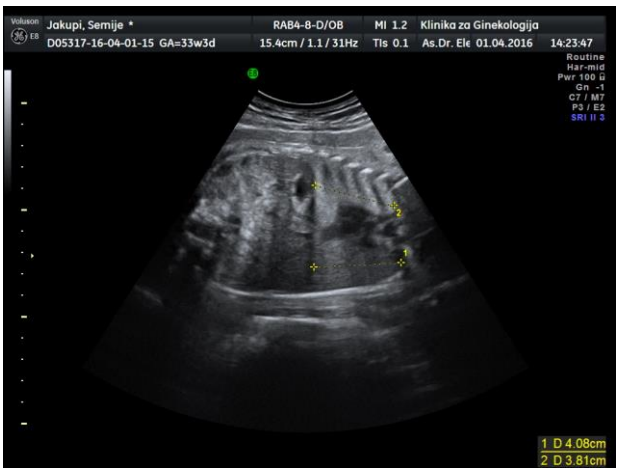

Fig. 2.
Fig. 1 and 2. Measurement of total fetal pulmonary volume

The measurements of the total fetal pulmonary volume were made considering that the fetal lungs ha-

ve a pyramid-like shape. The total pulmonary volume (ml) was calculated by determining the area of the base of both lung lobes and multiplying it by one-third of their height. In this process, a mathematical adaptation formula like 3D VOCAL (Virtual Organ Computer-Aided Analysis) was utilized (Figures 1 and 2).
The presence or absence of postnatal RDS was used as a method for comparison.

Results

A statistically significant difference was observed in the groups from 28-34 gestational weeks regarding the increase in the total fetal lung volume before and after the administered therapy for fetal lung maturation. On the other hand, the results indicated that the best fetal response to a single therapy occurred between 32-34 gestational weeks, while the least response was observed between 28-30 gestational weeks-necessitating a repeat therapy after at least 7 days.

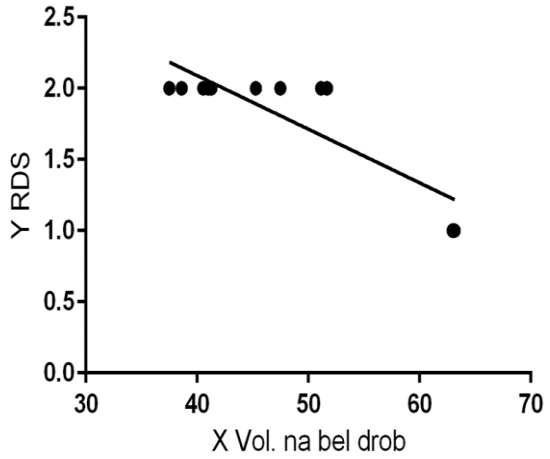

Fig. 3. Linear regression of total lung volume in prediction of RDS from 28-30 g.w.

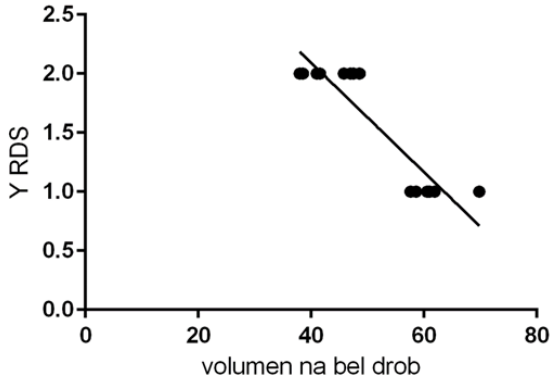

Fig. 4. Linear regression of total lung volume in prediction of RDS from 30 (+1 day) -32 g.w.

Additionally, a statistically significant difference of $p<0.05$ was found in predicting RDS based on the total fetal lung volume in all five groups according to gestational age with the best diagnostic accuracy from 30 (+1 day) -32 gestational weeks ($p<0.0001$) (Figures 3-7).

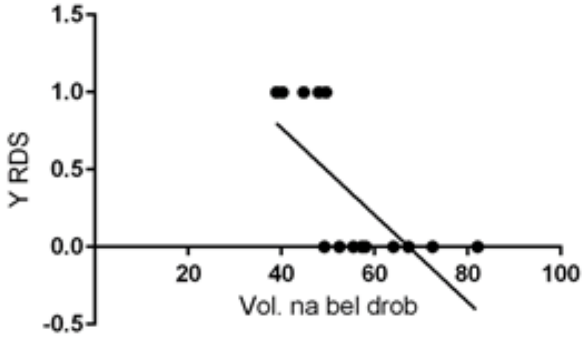

Fig. 5. Linear regression of total lung volume in prediction of RDS from 32 (+1 day) -34 g.w.

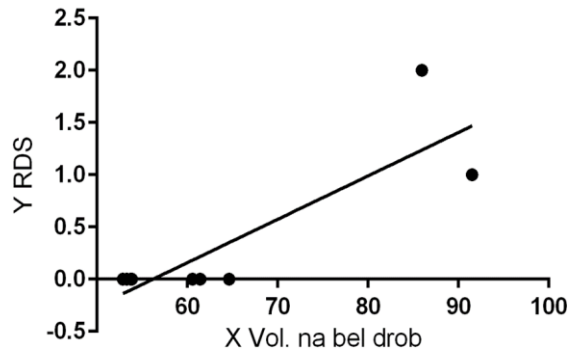

Fig. 6. Linear regression of total lung volume in prediction of RDS from 34 (+1 day) -36 g.w.

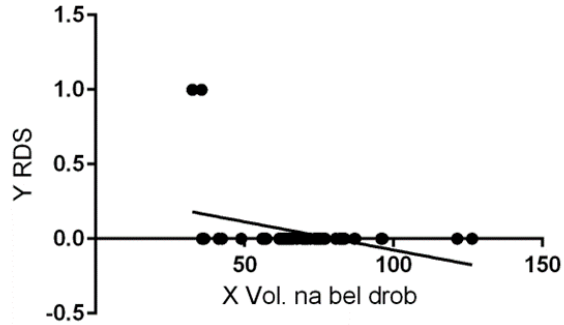

Fig. 7. Linear regression of total lung volume in the prediction of RDS from ≥ 37 g.w.

Furthermore, the measurement of the total fetal lung volume exhibited a high sensitivity of 71.4% and specificity of 75.9% for a cut-off value of ≤55.92, with an area under the curve (AUC) of 0.821 and statistical significance at $p<0.0001$ (Figure 8).

In addition, the method of measuring the total fetal lung volume demonstrated that the values of fetal lung volume linearly increased with the progression of gestational weeks (Figure 9).

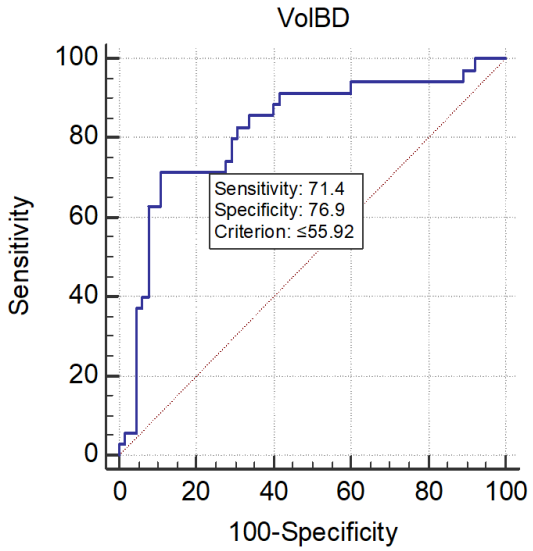

Fig. 8. ROC curve for total fetal lung volume (VolBD) in predicting RDS in all patients

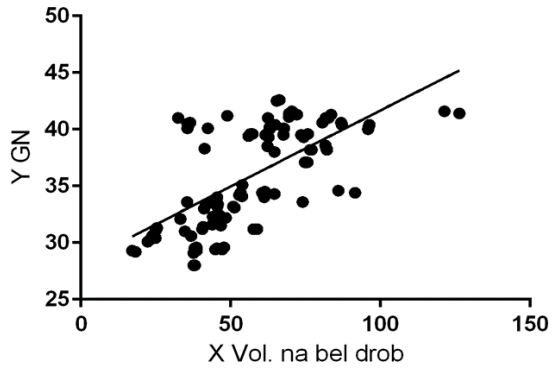

Fig. 9. Distribution of the total fetal lung volume by gestational weeks with statistical significance at $p<0.0001$

Discussion

It would prove highly beneficial to prenatally assess fetal maturity through a non-invasive method that is cost-effective, widely available, user-friendly, reliable, sensitive, specific, and applicable as a routine procedure in any healthcare facility equipped with quality ultrasound technology and trained personnel. This approach could facilitate timely predictions of fetal maturity and provide timely interventions when needed. Additionally, it would be advantageous to determine the most effective treatment group based on the gestational age of the fetus and identify cases where repeated intervention is necessary to ensure optimal fetal maturity. These objectives have inspired numerous researchers to conduct various studies aimed at achieving these goals.

Neonatal Respiratory Distress Syndrome (RDS) is a significant contributor to mortality and morbidity among premature infants. A widely recognized and established approach to mitigate this risk is the antenatal administration of corticosteroids, aimed at enhancing fetal pulmonary maturity. It is well-documented that the most advantageous impact of prenatal corticosteroid therapy occurs within the window of 24 hours to 7 days after the completion of administration [4]. Although corticosteroids have demonstrated a favorable impact on enhancing pulmonary maturity, the precise mechanism behind this beneficial effect remains a topic of ongoing investtigation.

Premature newborns, especially those with extreme prematurity, often develop neonatal respiratory distress syndrome (RDS) as a result of a lack of surfactant, high surface tension in the alveoli, and their collapse. Nowadays, there is a therapy with exogenous artificially prepared surfactant that mimics the fractions of surfactant A, B, and C. It can lead to a rapid and effective restoration of fetal lung function. However, even in the era of modern medicine and pharmacy, sometimes this treatment is ineffective and can result in high morbidity or, even worse, a fatal outcome for the newborn. Therefore, when premature birth is expected, it is crucial to determine the degree of fetal maturity, which has a significant impact on neonatal prognosis after birth. Today, there are various methods for assessing fetal maturity, such as determining the lecithin-to-sphingomyelin (L/S) ratio in amniotic fluid as predictors of synthesized surfactant necessary to reduce the surface tension of the alveoli, facilitating the breathing process. The L/S ratio is around 32/33 weeks of gestation and then increases, making the surfactant more effective, thereby improving the breathing process. It is essential for the difference between lecithin and sphingomyelin to reach a high value, so their ratio should be greater than 1.5, ideally 2 or more, for the fetus to have full lung and overall fetal maturity.

Recently, the method of determining lamellar bodies in amniotic fluid (LBC) has been described as a simple method with high sensitivity and specificity. This method is invasive but easier to perform in the laboratory and provides faster results. Gradually, this method is becoming the gold standard for prenatal assessment of fetal maturity and is almost completely replacing the method of determining the L/S ratio in amniotic fluid.

However this method is invasive and carry a certain level of risk, estimated at around 0.7% [5], for com-

plications such as premature rupture of the fetal membranes, pain, premature placental abruption, bleeding, and more. Additionally, it reduces the comfort of the mother and may induce fear and significantly increase the stress level, which, in patients with complicated pregnancies involving conditions like preeclampsia, diabetes, neurological disorders, etc., can worsen the mother's condition and, consequently, the fetus condition.

That is why the objectives of this study were as follows:

- To examine the validity of this new method for predicting fetal maturity, which should be non-invasive, cost-effective, accessible, simple, reproducible, and suitable for routine use in any hospital facility equipped with a good ultrasound device and educated staff. This was achieved by determining its sensitivity and specificity.
- To determine in which group, based on the gestational age of the fetus, the treatment works best by assessing fetal lung volume. Additionally, to identify cases where it is necessary to repeat the treatment to achieve better fetal maturity, ultimately reducing fetal mortality and morbidity, which are key goals outlined in the action plan of the World Health Organization from 2012 [6] and Healthy People 2020 [7].

The method we investigated in our study was the measurement of total pulmonary volume. This method was also performed with the mother in a semi-reclining position, following all the previously mentioned inclusion and exclusion criteria set for this study. The method was conducted trans-abdominally during routine ultrasound examinations. Since we couldn't use the VOCAL method on all available devices, we used a 2D method under the assumption that fetal lungs have a geometric pyramid shape. To measure their volume, we needed to measure the area of their base and multiply it by 1/3 of their height. Then, using the mathematical formula previously described in the Materials and Methods section, the results of the 2D measurements were transformed into 3D. This method was previously used by several authors, such as Gerards *et al*. in 2006 [8], to predict pulmonary hypoplasia in patients with premature rupture of fetal membranes. The goal was to determine impaired fetal lung function, which could be a cause of neonatal respiratory distress syndrome.

In our study, total pulmonary volume showed a significant difference in measurements before and after the administered therapy for fetal lung maturation according to the protocol.

We conducted the study in accordance with the inclusion and exclusion criteria of this study, including a healthy population where premature birth was expected, and in the absence of fetal anomalies. All measurements were performed with the mother in a semi-sitting position, which provided comfort during the examination, using a highly sophisticated ultrasound device, the GE Voluson E8 expert, equipped with a 3.5 MHz semi-convex probe.

As a gold standard, postnatal RDS was used. Therefore, the sensitivity and specificity of the new method were also compared to the gold standard, postnatal RDS.

In our study, we discovered a statistically significant difference in the change of total fetal lung volume before and after the administered therapy for fetal lung maturation among the groups with gestational ages between 28 and 34 weeks. Interestingly, the data showed that the therapy had the most significant impact between 32 and 34 gestational weeks, while its effectiveness was lower between 28 and 30 gestational weeks, necessitating a repeated therapy after a minimum of 7 days in these cases. Moreover, we found a statistically significant difference ($p<0.05$) in predicting RDS based on the total fetal lung volume across all five groups categorized by gestational age, with the highest diagnostic accuracy observed between 30(+1)-32 gestational weeks ($p< 0.0001$) (Refer to Figures 3-7 for more details.). Additionally, our measurement method for total fetal lung volume revealed a consistent trend where the values of fetal lung volume steadily rose as gestational weeks progressed (as illustrated in Figure 9).

In our study, the results indicated that the optimal cut-off value for total fetal lung volume was ≤ 55.9 (Figure 8).

Previous research has indicated that fetal heart movements can pose a significant challenge in 3D ultrasonographic lung volume measurement [9,10]. Some studies have proposed the necessity of a system that combines fetal electrocardiography with 3D ultrasonography to address this issue [11].

Kalache and his team employed relatively fast volume acquisition rates in their study, which appeared to mitigate the impact of fetal heart contractions on lung measurements through VOCAL. This was achieved even though the six measured slices encompassing the outer cardiac borders [12].

As highlighted by Peralta and his colleagues, one advantage of the VOCAL technique is its ability to include the lower regions of the lung that extend below the diaphragm dome. Additionally, it allows for adjustments to the lungs' contour in each plane, enhancing the accuracy of lung volume measurements [13].

In Laban S. *et al.* study [14], the Receiver Operating Curve (ROC) was employed to determine the cut-

off value for fetal lung volume (FLV) in predicting neonatal respiratory function outcomes. The ROC analysis revealed that the optimal FLV cut-off value was greater than 49.50, and the area under the ROC curve was calculated to be 0.936, signifying its excellent predictive capacity for neonatal respiratory function outcomes. The sensitivity, specificity, positive predictive value, and negative predictive value for FLV were found to be 87.80%, 88.20%, 92.90%, and 68.2%, respectively.

Laban *et al.* discovered that neonatal RDS is less likely to occur when FLV reaches a minimum of 32cm³ [15]. Prendergast and his team reported that 3D FLVs may serve as valuable predictors of neonatal respiratory outcomes in cases of abnormal lung growth, such as congenital diaphragmatic hernia and anterior wall defects [16]. Wang *et al.* determined that an FLV greater than 50 mL, when compared to amniotic fluid phospholipid analysis, indicated fetal lung maturity with high sensitivity and specificity [17]. These findings are similar to our results, but our cut-off values are a little bit higher ≤55.9 also comparable with other similar studies [18].

Conclusions

Our study showed that the measurement of the total fetal lung volume exhibited a high sensitivity of 71.4% and specificity of 75.9% for a cut-off value of ≤55.92, with an area under the curve (AUC) of 0.821 and statistical significance at $p<0.0001$.

Also, a statistically significant difference was observed in the groups from 28-34 gestational weeks regarding the increase in the total fetal lung volume before and after the administered therapy for fetal lung maturation. On the other hand, the results indicated that the best fetal response to a single therapy occurred between 32-34 gestational weeks, while the least response was observed between 28-30 gestational weeks - necessitating a repeat therapy after at least 7 days.

Conflict of interest statement. None declared.

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Мак Мед Прегled 2023; 77(1): 8-11

ММР

Original article

POSTOPERATIVE NAUSEA AND VOMITING AFTER LAPAROSCOPIC CHOLECYSTECTOMY - IS IT TIME TO REVISE THE ROUTINE ANTIEMETIC PROPHYLAXIS?

ПОСТОПЕРАТИВНО ГАДЕЊЕ И ПОВРАЌАЊЕ КАЈ ЛАПАРОСКОПСКА ХОЛЕЦИСТЕКТОМИЈА - ДАЛИ ТРЕБА ДА СЕ РЕВИДИРА РУТИНСКАТА АНТИЕМЕТИЧНА ПРОФИЛАКСА?

Bujar Osmani

University Clinic for Digestive Surgery, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

Introduction. Laparoscopic cholecystectomy is considered the golden standard in laparoscopic minimal invasive surgery. Possible side effects of laparoscopy are postoperative nausea and vomiting.
We conducted a cross-sectional study involving 35 patients who underwent laparoscopic cholecystectomy with an aim to observe the incidence of postoperative nausea and vomiting (PONV) and the need of antiemetic drugs.
Methods. This is a cross-sectional descriptive study which aim was toa analyze the incidence of PONV after laparoscopic cholecystectomy. The study took place at the University Clinic for Digestive Surgery, Clinical Centre Mother Teresa, Skopje, in duration of one month.
Inclusion criteria: Patients aged 18-65 from both sexes classified as ASA (American Society of Anesthesiologists) class 1 and 2 that underwent laparoscopic cholecystectomy according to ERAS protocols.
Exclusion criteria. Patients with metabolic disorders (diabetes mellitus, hypothyroidism); patients with neurologic disorders, patients on long-term corticosteroid therapy; patients on neuroleptic therapy; patients with history of malignant diseases and chemotherapy.
Methods. Observing the incidence of nausea and vomiting during 24 hours after the surgery (period prevalence) in patients who fulfilled the inclusion criteria, and who did not receive any antiemetic drug as prophylaxis.
Statistical methods. Descriptive statistical methods were used; numbers, percentages.
Results. Thirty-five patients that fulfilled the inclusion criteria were enrolled in this cross-sectional study; female 65%, male 35%; the mean age of patients was 52 years.
The incidence of nausea was 34%, while the incidence of vomiting was 17%, with predominantly females experiencing PONV.
Conclusion. In order to avoid polypharmacy, the routine antiemetic prophylaxis with antiemetic drugs in patients undergoing laparoscopic cholecystectomy adhering to Enhanced Recovery After Surgery principles is not necessary.

Keywords: laparoscopy, nausea, vomiting, prophylaxis

Correspondence to: Bujar Osmani, University Clinic for Digestive Surgery, 1000 Skopje, R. N. Macenonia;
E-mail: bujar.m.osmani@gmail.com

Абстракт

Вовед. Лапароскопската холецистектомија се смета за златен стандард во лапароскопската минимална инвазивна хирургија. Можни несакани ефекти на лапароскопијата се постоперативно гадење и повраќање.
Спроведовме студија на пресек врз 35 пациенти кои биле подложени на лапароскопска холецистектомија со цел да ја утврдиме инциденцата на постоперативно гадење и повраќање и потреба од антиеметички лекови.
Методи. Студија на пресек која има за цел да ја анализира инциденцата на постоперативно гадење и повраќање после лапароскопска холецистектомија. Критериуми за вклучување: Пациенти на возраст од 18-65 години од двата пола класифицирани како АСА (Американско друштво на анестезиолози) класа 1 и 2 кои биле подложени на лапароскопска холецистектомија според Протоколите на ЕРАС (забрзано опоравување после операција).
Критериум за исклучување. Пациенти со метаболички нарушувања (Дијабетес Мелитус, Хипотиреоидизам). Пациенти со невролошки нарушувања. Пациенти на долгорочна кортикостероид-

на терапија. Пациенти на терапија со невролептици. Пациенти со историја на малигни заболувања и хемотерапија.
Методи. Набљудување на инциденцата на гадење и повраќање во текот на 24 часа по операцијата (распространетост на периодот) кај пациенти кои ги исполниле критериумите за вклучување, и кои не добиваат никаков антиеметичен лек како профилакса.
Статистички метод. Користени се описни статистички методи; бројки, проценти.
Резултати. Вкупниот број на пациенти кои ги исполнија критериумите за вклучување во оваа кр студија на пресек е 35,од кои 65% жени и 35% мажи, средна возраст 52 години.
Инциденцата на гадење е 34%, додека инциденцата на повраќање е 17 %.
Заклучок. Со цел да се избегне полифармација, рутинската антиеметичка профилакса со антиеметички лекови кај пациенти кои се подложени на лапароскопска холецистектомија водена според принципите на забрзано опоравување после операција (ERAC препораки), не е неопходна.

Клучни зборови: лапароскопија, гадење, повраќање, профилакса

Introduction

Postoperative nausea and vomiting (PONV) refer to occurrence of nausea and vomiting in the early postoperative period. The most common causes of PONV are postoperative ileus, effects of opioids and muscle relaxants, patients' underlying comorbidities, such as metabolic disturbances, diabetes mellitus, neurologic disorders, long-term use of some medications, etc. Some authors report incidence of PONV in up to 35% of patients [1].
Laparoscopy as a minimally invasive surgical technique was introduced in 1985 by the German surgeon Erih Muhe, while the first cholecystectomy performed with video laparoscopy was realized in 1991 by Mouret [2]. This has marked the beginning of the era of minimal invasive surgery, with laparoscopic cholecystectomy as a golden standard.
But, besides all the advantages of minimal invasive surgery, some authors report increased incidence of PONV after laparoscopy, even up to 75% [3]. The factors that can trigger nausea and vomiting after laparoscopy are complex; they include age, obesity, changes in intraabdominal pressures due to pneumoperitoneum, duration of surgery, anesthetic drugs and comorbidities.
Although there is no general consensus whether antiemetic drugs should be used as prevention of PONV or as a rescue medicine if PONV occurs, many medical centres use antiemetic drugs as a routine prevention of PONV. Also, there is no consensus which antiemetic drug is the best choice for PONV. There are studies that compare effects of different antiemetic drugs, but most of the authors agree on using metoclopramide, ondansetron and dexamethasone as the safest and most efficient drugs [4]. Some authors suggest using neuroleptics, such as haloperidol and droperidol, or gabapentin, an antiepileptic drug [5]. It is worth noting that none of these drugs is completely safe and without side effects.
Metoclopramide has a good safety profile, but can cause neurologic side effects known as tardive dyskinesia and pyramidal syndrome. It can also cause confusion and delirium, especially in elderly individuals. Ondansetron is considered safe and well tolerated drug, but some of its rare side effects are headache and dizziness. Dexamethasone is corticosteroid with anti-inflammatory and immunosuppressive properties. Its primary use is not as antiemetic drug, but may be used in certain medical context to prevent nausea and vomiting, most commonly during chemotherapy, if prescribed by a medical professional. Haloperidol and droperidol may cause a prolonged sedation.
It is clear so far from the medical literature that the choice of antiemetic drug for prevention and treatment of PONV is an individual choice made by medical professionals, and it largely relies on the personal medical history of a patient and the medical context. But, in 1997, the ERAS (Enhanced Recovery After Surgery) protocols were introduced to the surgical community by the surgeon Henrik Kehlet. This "fast track" surgery refers to a multidisciplinary approach to every patient, in order to lower the length of hospital stay and improve the outcome. Cornerstones of ERAS protocols are educating the patient for the outcome, shortening the pre-operative fasting, allowing the intake of oral fluids up to two hours prior surgery, early mobilization of the patient, early post-operative feeding, applying multimodal opoid sparing anesthesia techniques or opioid-free anesthesia where applicable and avoidance of polypharmacy. Many medical centers have since implemented the ERAS protocols in many surgical fields [6].
After implementing the ERAS protocols at our Clinic, it was worth reevaluating the incidence of PONV after laparoscopic cholecystectomy and the need for routine antiemetic prophylaxis.

Material and methods

A cross-sectional descriptive study was conducted aimed at analyzing the incidence of PONV after laparoscopic cholecystectomy. The study took place at the University Clinic for Digestive Surgery, Clinical Centre Mother Teresa, Skopje, in duration of one month.

Inclusion criteria:

Patients aged 18-65 from both sexes classified as ASA (American Society of Anesthesiologists) class 1 and 2 that underwent laparoscopic cholecystectomy according to ERAS protocols.

Exclusion criteria:

- Patients with metabolic disorders (diabetes mellitus, hypothyroidism);
- Patients with neurologic disorders;
- Patients on long-term corticosteroid therapy;
- Patients on neuroleptic therapy;
- Patients with history of malignant diseases and chemotherapy.

Methods

Observing the incidence of nausea and vomiting during 24 hours after the surgery (period prevalence) in patients who fulfilled the inclusion criteria, and who did not receive any antiemetic drug as prophylaxis.

Statistical methods

Descriptive statistical methods were used; numbers, percentages.

Results

The total number of patients enrolled in the study was 35.

Table 1. Demographic data

Sex	Number	Percentage
Female	23	65%
Male	12	35%

Table 2. Incidence of nausea and vomiting during the first 24 hours postoperatively

	Number of patients	Percentage
Nausea	12	34 %
Vomiting	6	17%

The mean age of patients was 52 years.

Table 3. Distribution of incidence of PONV among sexes

	Nausea	Vomiting
Female	8(66%)	4(66%)
Male	4(34%)	2(34%)

Thirty-five patients that fulfilled the inclusion criteria were enrolled in this cross-sectional study; female 65%, male 35%; the mean age of patients was 52 years.
The incidence of nausea was 34%, while the incidence of vomiting was 17%, with predominantly females experiencing PONV.

Discussion

The incidence of PONV and its prevention after laparoscopy has been a subject of many studies over the last two decades. The reported incidence of PONV after laparoscopy according to some authors is as high as 75%. Many authors suggest different antiemetic drugs for prevention and treatment of PONV, but no general consensus has been made. In the era of "fast track' surgery and ERAS protocols, as well as multimodal anesthesia and opioid-free anesthesia, it is time to reevaluate the incidence of PONV and the need for routine antiemetic prophylaxis. For this purpose, we realized a cross-sectional study, to help us evaluate the incidence of PONV in patients subjected to laparoscopic cholecystectomy according to ERAS protocols. Patients included in the study did not have any significant comorbidities and did not receive medications with antiemetic properties that could interfere with the outcome. Our aim was to observe the influence of surgery and anesthesia over nausea and vomiting in "fast track" surgery settings. All of the patients were subjected to multimodal type of anesthesia (anesthesia technique that consists of a combination of different short-acting anesthetics and drugs in order to minimalize to use of opioids) customed for each patient individually to match the individual needs for pain relief, hypnotic effect and myorelaxation. None of the patients received opioid analgetic postoperatively.
The results of our study showed much lower incidence of nausea and vomiting compared to some other authors. In our study 34% of patients experienced nausea, while 17% vomited. Those patients received metoclopramide as a rescue antiemetic therapy. Some of the possible reasons for this outcome in percentage of patients with PONV might be the usage of anesthetics with antiemetic properties, such as propofol and sevoflurane, and the no-opioid policy for postoperative analgesia. These are all principles of ERAS protocols. Another principle of ERAS protocol is avoiding unnecessary polyphar-

macy [7-10]. In this manner and according to the results of our observational study, we suggest that antiemetic prophylaxis in patients classified as ASA 1 and 2 that undergo laparoscopic cholecystectomy is not necessary. If PONV do appear, we suggest a rescue antiemetic drug that has a safe pharmacological profile and does not have many side effects.
The shortcoming of this study is that it is a cross-sectional study with a small number of patients, and therefore, we recommend further long-term longitudinal studies to estimate the incidence of PONV in larger population of patients and thus reevaluate the usage of antiemetic drugs as prophylaxis.

Conclusion

In order to avoid polypharmacy, the routine antiemetic prophylaxis with antiemetic drugs in patients undergoing laparoscopic cholecystectomy adhering to Enhanced Recovery After Surgery principles is not necessary.

Conflict of interest statement. None declared.

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*Мак Мед Прегл*ед 2023; 77(1): 12-16

ММР

Original article

SEASONAL VARIABILITY OF (25OH) VIT D IN OBESE WOMEN IN NORTH MACEDONIA

СЕЗОНСКА ВАРИЈАБИЛНОСТ НА (25OH) ВИТ Д КАЈ ОБЕЗНИ ЖЕНИ ВО СЕВЕРНА МАКЕДОНИЈА

Argjent Mucha[1], Sonja Panovska[1], Iskra Bitovska[1], Igor Isjanovski[2], Petrit Izairi[5], Gazmend Amzai[3], Valbon Limani[1], Nikola Gjorgjievski[4] and Marija Toshevska Stefanoska[1]

[1]University Clinic for Endocrinology, Diabetes and Metabolic Disorders, Skopje; [2]University Clinic for Ophthalmology, Skopje; [3]University Clinic for Hematology, Skopje; [4]University Clinic for Nephrology, Skopje, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, [5]Polyclinic Florens, Tetovo, Republic of North Macedonia

Abstract

Introduction. Vitamin D is a steroid hormone that is synthesized and metabolized in humans through several pathways, including the skin, intestine, kidney, and liver. Vitamin D deficiency is directly associated with abdominal obesity (high waist circumference [WC]), obesity (high body mass index [BMI]), insulin resistance, and metabolic syndrome (MetS). The aim of this study was to investigate the vitamin D status and its seasonal dynamics in a random selection of adult obese women in North Macedonia.
Method. This cross-sectional study was performed at the University Clinic for Endocrinology, Diabetes and Metabolic Disorders in Skopje, North Macedonia. A total of 100 participants, women, were included in this study. The average BMI in the studied group was 37.1±5.6, ranging from 26 to 58. According to BMI, 21.0% of subjects belonged to the group overweight and 79.0% to the obesity group.
Results. The analysis of vitamin D (nmol/l) showed that the average value in the group was 33.3±14.5 nmol/l and it was within the range of reference values (25-110 nmol/l). 26.0% of subjects had reduced vit D values.
Discussion. This study observed variations in serum Vit D with the lowest values (below the reference values) in January 22.5±6.2 nmol/l, February 25.1±5.7 and March 20.8±7.1 nmol/l. The highest values within the reference values were registered in the summer period, June 50.1±23.7 nmol/l, July 45.1± 239.8.7 nmol/l and August 44.4±13.6 nmol/l. Vitamin D deficiency in obese individuals may be caused by storage of 25(OH)D in adipose tissue and high expression of vitamin D receptor in adipose tissue.
Conclusion. This cross-sectional study confirmed that obese women with a high BMI >30 had higher Vit D values in the summer months than in the winter period.

Keywords: 25-hydroxyvitamin D, waist circumference, body mass index, metabolic syndrome, waist-to-hip ratio

Correspondence to: Argjent Mucha University Clinic for Endocrinology, 1000 Skopje, R. N. Macedonia argjent_muca85@hotmail.com

Апстракт

Вовед. Витаминот Д е стероиден хормон кој се синтетизира и метаболизира кај луѓето преку неколку патишта, вклучувајќи ја кожата, цревата, бубрезите и црниот дроб. Недостатокот на витамин Д е директно поврзан со абдоминална дебелина (висок обем на половината [WC]), општа дебелина (висок индекс на телесна маса [BMI]), отпорност на инсулин и метаболички синдром (MetS). Целта на оваа студија е да се истражи статусот на витамин Д и неговата сезонска динамика во случаен избор на возрасни обезни жени во Северна Македонија.
Методи. Студијата на пресек е изведена во ЈЗУ Универзитетска клиника за ендокринологија, Скопје, Северна Македонија.
Во студијата на пресек земаат учество 100 испитаници, жени. Просечна вредност на БМИ во испитуваната група изнесува 37.1±5.6, во ранг од 26 до 58. 21.0% од испитаниците според БМИ припаѓаат во групата со зголемена ТТ(26-30), а 79.0% во обезната група.
Резултати. При анализата на вит.Д (nmol/l) утврдивме да просечната вредност во групата изнесува 33.3±14.5 nmol/l и е во граница на референтни вредности-25-110 nmol/l. 26.0% од испитаниците имаат намалени вредности на вит Д.

Дискусија. Оваа студија забележи варијации на серумскиот Вит Д со најниски вредности (под референтните вредности) во месец јануари 22.5±6.2 nmol/l, февруари 25.1±5.7 и месец март 20.8±7.1 nmol/l. Највисоки вредности во граница на референтните вредности се регистрираат во летниот период и тоа јуни 50.1±23.7 nmol/l, јули 45.1± 239.8.7 nmol/l и август 44.4±13.6 nmol/l. Недостатокот на витамин Д кај дебели лица може да биде предизвикан од складирање на 25(OH)D во масното ткиво и висока експресија на рецепторот на витамин Д во масното ткиво.

Заклучок. Оваа студија на пресек потврди дека обезните жени со висок БМИ >30 имаат повисоки вредности на Вит Д во летните месеци отколку во зимскиот период.

Клучни зборови: 25-хидроксивитамин D: 25(OH)D, обем на половината: WC, индекс на телесна маса: BMI, метаболички синдром: MetS, однос на струкот и колкот: WHR

Introduction

Vitamin D is a steroid hormone that is synthesized and metabolized in humans through several pathways, including the skin, intestine, kidney, and liver. Vitamin D concentration can be determined by measuring the serum concentration of 25-hydroxyvitamin D [25(OH)D] [1]. 1,25-dihydroxycholecalciferol [1,25-(OH)2D] is the active form of vitamin D. Vitamin D is participating in bone mineralization and thereby reduces the risk of rickets and osteoporosis [2]; however, it may also be connected with the prevention of cancer and cardiovascular disease. Vitamin D is also associated with length of hospitalization and mortality in critically ill patients [3]. There is a high prevalence of vitamin D deficiency and/or insufficiency globally.

Vitamin D deficiency is associated with an impaired glucose metabolism in adipocytes, which appears to be due to impairment of either insulin synthesis or secretion [5]. Therefore, the influence of vitamin D on glucose metabolism is related to several mechanisms, such as its effect on the interaction between 1,25-(OH)2D and vitamin D receptors on pancreatic b cells and expression of α-1- hydroxylase in β-cells [6]. Vitamin D deficiency is also directly associated with abdominal obesity (high waist circumference [WC]), obesity (high body mass index [BMI]), insulin resistance, and metabolic syndrome (MetS) [7,8,]. A systematic review showed an inverse association between 25(OH)D levels and impaired insulin release [7].

Vitamin D has two forms, D2 and D3. Vitamin D3 is synthesized in the skin upon exposure to ultraviolet B (UVB) radiation from sunlight [9]. The prevalence of vitamin D deficiency and insufficiency is often high in sunny countries [10,11]. Dietary sources of vitamin D include vegetables and orange juice as sources of vitamin D2 and eggs, fish oil, dairy products, and seafood as sources of vitamin D3 [9]. Some previous studies have evaluated the relationships between dietary habits and 25(OH)D concentration according to dietary sources of vitamin D [12]. Previous studies have shown that dietary foods high in dairy products, seafood, eggs, and vegetables were associated with higher serum 25(OH)D levels and dietary foods high in sweets, alcoholic beverages, fats, and soft drinks were inversely associated with serum concentrations of 25(OH)D [12].

The aim of this study was to investigate the vitamin D status and its seasonal dynamics in a random selection of adult obese women in North Macedonia.

Material and method

This cross-sectional study was performed at the University Clinic for Endocrinology, Diabetes and Metabolic Disorders in Skopje, North Macedonia.

A total of 100 participants, women, were included in this study, with an average age of 38.8±10.4 years, ranging from 19 to 60.

70.0% of them have completed secondary education and 30.0% higher education.

The average BMI in the studied group was 37.1±5.6, ranging from 26 to 58. According to BMI, 21.0% of subjects belonged to the group overweight (25-29.9), and 79.0% to the obesity group. The average value of waist circumference/cm was 113.1±15.9; hip circumference/cm 118.7±16.2; WHR 0.95±0.03. On average, the respondents took 2470 ±376.8 calories, ranging from 1800 to 3200 calories.

Laboratory measurements

Blood samples were taken individually (samples were taken in the period from January to October 2021). Serum was separated and samples were stored at -20°C until analyzed. Serum 25(OH)D level was measured in a Cobas e 411 speed machine (25 Hydroxivit D 25-110nmol/L).

Results

The analysis of vitamin D (nmol/l) showed that the average value in the group was 33.3±14.5 nmol/l, and it was within the range of reference values (25-

110 nmol/l). 26.0% of subjects had reduced vitD values (Table 1).

Table 1. Demographic and other parametric characteristics of subjects

Variables	Mean	Minimum	Maximum	Std.Dev.
Age	38.8	19.0	60.0	10.3979
BMI	37.1	26.0	58.0	5.5955
waist circ./cm	113.1	88.0	145.0	15.8956
hip circ./cm	118.7	89.0	152.0	16.2209
WHR	0 95	0.88	1.1	0.0328
calorie intake	2470.0	1800.0	3200.0	376.7887
VitD total (nmol/l/ref.25-110)	33.3	10.0	85.0	14.5097
education	***number***		***%***	
secondary	70		70.0	
higher	30		30.0	
BMI				
overweight (25-29.9)	21		21.0	
obesity (>30)	79		79.0	
VitD total				
< 25 nmol/l	26		26.0	
reference values	74		74.0	

The average values of vit.D (nmol/l) during the examined period of 10 months fluctuated. The lowest values (below the reference values) were registered in January 22.5±6.2 nmol/l, February 25.1±5.7 and March 20.8±7.1 nmol/l. The average values during April started to grow and amounted to 27.2±3.8 nmol/l, to reach an average value of 31.5±9.1 nmol/l in May. The highest values within the reference values were registered in the summer period, June 50.1 ±23.7 nmol/l, July 45.1±239.8.7 nmol/l and August 44.4±13.6 nmol/l (Table 2 and Figure 2). According to the analysis of variance test, the difference between the average values was significant for p <0.05 (F=8.020863, p=0.000000) (Table 2a).

There was a great selection of so-called post hoc tests that were performed after the ANOVA test presenting statistically significant results. Multiple comparison tests were also made. The purpose of these tests is to reveal the difference (between multiple samples) that is "responsible" for the overall statistically significant result. According to the post hoc test-Tukey HSD test, the difference was statistically significant for p<0.05 between June *versus* January, February, March, April, May and October (p=.000174, p=.000297, p=.000163, p=.000883, p= .014397, p=.023339) (Table 2b).

The difference was statistically significant for p<0.05 between July *versus* January, February, March and April (p=.001067, p=.005921, p=.000402, p=.022005) (Table 2b). The difference was statistically significant for p<0.05 between August *versus* January, February, March and April (p=.001695, p=.009293, p=.000581, p=.033013) (Table 2b).

Table 2. Presentation of the seasonal (monthly) average values of vit D

month	VitD total (nmol/l(ref.25-110) Means	VitD total (nmol/l/ref.25-110) N	VitD total (nmol/l/ref.25-110) Std.Dev.
Jan	22.5	10	6.20484
Fev	25.1	10	5.72422
March	20.8	10	7.13053
April	27.2	10	3.82390
May	31.5	10	9.05845
June	50.1	10	23.68520
July	45.1	10	9.83700
August	44.4	10	13.59085
Sept	34.5	10	7.13754
Oct	32.3	10	13.14914

Table 2a. Analyses of variance test

Variable	Analysis of Variance							
	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
VitD total (nmol/l/ref.25-110)	9276.850	9	1030.761	11565.90	90	128.5100	8.020863	0.000000

Table 2b. Analyses of the post hoc test - Tukey HSD test

	Tukey HSD test									
month	**{1} M=22.500**	**{2} M=25.100**	**{3} M=20.800**	**{4} M=27.200**	**{5} M=31.500**	**{6} M=50.100**	**{7} M=45.100**	**{8} M=44.400**	**{9} M=34.500**	**{10} M=32.300**
Jan {1}		0.999961	0.999999	0.995201	0.748686	0.000174	0.001067	0.001695	0.358006	0.646945
Fev {2}	0.999961		0.997564	0.999994	0.959356	0.000297	0.005921	0.009293	0.699133	0.917758
March {3}	0.999999	0.997564		0.959356	0.525217	0.000163	0.000402	0.000581	0.188748	0.419620
April {4}	0.995201	0.999994	0.959356		0.997564	0.000883	0.022005	0.033013	0.911117	0.991244
May {5}	0.748686	0.959356	0.525217	0.997564		0.014397	0.196797	0.259948	0.999872	1.000000
June {6}	0.000174	0.000297	0.000163	0.000883	0.014397		0.992426	0.980940	0.078094	0.023339
July {7}	0.001067	0.005921	0.000402	0.022005	0.196797	0.992426		1.000000	0.538772	0.269929
August {8}	0.001695	0.009293	0.000581	0.033013	0.259948	0.980940	1.000000		0.633606	0.346243
Sept {9}	0.358006	0.699133	0.188748	0.911117	0.999872	0.078094	0.538772	0.633606		0.999991
Oct {10}	0.646945	0.917758	0.419620	0.991244	1.000000	0.023339	0.269929	0.346243	0.999991	

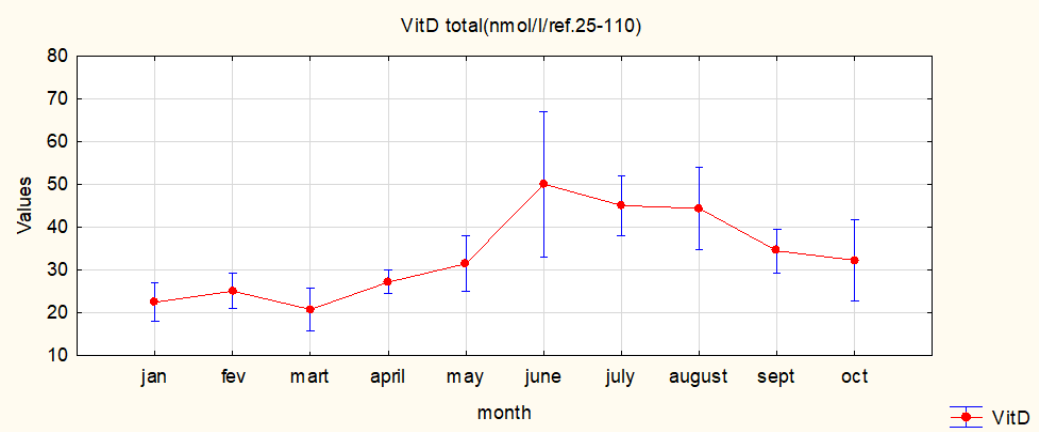

Fig. 2. Display of the seasonal (monthly) average values of vit D

Discussion

This study observed variations in serum Vit D with the lowest values (below the reference values) in January 22.5±6.2 nmol/l, February 25.1±5.7 and March 20.8±7.1 nmol/l. The average values during the month of April started to grow and amounted to 27.2±3.8 nmol/l, to reach an average value of 31.5±9.1 nmol/l in May. The highest values within the reference values were registered in the summer period, June 50.1±23.7 nmol/l, July 45.1±239.8.7 nmol/l and August 44.4±13.6 nmol/l. This study confirmed that high-calorie food does not affect the serum values of vitamin D.

Our results are in contrast to previous studies that showed a low prevalence of vitamin D deficiency in young adults and elderly Scandinavians, compared to the Mediterranean European population [13]. This is claimed to be due to the high intake of fatty fish, vitamin-fortified dairy products and vitamin D supplements in Scandinavia. In a previous Swedish study of women aged 61–86 years, only 19% had a serum 25(OH)D level below 50 nmol/L during winter; however, a high percentage, 25%, of the participants were on a sunny vacation during the winter [14]. Another explanation for the discrepancy may be greater awareness of osteoporosis and the need for calcium and vitamin D intake in postmenopausal women. Sweden and the Scandinavian countries top the ranking list of ten-year hip fracture probabilities, which can be used as an indicator of the prevalence of osteoporosis in a country [14]. Interpreting and taking measures of serum 25(OH)D values analyzed at different seasons of the year can present a problem

for the clinician. In most cases, the appointment time is not optimally chosen for the study of vitamin D levels. The results of the current study showed that the value of 25(OH)D measured in late spring can be expected to be about 35.1±16.3 nmol/L higher in late summer and *vice versa*.

The association between low serum 25(OH)D and high BMI found in this study is supported by several previous studies [15]. Vitamin D deficiency in obese individuals may be caused by storage of 25(OH)D in adipose tissue. Other proposed mechanisms are high expression of vitamin D receptor (VDR) in adipose tissue and vitamin D may play a role in the pathogenesis of metabolic syndrome [15]. Respondents who exercised regularly, at least once a week, had higher serum concentrations of 25(OH)D than those who were physically inactive during the summer period, but not during the winter. Many chronic diseases are associated with reduced physical function, less outdoor activity and, consequently, less UV-B exposure. Liver and intestinal diseases can further lead to malabsorption of vitamin D. Chronic diseases are also often associated with lower socioeconomic status, lower levels of education, and reduced economic resources to use summer months, multivitamins, and vitamin D-rich foods such as fish and shellfish.

Conclusion

This cross-sectional study confirmed that obese women with a High BMI >30 had higher Vit D values in the summer months than in the winter period. Fortification of dairy products with vitamin D supplementation can have an effect in ensuring the daily needs of vitamin D, especially in people with an increased BMI.

Conflict of interest statement. None declared.

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Мак Мед Прегled 2023; 77(1): 17-23

ММР

Original article

MENSTRUAL CYCLE DISORDERS AFTER COVID-19 VACCINATION

ПРОМЕНИ ВО МЕНСТРУАЛНИОТ ЦИКЛУС ПОСЛЕ COVID-19 ВАКЦИНАЦИЈА

Natasha Ilieva[1], Verica Popovska[2], Darko Iliev[3], Irena Aleksioska Papestiev[1], Viktorija Jovanovska[1] and Goran Dimitrov[1]

[1]University Clinic for Gynecology and Obstetrics, [2]Institute of Forensic Medicine and Criminalistics, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, [3]NGO Medicine and Ecology Research Center - MERC, Skopje, Republic of North Macedonia

Abstract

Introduction. COVID-19 vaccines were developed one year after the official first case of COVID-19 in China was reported by the WHO. More than 3 billion doses have been administered so far. Menstrual disturbances have also been reported, primarily by women, and confirmed in scientific research.
Aim. To evaluate menstrual cycle irregularities after COVID-19 vaccination among females in North Macedonia.
Methods. A cross-sectional study was conducted that used a questionnaire to collect data in the period 03.2022-03.2023, including women aged 18-45 and residents of the Republic of North Macedonia. Women who were referred for an examination at the University Clinic for Gynecology and Obstetrics in the capitol of the country, were invited to participate in the survey. The questionnaire was distributed electronically.
Results. A total of 191 women participated in this study, with a mean age of 34.31±8.05 years, 163 (85.3%) have been vaccinated, and 28 (14.7%) not vaccinated. Changes in menstrual cycle after COVID-19 vaccination were found in 89 (46.6%), predominantly after their second dose. The most frequent menstrual cycle changes were: heavy periods/ bleeding (menorrhagia), infrequent/irregular periods (oligomenorrhea) and painful menstruation (dysmenorrhea). Disorders were noticed right after vaccination (at the first menstrual cycle) in 30(15.7%) women, and in 35(18.3%) the menstrual cycle normalized, while in 63(33.0%) the changes still lasted.
Conclusion. In this study, an association of menstrual cycle changes and COVID-19 vaccination was found. More studies are needed and further follow-up in women with reported menstrual cycle changes.

Correspondence to: Natasha Ilieva, University Clinic for Gynecology and Obstetrics, 1000 Skopje, R. N. Macedonia; E-mail: nilieva.mk@gmail.com

Keywords: COVID-19, vaccine, menstrual cycle, disorders, menorrhagia

Апстракт

Вовед. Една година по означувањето на официјалниот случај со COVID-19 од страна на Светската Здравствена Организација, беше развиена вакцина против оваа заразна болест. Повеќе од три билиони дози од оваа вакцина се администрирани во светот во изминатиот период. Менструалните нарушувања после COVID-19 вакцинација беа пријавени, првично од самите жени, а асоцијацијата на овие две варијабли потоа беше и научно потврдена.
Цел. Да се евалуираат нарушувањата во менструалниот циклус после COVID-19 вакцинација кај жените во Република Северна Македонија.
Методи. Се работи за попречна студија која користи истражувачки прашалник и се одвива во периодот од март 2022 до март 2023 година. Учесничките во студијата се жени на возраст 18-45 години, државјани на Република Северна Македонија. Жените во наведениот временски интервал биле на преглед во Универзитетската гинеколошко-акушерска клиника во Скопје, кога се поканети да учествуваат во студијата. Прашалникот беше дистрибуиран и пополнуван електронски, со поддршка од истражувачкиот тим.
Резултати. Вкупно 191 учеснички во студијата, со просечна возраст 34.31±8.05 години, 163(85.3%) биле вакцинирани, а 28(14.7%) не биле вакцинирани. Промени во менструалниот циклус по вакцинација со COVID-19 вакцина биле пронајдени кај 89 (46.6%), претежно по нивната втора доза. Најчести промени во менструалниот циклус беа: пообилни менструации (менорагија), поретки циклуси (олигоменореја) и болни менструации (дисменореја). Промените беа забележани веднаш по

вакцинација (на првиот менструален циклус) во 30(15.7%) од случаите, а во 35(18.3%) менструалниот циклус се нормализирал, додека кај 63 (33.0%) промените сеуште перзистирале.
Заклучок. Поврзаноста на COVID-19 вакцинацијата со промените во менструалниот циклус е потврдена во оваа студија. Повеќе лонгитудинални студии се потребни за понатамошно следење на жените со присутни нарушувања во менструалниот циклус.

Клучни зборови: COVID-19, вакцина, менструален циклус, нарушувања, менорагија

Introduction

The COVID-19 pandemic has affected 697,032,526 people worldwide, of which 668,850,958 have recovered, and it is the cause of death of 6,932,236 people until 27th of October 2023. It has influenced every aspect of human's life and has changed the way of life in many instances [1]. The overwhelmming motivation of scientists to develop a vaccine that would protect against COVID-19 was the driving force behind the emergence of developing multiple vaccines against SARS-CoV-2 infection in less than a year since the first case [2]. Women's health with an emphasis on the menstrual cycle and the impact of the SARS-CoV-2 infection and COVID-19 vaccines have been a subject of numerous scientific research in order to scientifically evaluate short- and long-term consequences related to the pandemic [3]. Monthly menstruation in women represents a clear sign of health and fertility [4]. For evaluation, FIGO nomenclature includes the following parameters: frequency, regularity, duration and volume. Every disorder in the menstrual cycle affects a woman's psychological health and is the basis of further medical investigations, searching for its etiology [5]. Studies done so far have reported that COVID-19 vaccines are related to several menstrual cycle disturbances: increased menstrual cycle length, heavier menstrual bleeding, prolonged menstruation, delayed periods, breakthrough bleeding.
Vaccines that have been associated with menstrual cycle disorders, supported by scientific research include: typhoid vaccine, hepatitis vaccine and HPV vaccine [6]. Official bodies for registering adverse vaccine reactions do not collect data for menstrual cycle disturbances. Yet, women have self-reported menstrual cycle changes related to COVID-19 vaccination. Vaccine Adverse Event Reporting System (VAERS) from USA has received 13,118 self-reports and Medicines and Healthcare Products Regulatory Agency yellow card surveillance scheme from UK has received over 50,000 self-reports for menstrual disturbances following COVID-19 vaccination [4,7,8]. Reports from COVID-19 vaccinated women in USA were analyzed and 4,998 of them referred to irregular menstruation, and the rest of them to other disturbances as follows: delayed menstruation, intermenstrual bleeding, menstrual disorder, amenorrhea, metrorrhagia, hypomenorrhea, menorrhagia. A higher percentage of menstrual disorders related to COVID-19 vaccines has been noticed among women aged 30-49 years. Although the association of COVID-19 vaccines and menstrual irregularities has been confirmed, the findings are taken with caution due to the known limitations of self-reported data [8]. An online survey for COVID-19 vaccinated individuals in UK included 26,710 participants and affirmed menstrual disturbance in 20% of them. Smoking and previous history of SARS-CoV-2 infection were found to be risk factors, and oral contraceptives (estradiol containing) were a protective factor [4].
The aim of this study was to find out if there were menstrual cycle irregularities associated with COVID-19 vaccination in women from North Macedonia. This inquiry is particularly relevant given the widespread concerns among women about the impact of COVID-19 vaccine on their reproductive health. These findings would help in resolving women's anxiety and concerns and in providing high-quality medical services that will improve their quality of life.

Materials and methods

Design

This was a cohort study with cross-sectional design conducted in the period March 2022 until March 2023.

Sample

During this period of time, women with a scheduled examination at the University Clinic for Gynecology and Obstetrics in Skopje were invited to participate in this study. The questionnaire was modified and locally adapted, and based on reference questionnaires on the same topic. To participate in the study, it was necessary to meet the following inclusion criteria: residents of the Republic of North Macedonia and aged 18-45 years. Exclusion criteria included all patients with: hormone therapy, infertility, polycystic ovarian syndrome, endometriosis, pelvic in-

flammatory disease, primary ovarian insufficiency, as well as all pregnant and lactating women.

Research Instrument

A structured questionnaire was used as a research instrument, based on existing instruments that were previously validated in similar research. The questionnaire was modified and locally adapted, and based on reference questionnaires on the same topic [9-11]. It was compiled on the Google Forms platform, due to its accessibility and reliability [12,13]. It included 17 nominal questions for obtaining necessary data to answer the research objective: 10 for inclusion criteria, and 7 for menstrual cycle changes and COVID-19 vaccination status. Every patient was recruited by the research team, and detailed guidance was also provided at the beginning of the questionnaire together with the consent form. Patients were sent aa link to the questionnaire (by mobile short message, e-mail, viber and LinkedIn), which they filled out anonymously, having opportunity to contact the research team for any unclarity.

Ethical consideration

The Ethics Committee determined that formal approval was not required for this study due to its observational nature. The privacy and confidentiality of participants were upheld in accordance with the ethical standards prescribed by the Declaration of Helsinki. Informed consent was secured at the beginning of the questionnaire.

Statistical analysis

Data analysis was conducted with the software tools STATISTICA 8.0 and SPSS Statistics 23.0. A range of descriptive statistical methods (including average, standard deviation, 95% confidence intervals, median, and the range of minimum to maximum values) were applied for series characterized by numerical values. To evaluate the distribution of the data, tests such as Kolmogorov-Smirnov, Lilliefors, and Shapiro-Wilks were applied, noting the significance levels (p-value). Comparative analysis between numerical series was performed using the Wilcoxon Matched Pairs Test, indicating results with Z-scores and p-values, while the alignment of responses from study participants was assessed with reliability statistics, specifically Cronbach's Alpha (α). A p-value of less than 0.05 was considered to indicate a statistical significance.

Results

A total number of 191 respondents belonged to the adult group 18-45 years old. The results shown in Table 1 present the age of respondents. It varied in the interval of 34.31±8.05 years, the minimum age was 18 years and the maximum 45 years.

Table 1. Age of respondents

	N	Range	Minimum	Maximum	Mean	Std. Deviation
Age	191	27	18	45	34.31	8.045
Valid N (list wise)	191					

Table 2. Which COVID-19 vaccine have you been vaccinated with?

		Frequency	Percentage	Valid Percentage	Cumulative Percentage
Valid	Pfizer, BioNTech	97	50.8	50.8	50.8
	AstraZeneca, Oxford	6	3.1	3.1	53.9
	Sinopharm	23	12.0	12.0	66.0
	CoronaVac (Sinovac)	21	11.0	11.0	77.0
	Sputnik V	15	7.9	7.9	84.8
	I am not vaccinated	29	15.2	15.2	100.0
	Total	191	100.0	100.0	

In our survey of 191 respondents, the distribution of COVID-19 vaccine types varied. The majority, representing 50.8%, received Pfizer/BioNTech vaccine. Other vaccines administered included AstraZeneca/ Oxford (3.1%), Sinopharm (12.0%), CoronaVac/ Sinovac (11.0%), and Sputnik (7.9%). It is noteworthy that 15.2% of respondents reported they were not vaccinated. Detailed proportions of each vaccine type are illustrated in Table 2 and Figure 1.

A total of 82 (42.93%) respondents answered that they did not have any of the listed options in response to this question.

Table 3 shows the answers to the question: “Have

Table 3. Have you noticed any changes in menstrual bleeding since March 2020?

Category	Number	Percentage
Heavy bleeding	27	14.14
Changes in the color of vaginal discharge	7	3.66
Less frequent periods	19	9.95
None of the above	82	42.93
Other changes in vaginal discharge	4	2.09
Changes in menstrual bleeding in the closing days	8	4.19
Painful menstruation	12	6.28
More than 2 answers	32	16.76
Total	191	100.0

you noticed any changes in your menstrual bleeding since March 2020?"

We observed different changes in menstrual cycles of our respondents post-COVID-19 vaccination: 7.3% of participants reported changes after the first vaccine dose, 19.4% after the second dose, and 1.6% after the third dose (Table 4). Additionally, 18.3% of respondents indicated menstrual cycle changes irrespective of vaccination status. Among our study population, 14.7% were not vaccinated, and 38.7% did not experience any menstrual cycle changes post-vaccination (Table 4).

Table 4. Have the menstrual cycle changes appeared after COVID-19 vaccination?

		Frequency	Percentage	Valid Percentage	Cumulative Percentage
Valid	Yes, after the first dose	14	7.3	7.3	7.3
	Yes, after the second dose	37	19.4	19.4	26.7
	Yes, after the third dose	3	1.6	1.6	28.3
	No, the changes happened regardless of vaccination	35	18.3	18.3	46.6
	I'm not vaccinated against COVID -19	28	14.7	14.7	61.3
	I do not have changes	74	38.7	38.7	100.0
	Total	191	100.0	100.0	

The results shown in Figure 1 refer to the time when changes in the menstrual cycle occurred after COVID-19 vaccination. Of a total of 191 respondents, 30(15.7%) respondents had changes in the menstrual cycle immediately, during the first cycle; 16(8.4%) respondents had changes in the menstrual cycle after 2 months; 9(4.7%) respondents had changes in the menstrual cycle after 3 months; 25(13.1%) respondents had changes in the menstrual cycle after > 3 months; 86(45.0%) answered that there were no changes and 25(13.1%) respondents answered that they have not been vaccinated.

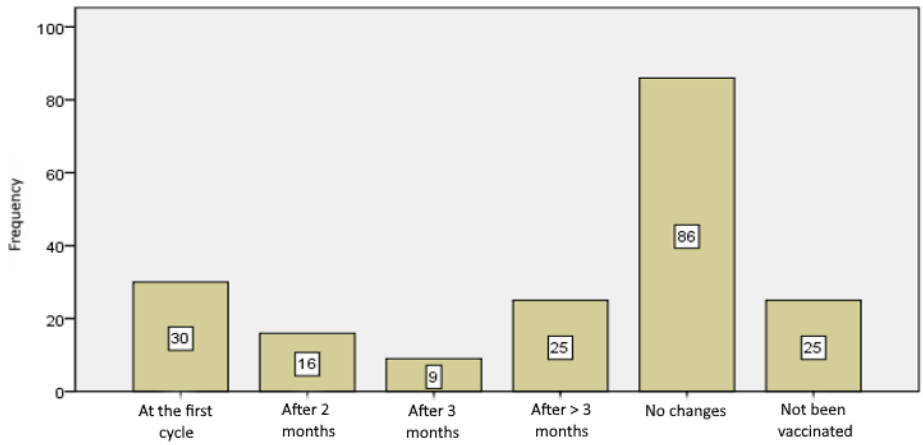

Fig. 1. How long after COVID-19 vaccination the menstrual cycle changes occurred?

Participants were also evaluated whether their menstrual cycle changes normalized and in which timeframe. Of a total of 191 respondents, in 13(6.8%) respondents the menstrual cycle normalized after 1-2

months, in 16(8.4%) respondents it normalized after 3-6 months, in 5(2.6%) respondents the menstrual cycle normalized after 7-12 months, in 1 (0.5%) it normalized after >1 year, 93(48,7%) respondents answered that there were no changes, and in 63(33.0%) the changes were still present.

Discussion

After initial vaccination of healthcare providers in North Macedonia, official COVID-19 vaccination process commenced on 31st of March 2021 in the country, after receiving 24,000 doses of AstraZeneca/Oxford vaccine, intended for risk groups of people (people with chronic conditions and older than 75 years) [14]. Then, other categories of citizens were included, and vaccine donations from Serbia, Italy, Turkey were welcomed to meet the needs. More than 1.85 million doses of COVID-19 vaccines have been administered and it is estimated that 44.4% of the population in North Macedonia has been vaccinated with the COVID-19 vaccine [15]. There are twelve COVID-19 vaccines with granted emergency use [16]. In North Macedonia, the following vaccines have been used: Pfizer/BioNTech (mRNA), Astra Zeneca/Oxford (Non-Replicating Viral Vector), Sputnik V (Adenovirus vaccine), Sinovac/CoronaVac (inactivated) and Sinopharm (inactivated). In our study, the greatest portion of vaccinated participants (50.8%) were vaccinated with Pfizer/BioNTech, and 15.2% were not vaccinated. During the pandemic, the discovery of the vaccine aroused positive and negative emotions, due to the given emergency in authorization processes [17]. Public health strategies were implemented to build trust regarding the safety of the vaccines and to ensure herd immunity [18].

In our study, 46.6% of women reported menstrual cycle changes after COVID-19 vaccination. A similar percentage was found in a systematic review of 14 studies that included more than 78,000 vaccinated females, with menorrhagia, metrorrhagia and polymenorrhea as the most commonly observed problems [19]. Women with menstrual cycle disorders after COVID-19 vaccination in our study reported heavy periods/bleeding-menorrhagia (14.4.%), infrequent/irregular periods (oligomenorrhea) (9.95%) and painful menstruation-dysmenorrhea (6.28%) as the most common menstrual cycle changes. A longitudinal cohort study involving 545 women from Arizona found a lower percentage of menstrual cycle changes after COVID-19 vaccination (25%) and in most of the cases the changes were reported as temporary (up to 6 months) [20]. The duration of menstrual cycle changes in our study varied; 15.2% of respondents resolved their regular menstrual cycle in a time interval of 6 months, 2.6% in 12 months and 0.5% in more than a year. In 33.0% of respondents, irregularities still lasted in the period of their participation in the study.

Our study found that 19.4% of women experienced changes after the second dose, which is in agreement with the extensive research indicating a potential association between COVID-19 vaccination and menstrual irregularities [21-23]. The findings of Laganà *et al.* are consistent with ours with reference to the most frequent changes in menstrual bleeding patterns that occurred after the second vaccine dose [24].

The mechanisms behind these menstrual changes remain under investigation. Recent hypotheses suggest a potential immune response triggered by the vaccine affecting hormonal regulation [25]. Turnbull and Rivier in their study explained that the menstrual cycle, regulated by the hypothalamic-pituitary-ovarian axis, may be influenced by stressors like COVID-19 pandemic stress or vaccination [26]. The immune response of the body to SARS-CoV-2 infection, and to COVID-19 vaccine is necessary in order to achieve immunization. While no menstrual changes were reported in the unvaccinated during the pandemic, the strong immune response to SARS-COV-2 infection or COVID-19 vaccination could disrupt this axis [27]. The COVID-19 spike protein and vaccine adjuvants are potential triggers for this immune reaction, which might lead to menstrual irregularities [28]. This response involving pro-inflammatory markers, can activate immune cells in the endometrium, possibly causing heavier or irregular bleeding, as Girardi *et al*. explained in their study [29]. Furthermore, vaccine-induced changes in hormone levels and immune-mediated phenomena like thrombocytopenia, previously observed with other vaccines, could also affect menstrual cycles [30-32]. However, as our study indicated, changes in a small proportion of unvaccinated individuals, other pandemic-related factors such as stress or lifestyle changes might also play a role [33-35].

Our study's limitation lies in its cross-sectional design, which precludes establishing a causal relationship. Longitudinal studies are needed to further elucidate the duration and severity of these changes over time. Additionally, exploring the impact of different vaccine types on menstrual cycles would be valuable [36,37].

In conclusion, while our study adds to the growing body of evidence linking COVID-19 vaccination to menstrual changes, these findings should be discussed carefully to avoid vaccine hesitancy. Healthcare

providers should be aware of these potential changes to better counsel and support their patients [38].

Conclusion

Our study has confirmed an association between menstrual cycle changes and COVID-19 vaccination. The most frequently reported changes were heavy periods/bleeding, infrequent/irregular periods, and painful menstruation. A significant portion of women experienced persistent changes, highlighting the potential long-term impact of vaccination on menstrual health. The likely mechanisms for these changes suggest that the vaccine-induced immune response may disrupt the hormonal balance, controlled by the hypothalamic-pituitary-ovarian axis. This suggests a complex relationship between the body's immune reaction to the vaccine's components and hormonal regulation.

While our findings are in line with existing research regarding the timing and nature of these menstrual changes, the cross-sectional design of our study presents limitations in establishing direct causality. This gap highlights the urgent need for longitudinal studies to gain a deeper and more comprehensive understanding of these changes over time.

Conflict of interest statement. None declared.

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Мак Мед Преѓлед 2023; 77(1): 24-28

ММР

Original article

CORRELATION OF PD-L1 GENE EXPRESSION WITH GRADE OF THE URINARY BLADDER CANCER

КОРЕЛАЦИЈА НА ЕКСПРЕСИЈАТА НА ГЕНОТ PD-L1 СО ГРАДУСОТ НА КАРЦИНОМОТ НА МОЧЕН МЕУР

Viktor Stankov[1], Oliver Stankov[1], Sasho Dochev[1], Stavridis Stavridis[4], Ognen Ivanovski[1], Josif Janculev[1], Martina Ambardjieva[3], Aleksandra Gavrilovska Brzanova[2], Sasho Panov[5] and Skender Saidi[1]

[1]University Clinic for Urology, Skopje, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje [2]University Clinic for Traumatology, Orthopedic Diseases, Anesthesia, Reanimation, Intensive Care and Emergency Centre, Skopje, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, [3]University Surgery Clinic "St. Naum Ohridski", Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, [4]Department of Urology, Acibadem Sistina Hospital - Skopje, [5]Faculty of Natural Sciences and Mathematics, Institute of Biology, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

Introduction. Bladder cancer (BC) ranks fourth in the prevalence of malignancies in developed countries and is the eighth leading cause of cancer-related mortality in men. PD-L1, known for its role in inhibiting immune responses against malignant cells, has garnered significant attention in BC research.
Methods. This study, comprising 45 patients with histopathologically confirmed urothelial carcinoma of the urinary bladder, analyzed the connection between histological grade and PD-L1 gene expression. The patient cohort was divided into 31 classified as low-grade and 14 as high-grade, with gender and age distribution well-balanced across the groups. PD-L1 expression was notably higher in the high-grade group (p=0.005), showing its potential clinical relevance as a biomarker.
Results. Univariate logistic analysis revealed a robust correlation between histological grade and PD-L1 expression, with high-grade patients exhibiting a 7.227-fold higher likelihood of increased PD-L1 expression. A predictive model for grade determination demonstrated commendable performance, boasting an area under the curve (AUC) of 0.788.
Conclusion. These findings provide compelling evidence of a strong association between PD-L1 gene expression and the histological grade of bladder cancer. PD-L1 emerges as a potential biomarker, shedding light on a disease pathological grade, offering a significant clinical value for precise prognosis, and guiding tailored treatment strategies. These insights hold promise for improved disease management and patient outcomes.

Keywords: bladder cancer, PD-L1 gene expression, polymorphisms rs861539, biomarker

Correspondence to: Viktor Stankov, University Clinic for Urology, 1000 Skopje, R. N. Macedonia; E-mail: viktor_stankov@yahoo.com

Апстракт

Вовед. Карциномот на мочниот меур се наоѓа на четвртата позиција во преваленцата кај малигни заболувања во развиените земји и е осма водечка причина за смртност предизвикана од карцином кај мажите. PD-L1, познат по својата улога во инхибирање на имунолошките одговори против малигните клетки, привлекол значително внимание во истражувањето на карциномот на мочниот меур.
Методи. Ова истражување, кое вклучуваше 45 пациенти со хистопатолошки потврден уротелен карцином на мочниот меур, ја истражуваше поврзаноста помеѓу хистолошкиот градус и експресијата на генот PD-L1. Кохортата на пациенти беше поделена на 31 пациент (пациенти со низок градус на карцином) и 14 (висок градус на карцином), при што дистрибуцијата на полот и возраста беше рамномерна помеѓу групите. Експресијата на PD-L1 беше значително поголема кај пациентите со висок градус (p=0.005), и ова го покажува неговиот потенцијалено клиничко значење како биомаркер.
Резултати. Направените анализи откриј сила корелација помеѓу хистолошкиот градус и експресијата на PD-L1, при што пациентите со висок градус покажаа 7.227-пати поголема веројатност

за зголемена експресија на PD-L1. Изработен беше предиктивен модел за одредување на градусот, кој прикажа добра предиктивност, со вредност за подрачјето под кривата (AUC) од 0.788.
Заклучок. Овие ноди обезбедуваат убедливи докази за јака поврзаност помеѓу експресијата на генот PD-L1 и хистолошкиот градус на карциномот на мочниот меур. PD-L1 се појавува како потенцијален биомаркер, овозможувајќи значителна клиничка вредност за точна прогноза и насочување на персонализирани стратегии за третман. Овие истражувања ветуваат напредок при менаџирање со болеста со цел подобар исходот за пациентите.

Клучни зборови: карцином на мочен меур, PD-L1 генска експресија, полиморфизам rs861539, биомаркер

Introduction

Bladder cancer (BC) is the fourth most common malignant disease in developed countries and the eighth most common cause of cancer death among men [1,2]. The diversity in tumor phenotype and the long duration of treatment make BC one of the clinical entities with the highest financial burden on the healthcare system and has a significant impact on patients' quality of life.
The histological evaluation of bladder cancer relates to tissue differentiation and tumor aggressiveness in relation to how abnormal the cells appear under microscopic analysis [3]. There are various systems for classifying the degree of tissue differentiation, but in the papers published over the last decade, the binomial system with two grades, low and high, is most commonly used.
At diagnosis, 75% of patients have non-muscle-invasive bladder cancer (NMIBC) [4]. However, when recurrences happen, muscle-invasive bladder cancers (MIBC) are diagnosed in some of these cases, which have a high risk of metastasizing the regional lymph nodes, as well as hematogenously in distant organs. Current molecular marker sets are not secure enough to enable accurate prediction of the potential for recurrence and disease progression.
PD-L1 gene (also known as B7-H1 or CD274) codes a ligand for the PD-1 receptor and is expressed in many malignant tumors, including BC. PD-L1 can inhibit immune responses either by binding to PD-1 or other receptors, resulting in significant effects on the susceptibility of malignant cells to immune recognition and their elimination [5]. The apoptotic pathway mediated by the PD-1/PD-L1 interaction inhibits T-cell activation and plays an important role in regulating the antitumor immunity of patients with malignant neoplasms.
Immune checkpoints have an important regulatory role, i.e., they prevent an excessive immune response that would damage normal cells and tissues. These checkpoints are activated when the proteins on the surface of T-cells recognize and bind to the appropriate receptors on other cells, such as some tumor cells. When the checkpoint and partner proteins bind mutually, they send a signal to inhibit T-cells. However, this can also prevent the immune system from recognizing and lysing malignant cells. Immunotherapeutic agents called immune checkpoint inhibitors are based on blocking checkpoint proteins, thereby preventing them from connecting with their receptor proteins [6]. This prevents the blockade signal from being sent and allows T-cells to kill malignant cells.
Modern treatment with targeted drugs is based on the inhibitory action of the immune control point protein called CTLA-4. Other immune checkpoint inhibitors act inhibitory on the protein called PD-1 or its receptor protein PD-L1. Namely, some tumor cells reduce the response of T-cells by producing an excessive number of PD-L1 protein molecules on their cell membrane.
The main aim of this scientific paper was to determine the correlation of the frequency of genotypes, i.e., alleles of the examined polymorphism with susceptibility to BC.

Materials and methods

The primary aim of this study was to correlate PD-L1 expression with the grade of BC, and for that purpose we analyzed 45 bladder tissue specimens obtained via transurethral resection of the bladder at the University Clinic for Urology. Patients were diagnosed with urothelial carcinoma of the urinary bladder, and were histopathologically confirmed based on the histopathological grade of differentiation (binomial system with two classes of differentiation: low and high grade).
Demographic data, histopathological findings, as well as relevant clinical data (cystoscopic, echographic, and CT findings, clinical course of the disease, etc.) were collected for each patient. Differences in terms of gender and age distribution between the two groups of patients were analyzed using the Student's t-test. Prior to that, normal distribution of these data was assessed using the Shapiro-Wilk test.

The quantitative expression of PD-L1 gene was determined by reverse transcription of RNA samples and real-time polymerase chain reaction (qRT-PCR) using fluorescent TaqMan probes on the OneStep Real-Time PCR system (Applied Biosystems). Total RNA was isolated from tissue fragments of tumor tissue (previously stored at -80°C) using TRI reagent, and cDNA was synthesized by reverse transcription. The levels of hTERT expression were calculated using the Livak method, relative to the expression of the housekeeping gene GAPDH. The relative quantitative values (RQ) were presented as 2-ΔCt. In this study, we present the final expression values of the PD-L1 gene for each patient as the logarithm of RQ with a base of 10, i.e., log10(RQ).

The correlation between the degree of tissue differentiation of the BC tissue sample from patients and the expression of PD-L1 gene was evaluated using the univariate logistic analysis. Receiver operating characteristic (ROC) curves were evaluated, and the area under the curve (AUC) value was obtained. The odds ratio was calculated within a 95% confidence interval (CI).

Values of $p<0.05$ were considered statistically significant, while values of $p<0.01$ were considered highly significant. Statistical calculations were performed using the XLSTAT 2016 and GenAlEx 6.5 software add-ins installed on Microsoft Excel 2016.

Results

A total of 45 patients with BC were included, of which 14 had a low grade (well-differentiated histologically) and 31 had a high grade (poorly differentiated) (Table 1 and Figure 1).

Table. 1. Distribution of BC grade

Grade	n	%
Low	14	31.11
High	31	68.89
Total	45	100.00

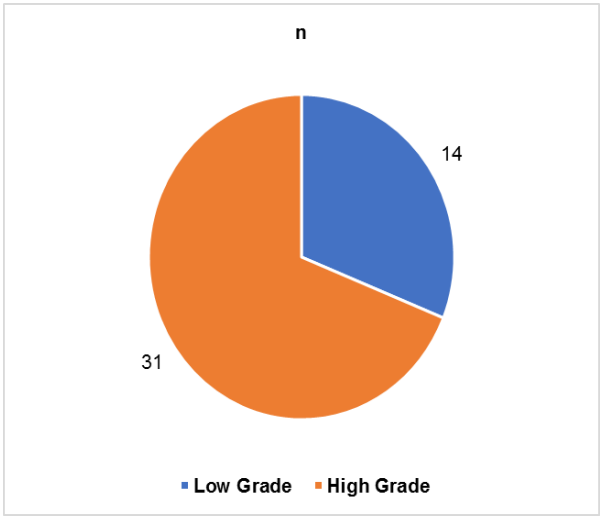

Fig. 1. Distribution of BC grade

The selected clinical data were evaluated and compared to PD-L1 expression in tumor cells.

The presented data and the results of the statistical analysis of the gender and age distribution in the two groups (patients with low and high grades) indicated statistically insignificant differences ($p>0.05$) (Tables 2 and 3 and Figures 2 and 3). The two groups were well balanced in terms of gender and age, which ensured a reliable comparison regarding gene expression.

Table. 2. Gender distribution

Gender	Low grade		High grade		Fisher exact test*
	n	%	n	%	*p*
male	12	85.71	26	83.87	
female	2	14.29	5	16.13	1.000
total	14	100.00	31	100.00	

* two-tailed

Table. 3. Age distribution

Parameter (years)	Low grade	High grade	Student's t-test *
n	14	31	
Average	65.14	62.16	
SD	11.27	7.87	0.311
min. age	47	46	
max. age	80	76	

* two-tailed

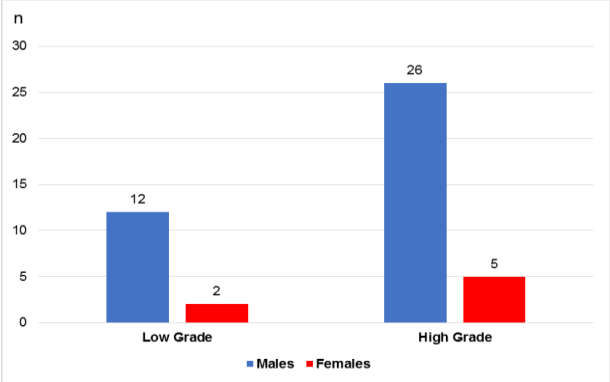

Fig. 2. Gender distribution

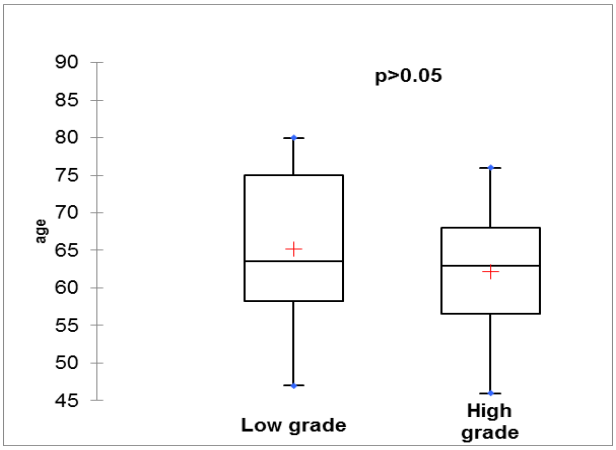

Fig. 3. Age distribution

Table. 4. Expression of PD-L1 gene according to BC grade

PD-L1	Low grade	High grade
n	14	31
average	0.779	1.398
SD	0.548	0.569
min. value	0.221	0.141
max. value	1.753	2.481

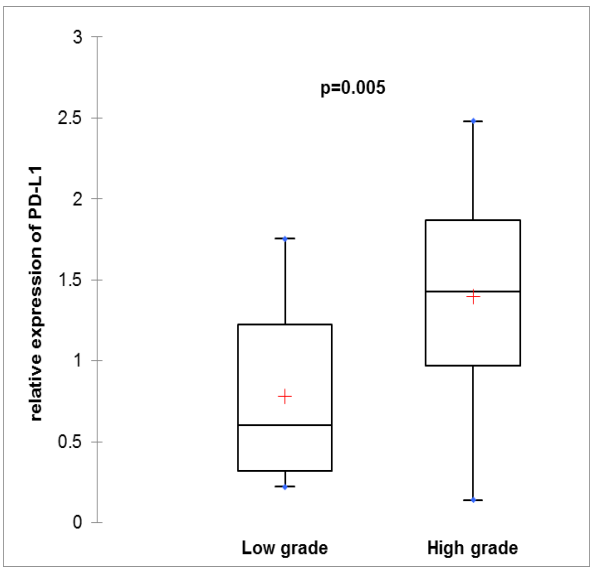

Fig. 4. Expression of PD-L1 gene according to BC grade

Table. 5. Univariant logistic analysis for correlation with expression of gene PD-L1 in relation with the grade

Parameter	β-coefficient	Standard error (SE)	Wald's χ^2	p	OR (95% CI)
Grade	0.676	0.241	7.879	0.005	7.227(1.816 - 28.757)

Furthermore, the data obtained from quantitative determination of PD-L1 gene expression were analyzed. The values referred to the expression of PD-L1 gene for each patient as log10(RQ). Descriptive results are presented in Table 4 and Figure 4.

It was evident that the expression of PD-L1 gene was statistically significantly higher in the group of patients with a high grade compared to the low grade (p=0.005).

From the results obtained, it can be seen that there was a correlation between the histological grade of differentiation (low and high) and the quantitative levels of PD-L1 gene expression. Patients with a high grade (poor differentiation) had a 7.227-fold higher probability of having increased levels of PD-L1 gene expression compared to patients with a low grade (good differentiation) in BC. The results of the univariate logistic analysis are presented in Table 5.

The constructed model for predicting the grade based on the expression levels of PD-L1 gene is depicted by the ROC curve in Figure 5.

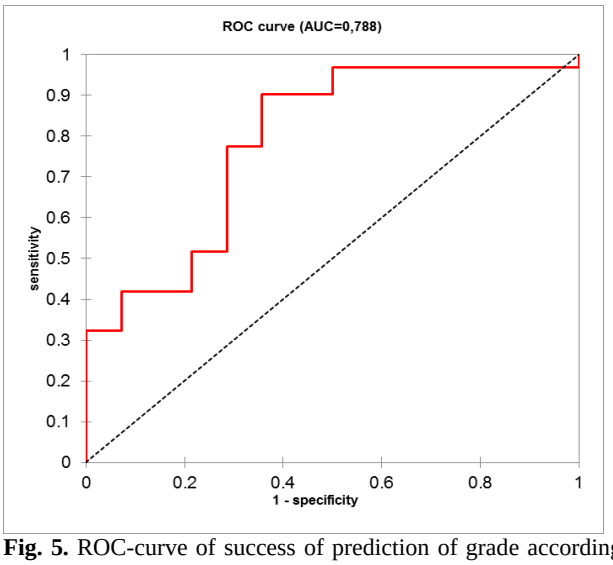

Fig. 5. ROC-curve of success of prediction of grade according to expression of PD-L1 gene in patients with BC

The value of the area under the curve (AUC) was 0.788, indicating a good predictive performance of the model.

Discussion

The results presented in this scientific paper indicated higher levels of transcriptional activity, specifically the expression of PD-L1 gene, in tissue samples from patients with BC with a higher histological grade (well-differentiated) compared to the lower grade (poorly differentiated), and the difference was statistically highly significant (p=0.005).

The expression of PD-L1 gene is present in antigen-presenting cells (APCs), such as monocytes, activeted dendritic cells, and others [7]. PD-L1 is a regulatory ligand that can inhibit immune responses by binding to the PD-1 receptor on the surface of T lymphocytes, leading to apoptosis or anergy of antigen-specific T cells. However, malignant cells can exploit these checkpoint pathways by overexpressing PD-L1, protecting themselves from detection and elimination by cytotoxic T cells of the immune system. Thus, excessive expression of PD-L1 is a mechanism by which tumors develop tolerance to immune responses.

The role of PD-L1 in the progression of bladder cancer, as well as many other malignancies, is undeniable. The correlation between PD-L1 expression in tumor cells and poor clinical outcomes was first reported in a study conducted by Nakanishi *et al.* that included 65 patients with BC [8].

Sharma *et al.* demonstrated that PD-L1 expression in tumor cells was not a good predictor of prognosis [9].

However, most published studies have demonstrated a correlation between elevated expression of PD-1/PD-L1 and a poor prognosis in bladder cancer. According to Kawahara (2018), PD-L1 expression may serve as a novel biomarker that correlates with the pathological grade of bladder cancer [10].

The results of this study also support the existence of a clear correlation and potential clinical utility of PD-L1 expression in predicting disease progression and selecting patients suitable for immune checkpoint inhibitor therapy.

Conclusion

The findings of this study, based on data and specimens from 46 patients, offer compelling evidence of the strong correlation between PD-L1 gene expression and the histological grade of bladder cancer. This correlation suggests that PD-L1 could potentially serve as a novel biomarker, shedding light on the pathological grade of bladder cancer. Such a biomarker could hold a significant clinical value, helping in establishing a more precise prognosis and guiding tailored treatment strategies.

Additionally, the link between PD-L1 and disease stage progression in bladder cancer adds a layer of importance to these discoveries. Elevated PD-L1 expression in high-grade tumors, often indicative of aggressive clinical behavior, implies that PD-L1 may influence the disease progression. This observation could pave the way for the development of targeted therapies aimed at modulating PD-L1 expression, potentially altering the course of bladder cancer at a crucial juncture.

Conflict of interest statement. None declared.

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Мак Мед Прегled 2023; 77(1): 29-32

ММР

Original article

PREDICTORS OF PATIENT SATISFACTION IN THE HEALTHCARE SYSTEM IN NORTH MACEDONIA

ПРЕДИКТОРИ НА ЗАДОВОЛСТВО НА ПАЦИЕНТИ ВО ЗДРАВСТВЕНИОТ СИСТЕМ ВО СЕВЕРНА МАКЕДОНИЈА

Aneta Petrusevska[1] and Vera Dimitrievska[2]

[1]Clinic Dr. Ancevski, MIT University – Skopje, [2]University of American College, Skopje, Republic of North Macedonia

Abstract

The aim of this study was to find service quality factors that are important to patients and to examine their links to general patient satisfaction in the Macedonian context. To achieve this goal, we provided insight, exploratory investigation and support for the strategical framework of hospital sectors as an initial strategy for marketing healthcare, increasing patients' number?, and improving patient satisfaction. Quantitative analysis was performed based on the overall patient satisfaction at national level to determine the importance of individual and national factors on healthcare system satisfaction of North Macedonia. The results have shown that overall satisfaction increases with age and other demographic variables are not significantly correlated with satisfaction. We made an Ordinal Regression Analysis. We found that the ordinal regression model on the medical infrastructure factor (independent variable) as a construct which includes "waiting rooms", "quality, hospital rooms", "lab services" and "drugs 14 availability" has shown a strong effect on overall patient satisfaction (dependent variable) with the health care system.

Keywords: patient satisfaction, health care system, factors of satisfaction

Апстракт

Целта на оваа студија е да се пронајдат факторите за квалитет на услугата кои се важни за пациентите и да се испитаат нивните врски со општото задоволство на пациентите во македонскиот контекст. За да се постигне оваа цел, обезбедуваме увид, истражувања и поддршка за стратешката рамка на болничките сектори како почетна стратегија за маркетинг на здравствена заштита, зголемување на пациентите и проширување на задоволството на пациентите. За потребите на студијата се направи квантитативна анализа врз основа на целокупното задоволство на пациентите на национално ниво за да се утврди важноста на индивидуалните и националните фактори за задоволството од здравствениот систем на Северна Македонија. Резултатите покажуваат дека целокупното задоволство се зголемува со возраста, а другите демографски варијабли не се значајно во корелација со задоволството. Ние користевме Ординална регресивна анализа. Откривме дека регресивен модел на факторот на медицинска инфраструктура (независна променлива) како конструкција што вклучува („чекални", „квалитет на болнички соби", „лабораториски услуги" и „достапност на лекови") покажа силен ефект на целокупното задоволство на пациентите (зависна променлива) со системот за здравствена заштита.

Клучни зборови: задоволство на пациентите, здравствен систем, фактори на задоволство.

Introduction

The literature data on patient satisfaction in Macedonia are scarce. The open-access database has generated a few articles on patient satisfaction in general, and patient satisfaction comparison between secondary tertiary and tertiary healthcare levels [1,2]. According to Lazarevik, the main predictors for patient satisfaction in healthcare services are associated with low users' satisfaction-waiting time for appointments, huge administrative procedures, and attitudes of the medical personnel towards patients

Correspondence to: Aneta Petrusevska, Clinic Dr. Ancevski, 1000 Skopje, R. N. Macedonia; E-mail: anetakokaleska@gmail.com

[1]. The top three indicators of patient satisfaction are trust and overall satisfaction with the attention of the doctors, as well as satisfaction with the outcome of the treatment. On the other hand, the study by Stefanovska shows that there is a difference in patient satisfaction between both healthcare levels [2]. Patients were significantly more satisfied in tertiary than in secondary outpatient healthcare facilities in almost all aspects of assessment related to general settings, nurse/administrative staff performance and physician performance. Patients in the secondary healthcare services (SHCS) were more satisfied than in the tertiary healthcare services (THCS) but only regarding the information on the location (83.9% *vs*.78.3%) and possibilities to enter and move inside the department (88.8% *vs*. 83.3%). Analysis of data for SHCS and THCS showed that there was no significant difference between the mean overall satisfaction scores about patients' gender, age, marital status, educational level, employment and number of visits.

The study attempted to understand the perceptions and experiences among patients from a bottom-up perspective, i.e., focusing on doctors, health workers, and counselors rather than on healthcare policies and ministers. It analyzed the work and attitudes of middle-level professionals directly involved in the care of the clients/patients. Moreover, the study also investigated the attitudes of the patients toward their interaction with professionals. What are the most common factors of patient satisfaction? The ultimate main goal addressed in this study was to explore the main factors and experiences of patient satisfaction in healthcare environments in North Macedonia.

Materials and methods

The study was a cross-sectional survey which maps perceptions of Macedonian healthcare users regarding the quality of experience offered by the healthcare system. This study went beyond descriptive statistics by employing multivariate analysis. We chose this design because the research body about satisfaction was done extensively but some factors were separately discussed that reflected our concept on service quality, trust, communication, and patient satisfaction. This enabled us to identify and categorize the variables that made our questionnaire easy and thus we could capture all the information we needed from our respondents. An online structured survey was employed to collect the primary data in the initial stage of the study. The internet website was utilized to get the data of respondents that were interested in providing information on patient satisfaction in North Macedonia. The sample consisted of 435 people who filled in the questionnaire. In three months of data collection, the research team with a response rate 139 of 75% finalized the sample to a total of 435 respondents. The survey was anonymous and no additional data was revealed about the respondent.

Analyses were conducted using the Statistical Package for Social Science (SPSS), version 26, and Amos (26). All results were based on data collected from the Macedonian population in 2019. We used both descriptive and inferential statistics to analyze variables in the study.

Results

The study sample was primarily comprised of female participants, i.e., they accounted for 360 respondents (78%) against 90 male participants (20%). As regards the respondents' age structure, the largest group included subjects aged 36 to 45 years (33%), followed by those aged 26 to 35 years (31.9%). The full demographic profile of respondents is given in Table 1. The sample was a mixture of different generational cohorts and could be assumed to be representative of the population. dents (78%) against 90 male participants (20%).

Table 1. Demographic profile

Gender	No.	%
Female	360	78.8
Male	91	20.0
Nationality		
Macedonian	412	93.8
Albanian	7	1.5
Serbian	7	1.5
Roma	2	0.4
Bosnian	2	0.4
Vlach	2	0.4
Turkish	3	0.7
Age		
18-25	60	13.1
26-35	146	31.9
36-45	152	33.2
46-55	66	14.4
56+	27	5.9
Education		
Higher education	278	60.8
Secondary education	91	20.0
Postgraduate (master/other)	80	17.2

The regression model was applied to analyze the overall satisfaction collected by the online survey addressing patients in the Republic of North Macedonia. The ordinal regression analysis aims to model dependence of polytomous ordinal response on a set of predictors, which are characterized as covariates. Under this model, predictor variables are

sub-aspects of patient satisfaction (demographic characteristics) and the outcome variable is overall satisfaction. Due to the ordinal polytomous nature of the outcome and predictor variables, the ordinal regression (OR) analysis was adopted to estimate the aspects of overall satisfaction. Namely, the OR model was designed and modelled to estimate the effects of some predictors and the outcome variable. The OR model was built to assess the influence of overall satisfaction and sub-items of satisfaction, whereas the second model of regression was performed to assess the influence of healthcare service quality against sub-items of satisfaction. To analyze the data, a numerical code (on the scale from 1 to 5) was assigned to each point on the ordinal scale, according to the methodology known as directly determined quantification [3]. In the models proposed, the outcome variables were named "overall satisfaction" and "quality of services", while the predictor variables represented the sub-aspects/dimensions of satisfaction.

Table 2. Ordinal regression analysis and overall satisfaction

	Estimate	Std. error	df	Sig.	95% confidence interval	
					Lower bound	Upper bound
[Overall satisfaction = 0]	2.960	625	1	.000	1.735	4.185
[Overall satisfaction = 1]	7.321	.791	1	.000	5.771	8.871
Med. administration	.040	.035	1	.259	-.029	.109
Med. competence	-.033	.029	1	.260	-.089	.024
Med. infrastructure	.008	.035	1	.040	-.060	.076
Interaction	.269	.047	1	.000	.177	.362
Sex	-.549	.272	1	.043	-1.083	-.016
Nationality	.006	.170	1	.970	-.327	.340
Age	.237	.100	1	.017	.042	.433
Education	.309	.136	1	.023	.043	.575
Income	.027	.101	1	.786	-.171	.226
Employment	.083	.085	1	.331	-.084	.249
Place	-.003	.023	1	.877	-.048	.041

The ordinal regression analysis revealed that five variables were associated with the overall patient satisfaction (Table 2): two sub-aspects of patient satisfaction and three demographic variables. The strongest predictors were those under the medical infrastructure construct ("lab services", "availability of medicines", "quality of waiting rooms", and "quality of hospital rooms") and the interaction construct ("listened to by doctors", "explanation by doctors", "trust in doctors" and" treatment effectiveness"), while in the group of demographic variables they included gender, age and education.

Discussion

the notion of patient satisfaction and its measures has shown that it is a very complex concept without clear meaning and/or definition about what satisfaction represents, which makes it even more difficult to be measured. This notion is a result of a multi-aspect concept and this study investigated patient satisfaction and its relationships in multi-factorial context. On that note, the study brought out some potential context-based factors that might influence patient satisfaction.

When discussing the results obtained and their implications, the study first presents main findings related to identified factors of satisfaction, main predictors and follows with elaboration of the study's future implications. The study sets an agenda for future research as follow-up, with the main focus on medical services and patient satisfaction. At the same time, some study limitations and next steps for future research are presented and discussed.

Based on assessment performed as part of the explanatory approach, the study identifies the regretssion model that best fits and influences patient satisfaction.

The framework of this study adds to previous work by suggesting medical administration a part of hospital healthcare setting as an indirect factor of patient satisfaction. Indicators of the medical administration construct include "waiting time", "patient admission", "clinical appointments", "patient discharge" [4,5]. In this study, medical administration was found to have indirect relationship with satisfaction and quality of services. This, in turn, influenced patient perceptions and values of healthcare providers. At the same time, these intangible aspects are part of the organizational culture within a healthcare unit [6].

Findings of this study have shown that organizational culture is important, but a complex element of evaluating patient satisfaction with services. Hence, to effectively address and meet patient needs it is important for organizational culture to be taken into consideration when designing healthcare business strategies.

Conclusion

The main conclusion of this study is that all independent dimensions of patient satisfaction are significant in explaining overall patient satisfaction. Results suggest multiple significant benefits for patient satisfaction by healthcare organizations paying attention to tangible and intangible, as well as interactive and structural characteristics of their service provision. Moreover, it reiterates the significance of sociodemographic characteristics in determining healthcare service quality and patient satisfaction. In particular, they continue to be important factors of substantial effect on use of healthcare services. Moreover, this study has identified a significant relationship with medical infrastructure ("lab services", "availability of medicines", "waiting rooms" and "hospital rooms") and satisfaction indicates that hospital managers might not have complete understanding and knowledge of their role to influence patient satisfaction by improving healthcare services.

Conflict of interest statement. None declared.

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MMP

Case report

ANTI-ANDROGEN THERAPY FOR METASTATIC TRIPLE-NEGATIVE BREAST CANCER – CASE REPORT

АНТИАНДРОГЕНА ТЕРАПИЈА ЗА МЕТАСТАТСКИ ТРОЈНО- НЕГАТИВЕН КАРЦИНОМ НА ДОЈКА-ПРИКАЗ НА СЛУЧАЈ

Deva Petrova[1,2], Katerina Kubelka Sabit[1,2] and Biljana Crcareva[2]

[1]Faculty of Medical Sciences, University Goce Delchev, Shtip, [2]Clinical Hospital Acibadem Sistina, Skopje, Republic of North Macedonia

Abstract

Breast cancer (BC) is the most common malignant disease in the female population. From a therapeutic point of view, the group of triple-negative breast cancer (TNBC) is the most difficult to treat, considering the reduced treatment options. TNBC cannot be treated as one homogenous disease. One such promising biomarker and target for therapy in TNBC is the androgen receptor (AR). The role of the AR in BC pathology is gaining clinical interest also in relation to the development of drugs that can modulate AR activity.

We present a case of triple-negative metastatic breast cancer with androgen receptor overexpression, successfully treated with anti-androgen therapy. The patient was diagnosed with bone metastasis 5 years after the initial diagnosis with breast cancer. At that point, we treated the patient with anti-androgen therapy involving Bicalutamide and bisphosphonate therapy. After 18 months of treatment, the patient is asymptomatic with disease regression.

Keywords: triple-negative breast cancer, androgen receptors, anti-androgen therapy

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Апстракт

Ракот на дојка е најчеста малигна болест кај женската популација. Од терапевтска гледна точка, групата на тројно негативни карциноми на дојка е најтешка за лекување, со оглед на намалените можности за терапија. Тројно негативниот карцином на дојка (ТНКД) не може да се третира како една хомогена болест. Еден таков ветувачки биомаркер и цел за терапија на ТНКД е андрогенскиот рецептор (АР). Улогата на андрогениот рецептор во патологијата на карциномот на дојка добива клинички интерес, исто така е значаени во врска со развојот на лекови кои можат да ја модулираат активноста на андрогените рецептори.

Во овој труд презентираме случај кога во третманот на тројно негативен метастатски карцином на дојка со прекумерно изразување на андрогени рецептори имавме успешно искуство со користење на антиандрогена терапија. На пациентот му била дијагностицирана коскена метастаза 5 години по првичната дијагноза на рак на дојка. Пациентот го третиравме со анти-андрогена терапија со Бикалутамид и бифосфонатска терапија.

По 18 месеци терапија пациентот е асимптоматски со регресија на болеста.

Клучни зборови: тројно-негативен рак на дојка, андрогени рецептори, антиандрогена терапија

Introduction

Breast cancer is the most common malignant disease in the female population. According to the immunohistochemical analysis that determines the expression of estrogen receptor, progesterone recaptor, HER2 receptors as well as the proliferative index Ki-67, there are four molecular subtypes of breast cancer: luminal A, luminal B, HER2-like, and basal-like (TNBC). This classification is useful for making therapy decisions in the routine clinical management of breast cancer patients and has impact on prognosis and overall survival [1].

From a therapeutic point of view, the group of triple-negative breast cancer is the most difficult to treat, considering the reduced possibilities for therapy. Until the beginning of the era of immunotherapy

Correspondence to: Deva Petrova, Department of oncology and radiotherapy, Clinical Hospital Acibadem Sistina, 1000 Skopje, R. N. Macedonia; E-mail: deva.petrova@acibademsistina.mk

and biological therapy, the only treatment option for these patients was chemotherapy.

Lehmann *et al.* identified six subclasses of TNBC based on transcriptomic analysis: basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal-like (ML), and luminal androgen (LAR) [2].

With such heterogeneity, lack of targeted therapy, reduced chemosensitivity, and poor prognosis, it is of vital importance different biomarkers, which can help to distinguish between different TNBCs and may present novel therapeutic targets, to be investigated [3].

TNBC cannot be treated as one homogenous disease. One such promising biomarker and target for therapy of TNBC is the androgen receptor.

The role of the androgen receptor in BC pathology is gaining clinical interest also in relation to the development of drugs that can modulate AR activity [4].

In this paper we present a case of triple-negative metastatic breast cancer with androgen receptor overexpression, successfully treated with anti-androgen therapy.

Case report

A 75-year-old patient with a history of invasive ductal carcinoma of the right breast was surgically treated in August 2017; a conservative surgery of the right breast and regional lymphadenectomy was performed.

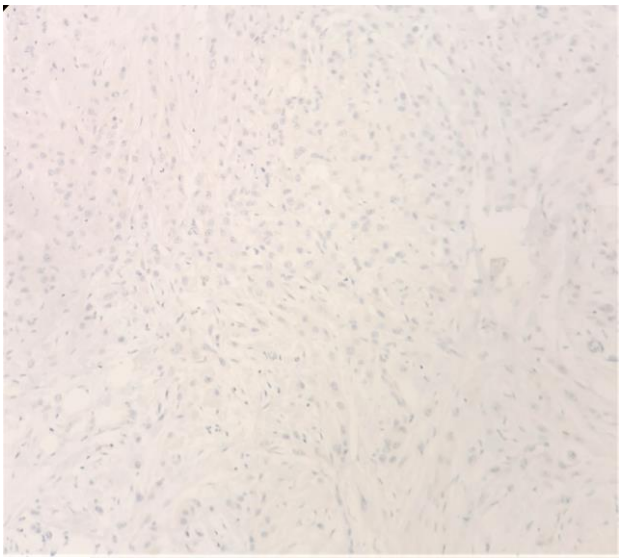

Fig. 1 a. Subsequent immunohistochemical analysis showed complete negativity for estrogen

Postoperative histopathological findings confirmed the diagnosis of moderately differentiated breast carcinoma with apocrine differentiation, measuring 1.4 cm (pT1a), no metastases were found in the 22 analyzed regional axillary lymph nodes (pN0). Surgical margins were clear and no lympho-vascular tumor emboli were present. The disease was post-operatively staged as IA. Subsequent immunohistochemical analysis showed complete negativity for estrogen (Figure 1a), progesterone (Figure 1b) and HER2 receptors (Figure 1c), placing the tumor in the triple-negative molecular subtype. Ki-67 proliferative index was 20%.

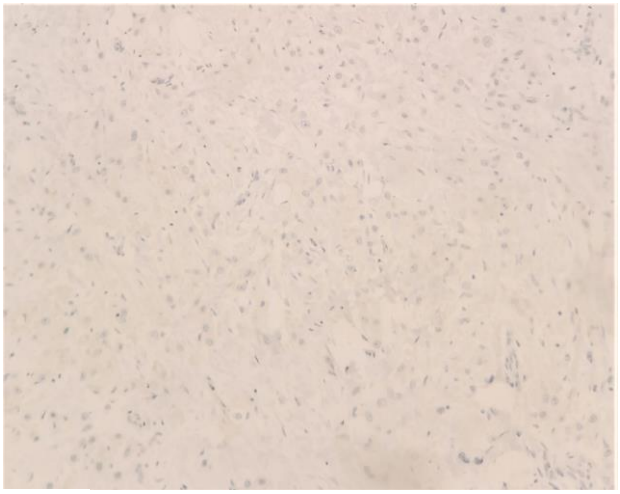

Fig. 1 b. Subsequent immunohistochemical analysis showed complete negativity for progesterone

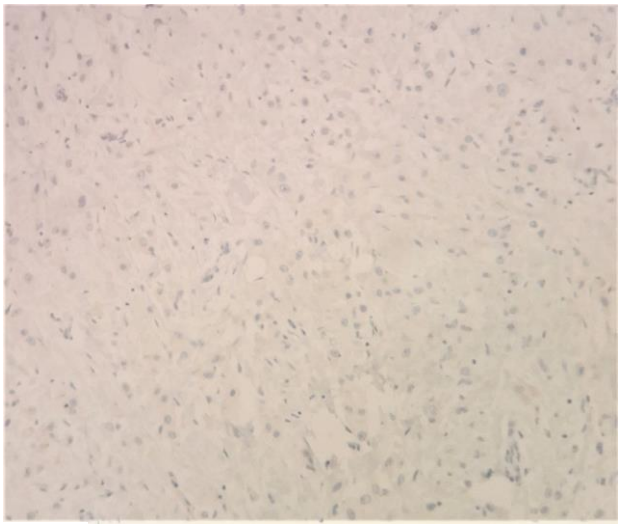

Fig. 1 c. Subsequent immunohistochemical analysis showed complete negativity for HER2 receptors

Fig. 1. Immunohistochemical analysis of the primary tumor shows negativity for estrogen (a), progesterone (b) and HER2 receptors (c).

Appendix 1

Postoperatively, the patient refused the proposed adjuvant chemotherapy and opted to attend regular check-ups with breast ultrasound every 6 months and mammography once per year. Five years after the initial diagnosis, the patient complained to the clinician of pain in the lower extremities, for which she was further examined. Clinical examinations were

performed to rule out vascular disease as a possible cause of pain. A Doppler ultrasound of the lower extremities was done, and in June 2022 a magnetic resonance imaging (MRI) of the spine was also performed. The MRI findings showed changes suspicious for osteosclerotic metastatic deposits (Figure 2).

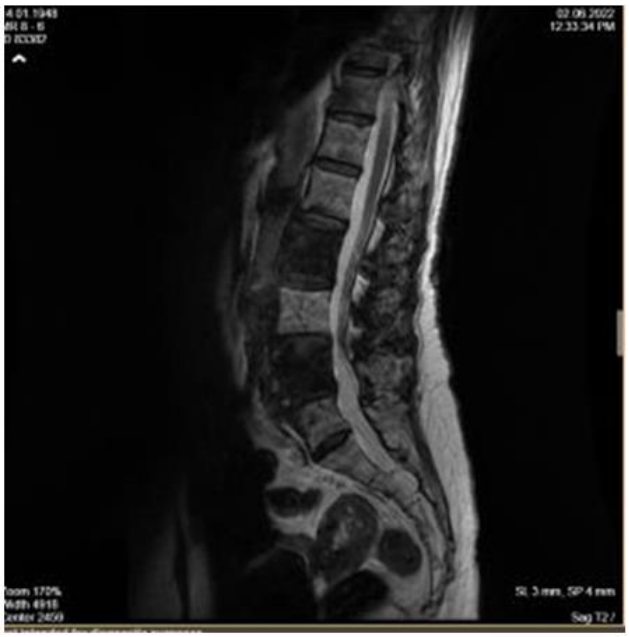

Fig. 2. MR of thoracolumbar spine, sagittal section

Appendix 2

Additionally, bone scintigraphy was carried out, which confirmed diffuse osteoblastic bone lesions suggestive of metastasis.
In order to evaluate the other organ systems and to precisely define the extent of the metastatic disease in this patient, a positron-emission tomography-computer tomography (PET-CT) was done, which confirmed the findings of the skeletal scintigraphy and ruled out presence of visceral metastatic changes.
In order to analyze the possibility of additional treatment options for the patient, BRCA1/2 mutation analysis was performed, which did not show pathogenic mutations in the BRCA1 and BRCA2 genes.
PDL-1 immunohistochemical analysis using the Ventana antibody anti-PD-L1 (clone SP142), was also negative, so the patient was not a candidate for immunotherapy. An immunohistochemical analysis of androgen receptors was also performed, which showed a strong nuclear positivity in 100% of the tumor cells (Figure 3).

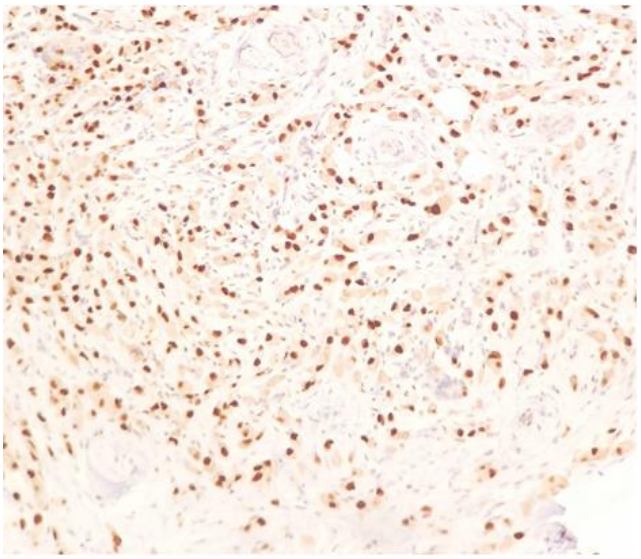

Fig. 3. Immunohistochemical analysis of the primary tumor shows a strong diffuse positivity for androgen receptor

Appendix 3

The patient was placed on hormonal anti-androgen therapy with Bicalutamide 50 mg daily and bisphosphonate therapy with Xgeva 120 mg monthly.
During therapy, the patient's condition was stable, and the first control to evaluate the response to the therapy was performed after 6 months of the treatment. In January 2023, the control bone scintigraphy was performed. Results from the bone scan confirmed disease regression (Figure 4).

Appendix 4

The patient continued with anti-androgen therapy and after 18 months of treatment, she is free of clinical symptoms and the disease is in regression.

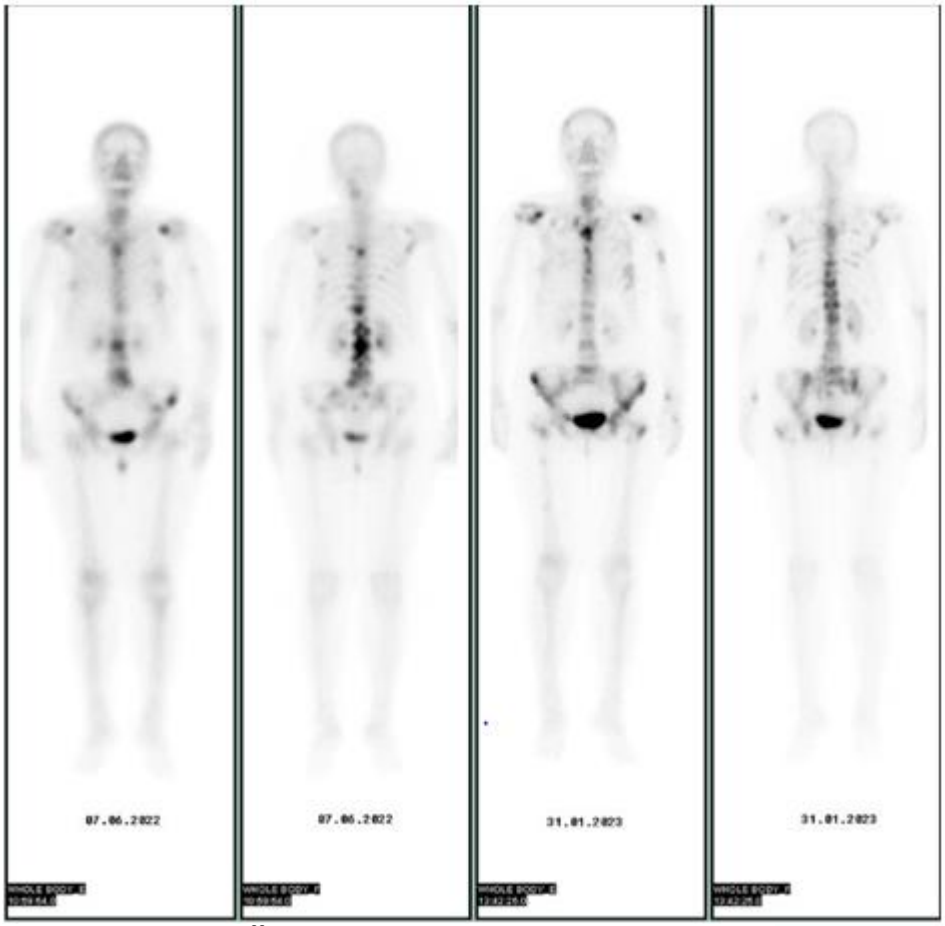

Fig. 4. Bone scan with $Tc^{99,}$ 5a before treatment, 5b. After treatment at 6 months

Discussion

Over the last decades, improved understanding of cancer biology led to the discovery of several actionable targets and enabled the development of new treatments that ultimately paved the way to personalized medicine. This paradigm shift from "one size fits all" to "to each his own" involved almost every tumor type, including breast cancer. However, while several targeted options have been developed for hormone positive and HER2 positive tumors in the last decades, TNBC has been considered an undruggable subtype for a long time. The seminal works by Lehman and Burstein dissected TNBC molecular profile, unravelling its profound heterogeneity and defining a new and unique entity of LAR (luminal androgen receptor positive) breast cancer [5].

The LAR subtype is enriched for hormonally regulated pathways and is dependent on androgen recaptor signaling. Although AR can be expressed in multiple molecular subtypes of TNBC, the LAR subtype has the highest level of AR expression. Distinct from unselected TNBC, the LAR subtype is predominantly subclassified in the non-basal subgroup and represents a novel subtype of TNBC with a distinct prognosis that offers an opportunity to develop targeted therapeutics [6].

There are certain characteristics of triple-negative breast cancer related to the age of a patient.

Age at diagnosis was greater among the LAR subtype and AR-positive TNBC in comparison to other subtypes and AR-negative TNBC, respectively [7,8]. Our patient was postmenopausal and seventy years old at initial diagnosis, which corresponded to literature data.

Interestingly, a recent study found significant associations between different degrees of AR expression and age of the patients. Older and postmenopausal patients had higher levels of AR in the tumors than younger and premenopausal patients. AR-negative tumors were observed just as much in pre- as in postmenopausal patients. Authors of the study speculated that AR-low and AR-high tumors were weakly and highly dependent on AR activity, respectively [9].

The differences in the results of studies on AR-positive TNBC tumors and LAR subtype TNBCs may be due to the absence of a clearly defined indicator of positive AR expression (AR cut-off) in the tumors. As previously noted, in most studies,

TNBC carcinomas were considered AR-positive, provided that the tumor has an AR expression of ≥10%. Meanwhile, the literature presented results for assessing the prognosis of the disease in cases of TNBC with an AR expression level of >0%. Such differences in the criteria for determining AR-positivity in TNBC tumors and LAR subtype TNBCs can have significant impact on the prognostic indicators [10].

Additionally, a very recent study hypothesized that TNBCs are entirely different entities when occurring in young or old patients, as they exhibited differences in subtypes, fibrosis, Ki-67 index, and somatic mutations. [11].

Knowledge about differences between TNBCs in young and elderly patients could clarify the differrences in therapeutic approaches, as older patients could be less responsive to conventional chemotherapy and might benefit more from a more personalized therapeutic approach (e.g., anti-androgen therapy) [12].

Santonja *et al*. showed that patients with the LAR TNBC subtype had the highest resistance to neoadjuvant anthracyclines and/or taxane-based chemotherapy with a complete tumor response rate of just over 14% [13].

All the abovementioned data were taken into account when making the decision for our patient's treatment. The patient met the criteria of an elderly patient, over 70 years of age, with triple-negative metastatic breast cancer and high expression of androgen receptors. Of course, when making the decision, we also took into account the expectations of the patient, who refused chemotherapy treatment. Additional important factors were absence of visceral metastasis and location of metastatic changes in skeletal structures only, as previously shown in the bone scan findings.

Metastatic TNBC usually shows aggressive behavior and displays predominantly visceral metastases; most metastatic events occur without intervening local recurrences. A combinational phenotype of AR-ERβ+p53+ was significantly associated with poorer overall survival (OS) [14].

Interestingly, studies found an increased AR status in bone metastasis and a decreased status in brain metastasis, which is of great interest, as TNBCs usually metastasizes toward the brain, and association between AR expression and brain metastasis should be further evaluated. Some authors suggested that AR loss could be associated with the metastatic tumor potential but these assumptions need further evaluation.

Both androgens and anti-androgens have demonstrated variable inhibitory and stimulatory effects in AR-positive breast cancer depending on estrogen receptor and HER2 co-expression. Androgen signaling pathways interact with other critical cellular pathways, such as the PI3K/AKT/mTOR, Ras/Raf/MAPK/ERK, Wnt/β-catenin, and estrogen signaling pathways [15]. Therapeutic exploitation of AR has been crucial in management of prostate cancer [16].

Androgens have different effects among breast cancer subtypes and AR agonists have been considered a possible therapeutic strategy in breast cancer [17]. In different breast cancer models, they could have anti-proliferative effects when in co-expression with estrogen receptors and pro-proliferative in absence of estrogen receptor positivity. In the latter context, AR promotes cell proliferation by acting at different levels indicating a potential unfavorable role of AR in hormone receptor (HR)+ BC. Conversely in TNBC, AR may have both favorable prognostic and predictive value, since increasing evidence suggests that AR positive TNBC patients may respond to AR targeting agents [17]. Corresponding to this data, our patient had triple-negative breast cancer, with overexpression of androgen receptors and is supposed to have better prognosis, so we could expect a good response to anti-androgen treatment in her case. The patient in our case had partial response to anti-androgen therapy with Bicalutamide. During the treatment, the patient had a good quality of life; there were no adverse reactions to the treatment, and a stable disease was achieved after the partial response to the therapy. The advantage of hormone therapy is the good quality of life without pronounced toxic drug effects.

Cyclin-dependent kinase (CDK) 4/6 inhibitors have been shown to effectively antagonize AR F876L function and restore sensitivity to anti-androgen therapy. Although this was demonstrated in prostate cancer cell lines, the effect could be present across AR-dependent tumor types, including AR-positive TNBC [18].

Conclusion

Our case report could help clinicians' decision-making process when treating patients with triple- negative metastatic cancer. We believe that the analysis of AR expression in these tumors can be a significant factor in the decision on therapy in these patients. Anti-androgen therapy in older patients can be an effective and easily acceptable treatment method given the significantly lower number of side effects when compared to chemotherapy. This treatment option should be considered especially when there is oligo-metastatic disease and when there is no life-

threatening situation and no visceral metastases, as was the case in our patient.

Conflict of interest statement. None declared.

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*Мак Мед Прегл*ед 2023; 77(1): 39-44

ММР

Case report

OCULAR MYASTHENIA GRAVIS IN CHILDREN: A CASE REPORT

ОКУЛАРНА ФОРМА НА МИЈАСТЕНИЈА ГРАВИС КАЈ ДЕЦА: Приказ на случај

Danilo Nonkulovski, Karolina Ristova Stefanovska, Daniela Georgievska Kapushevski and Jakim Stojcevski

University Children's Hospital, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

Introduction. Myasthenia gravis is a chronic, autoimmune, neuromuscular disease that is characterized by skeletal muscle weakness and fatigue. It occurs when normal neurotransmission between the nerve and muscle is interrupted at the neuromuscular junction. In case of myasthenia gravis, antibodies block, alter or destroy the AChR receptors for acetylcholine (which is a neurotransmitter) at the neuromuscular junction, which prevents the muscle from contracting. The clinical presentation of myasthenia gravis is certain muscles weakness, such as those that control eye and eyelid movement, facial expression, chewing, talking, and swallowing, weakness in the arms, hands, fingers, legs, and neck. The weakness worsens after periods of activity and improves after periods of rest.
Aim. To present a case of myasthenia gravis in a 6-month-old child; it is a very rare disease encountered in children. It is very important to early recognize and diagnose the disease, in order to determine a timely and adequate therapy approach and improve the child`s quality of life.
Methods. Anamnesis, neurological examination, blood samples from the patient and the mother for genetic analysis, laboratory analysis, imaging diagnostic methods (x-ray, CT and MRI), prostigmin test.
Results. We present a case of a 6-month-old boy, diagnosed with myasthenia gravis. The suspicion for the disease was based on the only symptom present-ptosis of the right eyelid. After the performed examinations, we got positive results for myasthenia gravis. Therapy with anticholinesterase medication (Mestinon) was recommended.
Conclusion. Myasthenia gravis as a very rare disease in children is a big challenge for diagnosing, due to the variety of clinical presentations and similarity with other neuromuscular diseases. Early diagnosis allows a multidisciplinary approach for timely treatment that will improve the muscular weakness and the quality of life.

Keywords: myasthenia gravis, AChR for acetylcholine, HLA typing class 1 and 2, prostigmin test

Correspondence to: Danilo Nonkulovski, University Children's Hospital, 1000 Skopje, R. N. Macedonia; E-mail: danilo_non@yahoo.com

Абстракт

Вовед. Мијастенија гравис е хронична, автоимуна, невромускуларна болест, која се карактеризира со слабост на скелетната мускулатура и замор. Се јавува кога нормалната невротрансмисија помеѓу нервот и мускулот е прекината на ниво на невромускулара синапса. Во случај на мијастенија гравис, антитела ги блокираат, менуваат или ги уништуваат рецепторите за ацетилхолин (AChR) кој е невротрансмитер во невромускуларната синапса и на тој начин се оневозможува мускулна контракција. Клиничката презентација на мијастенија гравис е слабост на одредена мускулна група, како на пример окуларните мускули, мускулите кои ја контролираат фацијалната експресија, џвакање, зборување и голтање, слабост во рацете, дланките, прстите, нозете и вратот. Слабоста се влошува по периоди на активност и се подобрува по периоди на одмор.
Цел. Да се прикаже случај на мијастенија гравис кај 6 месечно дете-многу ретка болест кај децата. Навремено препознавање на симптоматологијата на болеста и навремена дијагноза е предуслов за што порано да се пристапи кон спроведување на адекватна терапија, со цел да се подобри квалитетот на животот на детето.
Методи. Анамнеза, невролошки преглед, примерок од крв на пациентот и мајката за генетска анализа, лабораториски анализи, серолошки испитувања на специфични anti AChR at, радиолошка дијагностика РТГ, КТ и МРИ на граден кош, простигминski тест.

Резултати. Презентираме случај за машко доенче на 6 месечна возраст со дијагноза на мијастенија гравис. Сомневањето за болеста се базира на единствениот симптом, птоза на десен очен капак. Направените дијагностички иследувања се во прилог на мијастенија гравис. Кај пациентот е препорачана терапија со Sir. Mastinon.
Заклучок. Мијастенија гравис како ретко заболување во детска возраст е голем предизвик за дијагностицирање, поради разноликоста на клиничката презентација и сличноста со други невромускуларни болести. Брза и навремена дијагностика на болеста овозможува мултидисциплинарен пристап за навремено започнување на терапија, која ја подобрува слабоста во мускулите и го подобрува квалитетот на животот.

Клучни зборови: мијастенија гравис, AChR за ацетилхолин, типизација на ХЛА класа 1 и 2, простигмински тест

Introduction

Myasthenia gravis is a chronic neuromuscular disease, caused by an error in the transmission of nerve impulses to muscles [1,7]. Normally, the binding of the neurotransmitter acetylcholine to its receptor AChR activates the muscle and causes a muscle contraction. In case of myasthenia gravis, antibodies block, alter, or destroy the AChR receptors for acetylcholine at the neuromuscular junction, which prevents the muscle from contracting. This is most often caused by antibodies attaching to the acetylcholine receptor itself AChR, but antibodies attaching to other proteins, such as MuSK (Muscle-Specific Kinase) protein, can also decrease transmission at the neuromuscular junction [1-3].
The thymus gland which controls immune function may be associated with myasthenia gravis.[5] It grows gradually until puberty, and then gets smaller and is replaced by fat. It plays an important role in the development of the immune system. It is responsible for producing T-lymphocytes and a specific type of white blood cells. In many adults with myasthenia gravis, the thymus gland remains large and may develop thymomas (tumors of the thymus gland). Thymomas are most often harmless, but they can become cancerous. Scientists believe the thymus gland may cause the immune system to attack its own cells and tissues and produce anti-AChR antibodies. "Myasthenia" is a Latin word that means muscle weakness, and "gravis" means seriously, tough. The first description of a patient with myasthenia gravis was made in 1672 byTomas Vilis (1621-1675), an English doctor, famous neurologist [1-3].
Myasthenia gravis affects both men and women and occurs across all racial and ethnic groups. It most commonly impacts young women (20-40) and men (50-80), but it can occur at any age, including childhood. Myasthenia gravis is contagious.

Epidemiology

Incidence: 10-20/1,000,000 to 3-30/10,000,000; *Prevalence:* 150-200/1, 000,000; *Mortality:* 0.06-0.89/ 100,000 [6]. In the pediatric population, the incidence is estimated to be between 1.0-5.0/1,000,000 [9]. It is estimated that between 10% and 15% of all cases of myasthenia occur in the pediatric population [10]. Ocular myasthenia gravis (OMG) accounts for approximately 10%-35% of cases of MG in childhood.
Myasthenia gravis is rarely seen in infants. The fetus may acquire antibodies from a mother affected with myasthenia gravis - this form is called neonatal myasthenia gravis [11]. It is generally temporary, and the child's symptoms usually disappear within two to three months after birth [1].
Rarely, children of a healthy mother may develop congenital myasthenia. This is not an autoimmune disorder. It is inherited as an autosomal recessive disease. It is caused by defective genes that produce abnormal proteins in the neuromuscular junction [14,15]. The symptoms are similar to neonatal myasthenia gravis and are lifelong.
There is also a juvenile myasthenia gravis**.** This is an autoimmune disorder manifested between the 2nd and 20th year, mostly often after the 10th year of life [12].

Classification of the disease according to:

1. Age of onset
- Transient neonatal MG
- Adult autoimmune MG

2. Presence or absence of anti-AChR antibodies:
- Seropositive MG
- Seronegative MG

3. According to the etiology of the disease, 4 groups are distinguished:
- Acquired autoimmune MG
- Transient neonatal MG caused by a passive transfer of antibodies (anti-AChR) to the mother
- MG induced by drugs
- Congenital myasthenic syndromes in which there is AChR deficiency.

Clinical classification (by Osserman)
- Group I of MG-ocular myasthenia gravis;

- Group IIA of MG-mild generalized myasthenia gravis;
- Group IIB of MG-moderate to severe generalized myasthenia gravis;
- Group III of MG-acute (sudden) or severe generalized myasthenia gravis with respiratory failure;
- Group IV-late, severe myasthenia gravis with a significant bulbar symptomatology;
- Group V-intubation.

Clinical presentation

The core trait of myasthenia gravis is muscle weakness and fatigue. Certain muscles such as those that control eye and eyelid movement, facial expression, chewing, talking, and swallowing are often (but not always) involved in the disorder. People with myasthenia gravis may experience the following symptoms [1-3]:

- weakness of the eye muscles (called ocular myasthenia)
- drooping of one or both eyelids (ptosis)
- blurred or double vision (diplopia)
- a change in facial expression
- difficulty swallowing
- shortness of breath
- impaired speech (dysarthria)
- weakness in the arms, hands, fingers, legs, and neck.

The beginning of the disease may be sudden, and often symptoms are not immediately recognized as myasthenia gravis. Ocular motor disturbances, ptosis, diplopia or strabismus are the initial symptoms of myasthenia gravis in two-thirds of patients; these symptoms appear within 2 years. In ocular MG only the extraocular muscles and levator palpebrae are involved [16,17]. Other muscle weakness rarely appear as initial symptoms. Symptoms fluctuate throughout lifetime (active and inactive stage), but the weakness is progressive. After 15 to 20 years, weakness usually becomes constant and the most severely involved muscles are frequently atrophic (burnt-out stage). The severity of weakness also fluctuates during the day, usually being least severe in the morning after periods of rest, and worse after periods of activity, as the day progresses, especially after prolonged use of affected muscles. The degree of muscle weakness varies greatly among individuals [1-3]. Patients presenting with the ocular subtype may convert to generalized myasthenia gravis [8].

Sometimes the severe weakness of myasthenia gravis may cause respiratory failure. This condition is called myasthenic crisis and it requires immediate emergency medical care [1,3].

Diagnosis

Other conditions can cause muscle weakness, and hence myasthenia gravis can be difficult to diagnose. A doctor may perform or order several tests to confirm the diagnosis of myasthenia gravis:

• *A physical and neurological examination.*

• *An edrophonium chloride test.* This test uses injections of edrophonium chloride (TENSILON) at a dose of 0.2 mg/kg to briefly relieve weakness in people with myasthenia gravis. The drug blocks the breakdown of acetylcholine and temporarily increases the levels of acetylcholine at the neuromuscular junction. It is usually used to test ocular muscle weakness. The same effect gives NEOSTIGMIN at a dose of 0.04 mg/kg.

• *A blood test.* Most individuals with myasthenia gravis have abnormally elevated levels of acetylcholine receptor AChR antibodies. A second antibody, called anti-MuSK antibody, has been found in about half of patients with myasthenia gravis who do not have acetylcholine receptor antibodies. A blood test can also detect this antibody.

In some patients with myasthenia gravis, neither of these antibodies is present. These individuals are said to have seronegative (negative antibody) myasthenia.

• *Electrodiagnostics*. EMG is considered the most sensitive test for myasthenia gravis; it detects the impaired nerve-to-muscle transmission. Diagnostic tests include repetitive nerve stimulation with small pulses of electricity to tire specific muscles. Muscle fibers in myasthenia gravis do not respond to repeated electrical stimulation compared to muscles of normal individuals.

• *Diagnostic imaging*. Diagnostic imaging of the chest: CT or MRI may identify the presence of a thymoma.

• *Pulmonary function testing***.** Measurement of the breathing strength can help in predicting respiration failure and lead to a myasthenic crisis.

Because weakness is a common symptom of many other disorders, the diagnosis of myasthenia gravis is often missed or delayed (sometimes for more years) in people with mild weakness or in those individuals whose weakness is restricted to only a few muscles [1-3].

Treatment

Myasthenia gravis can generally be controlled but not cured. The aim of the therapy is to help reduce and improve muscle weakness. With treatment, the muscle weakness often gets much better [1,2].

• *Anticholinesterase medications.* Medications which slow the breakdown of acetylcholine at the neuromuscular junction and thereby improve neuromuscular transmission and increase muscle strength include anticholinesterase agents such as: Neostigmin (Prostigmin), Pyridostigmin (Mestinon), Physostigmin (Eserin), Amberonium-chloride (Mytelase), Edrophonium chlorid (Tensilon).
• *Monoclonal antibody.* This therapy targets the process by which acetylcholine antibodies injure the neuromuscular junction. In 2017, the U.S. Food and Drug Administration approved the use of Eculizumab for the treatment of generalized myasthenia gravis in adults who have positive test for the anti-acetylcholine receptor (AchR) antibody.
• *Immunosuppressive drugs.* Improve muscle strength by suppressing the production of abnormal antibodies. They include Prednisone, Azathioprine, Mycophenolate mofetil, and Tacrolimus.
• *Plasmapheresis and intravenous immunoglobulin*. These therapies may be options in severe cases of myasthenia gravis. Individuals can have antibodies in their plasma that attack the neuromuscular junction. These treatments remove the destructive, harmful antibodies, although their effectiveness usually only lasts for a few weeks to months. Intravenous immunoglobulin is a highly concentrated injection of antibodies that temporarily changes the way the immune system operates. It works by binding to the antibodies that cause myasthenia gravis and removing them from circulation.
• *Thymectomy.* This procedure of removal of the thymus gland (which often is abnormal in individuals with myasthenia gravis) can reduce symptoms and may cure some people, possibly by rebalancing the immune system. A NINDS-funded study found that thymectomy is helpful both for people with thymoma and those with no evidence of tumors. The surgery reduces muscle weakness and the need for immunosuppressive drugs [1-4].

Prognosis

With treatment, most patients with myasthenia gravis can achieve significant improvement of their muscle weakness and have normal or nearly normal lives. Remission, either temporary or permanent, can be achieved. Muscle weakness may disappear completely so that therapy can be discontinued. Stable, long-lasting complete remissions are the goal of thymectomy and may occur in about 50% of patients who undergo this procedure [3].
Factors of exacerbation: infective diseases, systemic illnesses (especially viral respiratory infections), high body temperature, fever, physical injury, emotional upset, hypo- or hyperthyroidism, some medicines, allergic reaction, surgery procedures [3].
Differential diagnosis: mitochondrial myopathy, myotonic dystrophy, thyroid ophthalmopathy (hypo- or hyperthyroidism), progressive external ophthalmoplegia, lesions in the brain stem (tumors, vascular lesions), oculopharyngeal dystrophy and other myopathies, encephalitis of different etiology and clinical course, amyotrophic lateral sclerosis, acute polyradiculoneuritis-Guillain-Barre, congenital myasthenic syndromes, botulism [4].
Association with other autoimmune diseases: 15-20% of patients with myasthenia gravis can have another autoimmune disease at the same time: polymyositis, SLE, idiopathic thrombocytopenic purpura, chronic thyroiditis, rheumatoid arthritis, Chron's disease, Sjogren's syndrome, ulcerative colitis, psoriasis [4,13].

Case presentation

We present the case of a 6-month-old boy, from a normal pregnancy. The baby was delivered by caesarean section at 37 gestational weeks (weight 3320 g, body length 47 cm.). The patient was examined in neurological outpatient clinic because of the only symptom of ptosis of the right eyelid. Family health history revealed thyroid gland disease and epilepsy of the grandmother and cerebral paralysis of mother's cousin. The physical and other neurological findings were normal.
Laboratory analyses, IERH, C3, C4 and thyroid hormones were unremarkable. AChR antibodies were positive. X-ray and CT of the chest were normal. Development tests: result Q.A=100%.

Fig. 1. Before therapy

Fig. 2. After 6 months

Results from HLA typing class 1 and 2: genomic DNA was isolated from white cells in peripheral blood, of which HLA genes in loci HLA-A, -B, -C, -DRB1, -DQA1 and -DQB1 were typed using xMAP technology (Luminex), shown according to NMDP CODE.

The specific antibodies for myasthenia gravis were positive, and the patient was hospitalized for making the prostigmin test. The test was positive, hence the diagnosis of myasthenia gravis was confirmed.

After establishing the diagnosis, the treatment with anticholinesterase medication (Mestinon) was recommended and we achieved a satisfying response, resulting in clinical improvement.

Discussion

Myasthenia gravis in children is a rare disease. Symptoms may start showing slowly over weeks or months and can manifest differently in each child. They vary by the type of myasthenia gravis. A child may become very tired after very little activity. He or she may start having trouble with chewing and swallowing. Drooping eyelids may be so severe that the child cannot see or may have double vision. Babies may have delays in their motor skills such as crawling, sitting, and walking. In ocular myasthenia gravis (OMG) ptosis, diplopia or strabismus are the initial symptom. Extraocular muscles and levator palpebrae are involved [16,17]. Ocular myasthenia gravis accounts for approximately 10%-35% of cases of myasthenia gravis in childhood.

In our case, the presence of ptosis of the right eyelid was the only neurological symptom. Having in mind the fact that ptosis is one of the earliest and most common symptom of myasthenia gravis, there was a suspicion for this disease. All diagnostic procedures were done (including specific diagnostic examinations like specific AChR antibodies, x-ray, thoracic CT and prostigmin test, genetic analysis). The results were positive for myasthenia gravis, so the diagnosis was made. A first-line symptomatic therapy with anticholinesterase medication (Mestinon) was recommended.

After initiation of the therapy, the patient was regularly controlled in a neurology outpatient clinic. Significant clinical improvement was achieved as well as decreasing of the antibody level in the blood. In order to achieve a satisfying effect of this therapy, it was recommended to keep applying the same therapy and to make regular check-ups.

Patients with ocular myasthenia gravis must be monitored for development of amblyopia from ptosis or strabismus and have to be treated appropriately. Amblyopia may develop in 25% to 50% of patients with OMG [16,17]. Ptosis may remain present, followed by stabilization of the disease in 46% to 66% of patients [16,17]. Complete resolution of manifestations of OMG can occur in 13% to 50% of patients, and may occur in 31% of all juvenile myasthenia patients [16].

Conclusion

Pediatric ocular myasthenia gravis (OMG) is difficult to diagnose and manage, owing to its rarity and low index of suspicion in the early stage of the disease. Also, many other conditions with similar presentation cause further delay in providing diagnosis. The course of myasthenia gravis is variable in all patients but it is usually progressive. In some pa-

tients, it can significantly impact the quality of life. There is no curative treatment for this disease. The recommended therapeutic medications and interventions implemented timely and adequately can help in improving the outcome and the quality of life. According to the literature and experience, anticholinesterase medication is recommended as a first-choice drug. We recommended Mestinon for our patient. The goal of early diagnosis and treatment is to achieve the best outcome.

Conflict of interest statement. None declared.

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Мак Мед Прегled 2023; 77(1): 45-48

ММР

Case report

SEMAGLUTIDE AS A FACTOR FOR INSULIN DISCONTINUATION IN A PATIENT WITH TYPE 2 DIABETES - CASE REPORT

СЕМАГЛУТИД КАКО ПРИЧИНА ЗА ПРЕКИН НА ИНСУЛИНСКА ТЕРАПИЈА КАЈ ПАЦИЕНТ СО ДИЈАБЕТ ТИП 2 - ПРИКАЗ НА СЛУЧАЈ

Marija Bajdevska Spirkovska

General Hospital Kumanovo, Kumanovo, Republic of North Macedonia

Abstract

Introduction. Type 2 diabetes (T2D) is associated with a high cardiovascular risk, and besides treating hyperglycemia, we must also treat comorbidities. T2D prevalence is rising exponentially, along with sedentary lifestyle and obesity. Modern diabetes therapy is focused on cardiovascular benefit, and glucagon-like peptide 1 receptor agonists (GLP-1 RA) are stepping out to be one of the first choices for treatment, leading to reduced insulin dose and insulin discontinuation in some patients.

Case report. We present a case of a 66-year-old male, T2DM diagnosed in 2012, poorly controlled, on metformin and premix insulin regiment, GLP-1 RA semaglutide once weekly was added. We aimed to give an answer if it was possible to improve glycoregulation and to stop insulin therapy. Change in glycosylated hemoglobin (HgA1c) and fasting glycemia was measured on six-month intervals, from baseline when semaglutide was initiated, to 12 months after insulin discontinuation. The patient first visited my office in August 2021, and semaglutide once weekly was added to his standard therapy. His HgA1C was 12.6%, and fasting blood glucose 8.5 mmol/l. After 6 months, the patient had lost 6 kg and HgA1C was 6.7%. I decided to start reducing the insulin dose gradually for one month, and then to discontinue insulin therapy. After six mounts, his Hga1c was 5.7%, receiving semaglutide 1 mg once weekly, and metformin 2000 mg daily. His last visit was in February 2023, with HgA1C 5.8%. He stopped insulin therapy 1 year ago, after 8 years of continuous use. We managed to simplify the patient's therapy regimen, while preserving excellent glycemic control.

Conclusion. Insulin discontinuation is possible and can be advised by healthcare professionals when improved glucose control and weight loss is achieved, or when non-insulin diabetes medication is initiated.

Keywords: semaglutide, insulin discontinuation, T2D

Correspondence to: Marija Bajdevska Spirkovska, General Hospital Kumanovo, 1300 Kumanovo, R. N. Macedonia; E-mail: bajdevska@gmail.com

Апстракт

Вовед. Дијабетес тип 2 (ДТ2) е асоциран со висок кардиоваскуларен ризик и покрај третманот на хипергликемијата, важно е и да ги лекуваме и коморбидитетите. Преваленцата на ДТ2 расте експоненцијално со дебелината и седентарниот животен стил. Современиот третман на дијабетесот е фокусиран на кардиоваскуларниот бенефит и токму аналозите на хуманиот глукагонсличен пептид-1 (ГЛП-1 РА) стануваат една од првите опции за третман, овозможувајќи намалена потреба од инсулин, дури и прекин на инсулинската терапија кај некои пациенти.

Приказ на случај. 66 годишен маж, ДТ2 дијагностициран во 2012, лошо контролиран на терапија со метформин и премиксен инсулин, додадовме ГЛП-1 РА семаглутид еднаш неделно, на неговата стандардна терапија. Беше следена промена на гликолизираниот хемоглобин (ХГА1Ц) и гликемијата на гладно, на шест месечни интервали, од моментот на иницирање на семаглутид, до 12 месеци по прекинување на инсулинската терапија. На првата посета во август 2021, ХГА1Ц беше 12,6%, гликемијата на гладно 8.5 ммол/л. После 6 месеци од иницирањето на семаглутид, пациентот имаше изгубено 6 кг, ХГА1Ц беше 6,7% и одлучивме постепено да ја намалуваме инсулинската доза во тек на еден месец и ја прекинавме инсулинската терапија. На следната контрола, ХГА1Ц беше 5,7%, само со метформин 2000мг дневно и семаглутид 1мг неделно. На последната контрола, во февруари 2023 ХГА1Ц беше 5,8%, а гликемијата на гладно 6,2ммол/л. Пациентот не зема инсулин една го-

дина, после осум години континуирана инсулинска терапија. Успеавме да го олесниме терапискиот режим на пациентот, воедно зачувувајќи одлична гликорегулација.
Заклучок. Прекин на инсулинската терапија е можен, и може да биде препорачан од лекар кога е постигната добра гликорегулација, редукција на телесната тежина или кога е воведена соодветна антидијабетична терапија.

Клучни зборови: семаглутид, прекин на инсулин, дијабетес тип 2

Introduction

Type 2 diabetes (T2D) is a chronic complex disease, associated with a high cardiovascular risk and multiple comorbidities, and besides treating hyperglycemia, we must also treat other comorbidities like hypertension, dyslipidemia and renal disease.
According to the 2021 IDF Atlas, 537 million people worldwide live with diabetes, and 541 million people live with prediabetes [1]. A high prevalence of T2D has been observed in developing countries, and in populations adopting the western lifestyle, and its prevalence is rising exponentially, along with sedentary lifestyle and obesity [1].
T2D is the most common type of diabetes (>90%), and there is evidence that it can be prevented or delayed [2]. Also, there is evidence that remission of T2D is possible [3].
There are several pharmacological options available for T2D treatment, from lifestyle change and metformin as the first step, but due to the progressive nature of the disease, other medications and insulin are included in the next steps of therapy intensification [4]. However, optimal glycemic control is not achieved in many patients; therefore, new therapeutic strategies are needed [5].
Modern diabetes therapy is focused on cardiovascular benefit of the antidiabetic drugs, and glucagon-like peptide 1 receptor agonist (GLP-1 RA) and sodium-glucose co-transporter-2 inhibitors (SGLT 2) are stepping out to be the best first choice of therapy intensification in patients, especially patients with comorbidities [6].
In 2005, the first GLP-1 RA were approved for T2D treatment; in the beginning, they were injected twice daily, and at present once daily, once weekly, and even oral daily preparations are available for patients' treatment [4].
Using GLP-1 RA reduces the need of insulin therapy in patients, leading to reduced insulin dose and insulin discontinuation recommended by healthcare professionals in some patients [5].

Aim

To evaluate the impact of adding once weekly GLP-1 RA semaglutide in a poorly controlled patient with T2D, on metformin and premix insulin regiment. We aimed to give an answer if it was possible to improve glycoregulation and to stop insulin therapy. Changes in glycosylated hemoglobin and fasting glucose were measured on six-month intervals, from baseline when semaglutide was initiated, to 12 months after insulin discontinuation.
Written informed consent was obtained from the patient, and data was collected retrospectively from the electronic healthcare data base from 2012, when his diabetes type 2 was diagnosed.

Case report

We present the case of a 66-year-old male, T2DM diagnosed in 2012; first presentation with diabetic ketoacidosis and hospitalization in the General hospital Kumanovo, when basal insulin was prescribed by a diabetologist. The following comorbidities were present: hypertension (2014), dyslipidemia (2013) and diabetic maculopathy (2017), regularly controlled by a cardiologist and an ophthalmologist. Records of glucose control were taken from the electronic medical base of the patient. Over the years, the patient was poorly controlled, and insulin therapy and metformin were the only option for him. Different insulin regimens were used throughout/ during the course of the years (firstly, basal insulin, then bolus on lunch was added, and then premix insulin was used). Fasting glucose, postprandial glucose and glycosylated hemoglobin (HgA1C) were performed during regular control visits in the Center for diabetes, but a poor glucose control was observed all the time. After a few years, the patient was disappointed that something could be done for him, and refused to even check his blood glucose.
The patient first visited my office in August 2021, and semaglutide once weekly was added to his insulin therapy-premix insulin (insulin degludec, insulin aspart) twice daily and metformin 2000 mg daily. His HgA1C was 12.6%, and fasting blood glucose 8.5 mmol/l. After six months, in February 2022, the patient had lost 6 kg of weight; HgA1C was 6.7%, postprandial glucose was 7.8 mmol/l, and I decided to start reducing the insulin dose gradually for one month, and then to discontinue insulin therapy, but the patient was advised to measure his

capillary glucose in the mornings and two hours after lunch.

After six months, in August 2022, his HgA1C was 5.7%, only taking semaglutide 1mg sc weekly, and metformin 2000 mg daily. The patient was satisfied with his glycoregulation, and his last visit was in February 2023, with excellent glycoregulation, HgA1C 5.8%, fasting glucose 6.2 mmol/l, postprandial glucose 7.4 mmol/l. He stopped insulin therapy 1 year ago, after 8 years of continuous insulin therapy, and now he has excellent glycoregulation.

The patient's glycoregulation during the years living with diabetes, is presented with HgA1C in Figure 1.

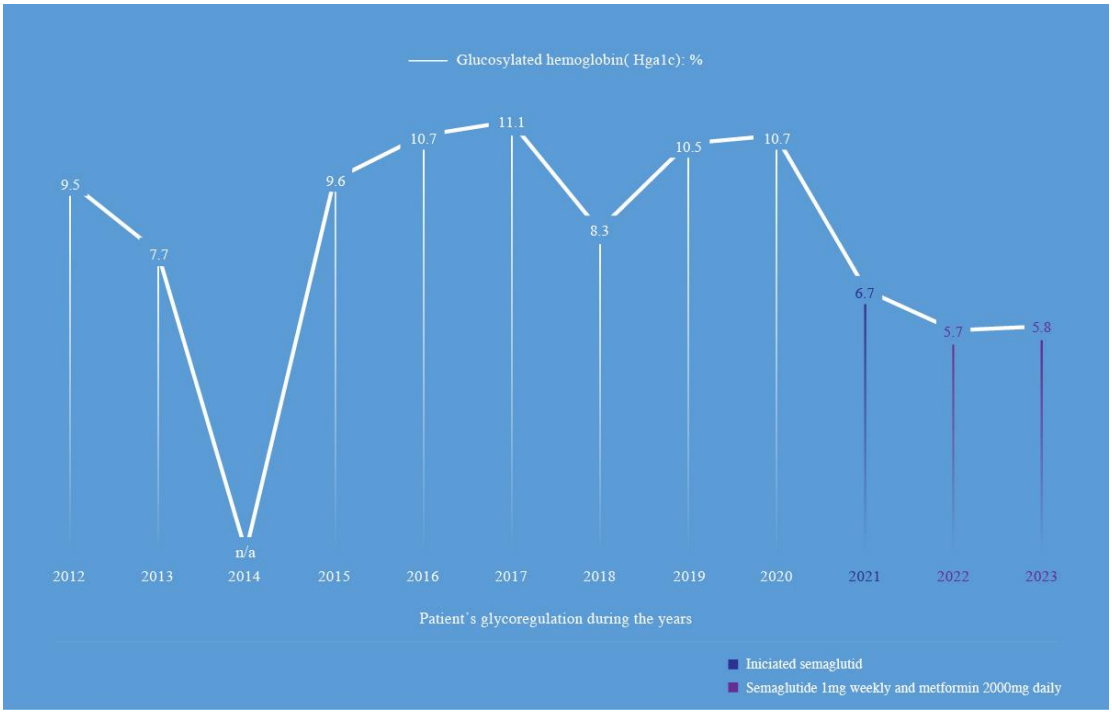

Fig. 1. The patient's glycoregulation presented with HgA1C

After introducing semaglutide, HgA1C fasting glucose and body weight were significantly lower and remained stable up to 1 year after insulin discontinuation. Also, semaglutide was well tolerated without hypoglycemic episodes. We managed to simplify the patient's therapy regimen, while preserving excellent glycemic control.

Conclusion

Today, patient-centered approach, according to evidence-based medicine is baseline in treating patients with diabetes [6]. Insulin discontinuation is possible and can be advised by healthcare professionals when improved glucose control and weight loss is achieved, or when non-insulin diabetes medication is initiated [2]. T2DM has serious economic and medical impact on the population worldwide. Developing new efficient drugs for treatment of diabetes and preventing and delaying its complications is a very important part of global diabetes treatment, because despite all we know about diabetes and its complications, we still start treating diabetes late in many patients, when its complications are already present [3]. Improvement of glucose control, weight loss, and decreased food intake are all effects of adding GLP-1 agonist, resulting in reduced need of insulin therapy [7]. Replacing daily insulin injections with once-weekly injections improves quality of life in patients and increases their satisfaction. Also, it is important to mention that GLP-1 receptor agonists reduce cardiovascular events (stroke, myocardial infarction and associated mortality) in patients with T2D [8].

Conflict of interest statement. None declared.

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Мак Мед Прегled 2023; 77(1): 49-51

MMP

Case report

PORTAL VEIN THROMBOSIS AS A RARE FORM OF VENOUS THROMBOEMBOLISM IN A PATIENT WITH JAK2V617F POSITIVE MYELOPROLIFERATIVE NEOPLASMS

ТРОМБОЗА НА ПОРТНА ВЕНА КАКО РЕТКА ФОРМА НА ВЕНСКИ ТРОМБОЕМБОЛИЗАМ КАЈ ПАЦИЕНТКА СО JAK2V617F МИЕЛОПРОЛИФЕРАТИВНА НЕОПЛАЗМА

Mario Jakimovski[1], Marica Pavkovic[1], Viktorija Calovska[2], Sonja Genadieva-Stavric[1], Irina Panovska-Stavridis[1] and Aleksandar Stojanovic[1]

[1]University Clinic for Hematology, [2]University Clinic for Gastroenterohepatology, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

The incidence of venous thromboembolism (VTE) in patients with myeloproliferative neoplasms (MPNs) is higher than in controls and it is 0.6-1.0 per 100 patients-years, and the rate of recurrence after VTE is 6.0-6.5 per 100 patients-years. Patients with myeloproliferative neoplasms are at a high risk of rarer form of thrombotic complications like portal vein thrombosis (PVT).

We present a case of a young female patient with JAK2V617F positive MPN that developed a portal vein thrombosis during the course of the myeloproliferative disease. She was treated with low molecular weight heparins and vitamin K antagonists (VKA) with partial resolution of the PVT and consecutive successful pregnancy.

Portal vein thrombosis is rare but serous complication in patients with MPN. Long-term oral anticoagulation with vitamin K-antagonists (VKA) is recommended in all PVT patients with the MPN-related permanent prothrombotic state; the benefits of adding aspirin to VKA are uncertain. Cytoreduction is warranted in all PVT patients with an overt MPN, but its appropriateness is doubtful in those with molecular MPN without hypercythaemia.

Keywords: myeloproliferative neoplasms, portal vein thrombosis, JAK2V617F, anticoagulation treatment

Апстракт

Инциденцата на венски тромбоемболизам (ВТЕ) кај пациенти со миелопролиферативни неоплазми (МПН) е повисока отколку кај контролната популација, истата е 0,6-1,0 на 100 пациентски-години, а стапката на повторување на ВТЕ е 6,0-6,5 на 100 пациентски-години. Пациентите со миелопролиферативни неоплазми имаат повисок ризик од поретки форми на тромботични компликации како тромбоза на порталната вена (ПВТ).

Презентираме случај на млада пациентка со JAK2V617F позитивна МПН која развила тромбоза на порталната вена во текот на миелопролиферативната болест. Пациентката беше третирана со ниско молекуларен хепарин и антагонисти на витамин К (ВКА) со делумно подобрување на ПВТ и последователна успешна бременост.

Тромбозата на порталната вена е ретка, но серозна компликација кај пациенти со МПН. Се препорачува долготрајна орална антикоагулантна терапија со витамин К-антагонисти кај сите пациенти со ПВТ со пропратна протромботична состојба како МПН; придобивките од додавањето на аспирин на ВКА се неизвесни. Циторедукцијата е оправдана кај сите пациенти со ПВТ и МПН, но нејзината успешност е сомнителна кај болните со МПН без хиперцитемија.

Клучни зборови: миелопролиферативни неоплазми, тромбоза на порталната вена, JAK2V617F, антикоагулантен третман.

Introduction

Myeloproliferative neoplasms (MPNs) according to the latest classification include polycythemia vera (PV), essential thrombocythemia (ET), prefibrotic/ early myelofibrosis (prePMF), and primary myelofibrosis (PMF) [1]. Myeloproliferative neoplasms (MPN) are a heterogeneous group of clonal neoplastic disorders characterized by the overproduc-

Correspondence to: Marica Pavkovic, University Clinic for Hematology, 1000 Skopje, R. N. Macedonia; E-mail: pavkovicm@yahoo.com

tion of erythrocytes in polycythemia vera (PV), the overproduction of platelets in essential thrombocythemia (ET) and bone marrow fibrosis in myelofibrosis (PMF).

The most common molecular marker in MPN is JAK2V617F mutation identified in 95% of PV patients and around 50% of patients with ET and PMF [1,2]. This mutation acts as a driver for clonal proliferation through constitutive activation of the JAK/STAT pathway. Other mutations present in patients with MPN are mutations in CALR gene, MPL gene or other JAK2 mutations [2,3].

The clinical course of MPNs can be complicated by thrombotic complications or progression into secondary myelofibrosis or acute leukemia [2]. The risk for thrombosis can be modified by a medical intervention, and special guidelines have been developed to control and lower the number of thrombotic complications in patients with MPNs [3]. The most common thrombotic complications are venous thromboembolism (VTE) that develop in about one-third of cases. The ncidence of overall thrombosis per 100 patients-years was 2.6 in PV, 1.9 in ET and prePMF [4-6], compared to the lower annual incidence of major VTE between 0.1 and 0.2% in the general population in Western countries [7]. A rare form of thrombosis like splanchnic venous thrombosis (SVT) and cerebral venous thrombosis (CVT) are greatly overrepresented in patients with MPNs in comparison with the general population [8,9]. Venous thrombosis at usual sites (i.e., deep venous thrombosis of the legs with or without pulmonary embolism) and isolated pulmonary embolism (PE) have been reported to account for 54-77% of venous thromboembolisms in PV (10), 41% in ET [11], and 89% in PMF [6].

Portal vein thrombosis (PVT) is a severe complication, which in many cases appears at the onset of the disease; the risk factors are related to the presence of qualitatively altered thrombocytes and leukocytes, leading to their activation and appearance of leukocytes-platelet-aggregates; anomalies of portal vein endothelial cells are also implicated [8,11]. The presence of JAK2V617F mutation increases the risk for splahnic thrombosis [11].

Case report

We present a case of a young female patient born in 1984, diagnosed with JAK2V617F positive MPN at the age of 24 years with hemoglobin level of 151 g/L, hematocrit 0.46, white blood cells (WBC) 11,4x 10^9/L and platelet count 1022x10^9/L. Blood smear presented with increased platelet number, platelet aggregates with normal features of leukocytes and erythrocytes. Bone marrow biopsy confirmed the diagnosis of thrombocythemia and molecular analysis confirmed the presence of JAK2V617F mutation. Abdominal ultrasound at the time of diagnosis was normal.

We started the initial treatment with Interferon alpha at a dose of 3 million units, daily, 3 times per week and Aspirin 100 mg daily. Due to insufficient control of platelet count with IFN, we continued with Anagrelide treatment at a dose of 1 mg twice daily with occasional phlebotomies for hematocrit control and Aspirin 100 mg daily. Platelet level during Anagrelide treatment varied between 404 and 916x10^9/L. Interferon alpha therapy was started again due to suboptimal control of platelet count with Anagrelide. Eight years after the diagnosis of thrombocythemia, the patient developed portal vein thrombosis (PVT) while she was on Interferon alpha and Aspirin therapy for MPN. At the time of diagnosis of PVT, hemoglobin level was 156 g/L, WBC 10.2, platelet count 543x109/L. The diagnosis was confirmed with CT scans and Doppler ultrasound. Biochemistry tests confirmed elevation of hepatic enzymes AST 141, ALT 109, hyperbilirubinemia of 39. Anticoagulation treatment of PVT was started immediately with low molecular weight heparins (Enoxaparin 2x40 mg). Treatment of underlying thrombocytosis continued with Interferon alpha 3 million units, 3-5 times per week with platelet count between 288 and 376x10^9L. Oral anticoagulant therapy with Acenocoumarol was started 3 weeks after the diagnosis overlapping with LMWH until therapeutical INR was achieved (INR 2.3 up to 4.0). She also needed a supportive treatment with two consecutive evacuations (EAP) of 3+2 L of peritoneal fluid; 20% albumin and fresh frozen plasma; diuretics and hepatoprotective therapy.

Control ultrasounds were performed monthly in the first six months, with slow improvement and uncomplete resolution of thrombotic formations in the left and right hepatic veins, as well as development of signs of hepatic cirrhosis. In 2019 due to pregnancy, treatment with VKA was stopped and LMWH was used during the pregnancy and breast-feeding period. After that, she continued taking VKA. Interferon treatment was stopped during pregnancy due to low number of platelets between 100-150x10^9/L and normal blood cell count. In September 2020, due to occlusion of hepatic veins, a surgical intervention with transjugular intrahepatic portosystemic shunt (TIPS) was performed.

Last blood count in March 2023 was normal with Hgb 131 g/L, WBC 6.7, Plt 164, INR 2.9 and she is

on regular treatment with VKA without any treatment for MPN due to normal blood cell counts.

Discussion

VTE is a common complication in patients with MPNs, with a reported incidence ranging from 6 to 39% [5,6]. However, PVT is a rare form of VTE, accounting for approximately 2-5% of all cases of VTE [8,9]. PVT can lead to severe complications, such as portal hypertension, liver failure, and gastrointestinal bleeding.

The pathogenesis of PVT in patients with MPNs is not fully understood, but it is believed to be multifactorial, with factors such as endothelial dysfunction, increased blood viscosity, and hypercoagulability playing a role. The JAK2 V617F mutation has been implicated in the pathogenesis of MPNs and is also associated with an increased risk of VTE [12].

The management of PVT in patients with MPNs is challenging and requires a multidisciplinary approach. Anticoagulation therapy is the cornerstone of treatment, and the duration of therapy depends on the underlying condition and the risk of recurrence. In patients with PV, the use of cytoreductive therapy, such as hydroxyurea, can reduce the risk of thrombosis. In cases of recurrent or refractory PVT, alternative therapies such as thrombolytic therapy or transjugular intrahepatic portosystemic shunt (TIPS) may be considered [13].

While the underlying mechanisms for the increased thrombotic risk in MPNs are not fully understood, current evidence suggests that cytoreductive therapy and phlebotomy may play a role in reducing the risk of thrombosis [12].

Further research is needed to elucidate the pathophysiology of thrombosis in MPNs and to develop targeted therapies for these patients [12,14]. Clinicians should be aware of the potential for PVT in patients with MPNs and monitor them closely for signs and symptoms of thrombosis. A standard VTE prophylaxis, such as anticoagulation, should be used for patients with acute VTE, while low-dose aspirin may be beneficial for those with a history of thrombosis or cardiovascular risk factors.

Conclusion

PVT is a rare but serious complication of MPNs, particularly in patients with JAK2 positivity. Early recognition and management of PVT in these patients is crucial to prevent further thrombotic events and improve outcomes. Cytoreductive therapy and phlebotomy may also be considered in patients with PV and other MPNs to reduce the risk of thrombosis. A multidisciplinary approach involving hematologists, gastroenterologists, and hepatologists may be necessary for the optimal management of PVT in patients with MPNs.

Conflict of interest statement. None declared.

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Мак Мед Прегледд 2023; 77(1): 53-63

ММР

Case report

MULTI-ORGAN INVOLVED BENIGN METASTASIZING LEIOMYOMA (BML) INVESTIGATED WITH ^{18}F-FDG PET/CT

МУЛТИ-ОРГАНСКИ БЕНИГНЕН МЕТАСТАЗИРАЧКИ ЛЕОМИОМ (БМЛ) ИСПИТУВАН СО ^{18}F-FDG ПЕТ/КТ

Slavko Tasevski, Dusica Todorova - Stefanovski and Ana Ugrinska

University Institute for Positron Emission Tomography, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

Benign metastasizing leiomyoma (BML) is a rare condition characterized by the formation of lesions that may occur in various parts of the body, exhibiting similar pathological characteristics to uterine leiomyoma. Generally, it occurs in the lungs, but it can also affect other organs including the heart, brain, lymph nodes. We present a case of a 74-year-old patient with a history of hysterectomy due to leiomyoma. As a result of a severe abdominal pain, eight years later, she underwent a computed tomography (CT) scan, which showed lesions in the lungs, liver and in the epigastric region. One of the lung lesions was biopsied and the finding was consistent with BML. Consequently, she was referred to our institution for positron emission tomography/computed tomography (PET/CT) using 18-fluoro-2-deoxy-D-glucose (^{18}F-FDG). The findings pointed toward multi-organ lesions with mild ^{18}F-FDG uptake in the thorax and abdomen, one of them infiltrating the ribs. Since there is no standardized treatment for BML, personalized treatment strategy should be involved, based on the hormonal receptor status and the size and location of the tumor. Further investigations are needed to determine the effectiveness of using ^{18}F-FDG PET/CT in the diagnosis and management of BML.

Keywords: ^{18}F-FDG -PET/CT, leiomyoma, metastasizing, multi-organ

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Апстракт

Бенигнен метастазирачки леомиом (БМЛ) е ретка состојба карактеризирана со формирање на лезии што можат да се јават на различни делови од телото, со слични патолошки карактеристики како леомиомот на утерус. Генерално се јавува во белите дробови, но исто така и во други органи вклучувајќи го срцето, мозокот, лимфните јазли. Презентираме случај на 74-годишна пациентка со историја на хистеректомија поради леомиом. Како резултат на силна стомачна болка, осум години подоцна направена е компјутерска томографија (КТ), кој прикажа лезии на белите дробови, црниот дроб и на епигастрично. Една од лезиите на белите дробови беше биопсирана и резултатот беше во прилог на БМЛ. Следователно, таа беше препратена во нашата институција за Позитронско-емисиона томографија/компјутерска томографија (ПЕТ/КТ) користејќи 18-флуоро-2-деокси-D-гликоза (^{18}F-FDG). Резултатите беа во прилог на мултиорганski лезии со умерен прифат на ^{18}F-FDG во торакс и абдомен, каде една од нив ги инфилтрира ребрата. Бидејќи не постои стандардизиран третман за БМЛ, потребно е да се вклучи персонализирана стратегија за третман, базирана на статусот на хормоналниот рецептор, како и големината и локацијата на туморот. Потребни се дополнителни истражувања за да се утврди ефективноста на користењето на ^{18}F-FDG ПЕТ/КТ во дијагнозата и менаџирањето на БМЛ.

Клучни зборови: ^{18}F-FDG -ПЕТ/КТ, леомиом, метастазирачки, мулти-органски

Introduction

Benign metastasizing leiomyoma (BML) is a rare condition, which manifests itself in lesions throughout the body, whose pathological features are identical to uterine leiomyoma. It usually appears in patients who previously had hysterectomy due to leiomyoma [1]. The time interval between hysterectomy and BML diagnosis is around 8.8 years and it can occur both

Correspondence to: Slavko Tasevski, University Institute for Positron Emission Tomography, 1000 Skopje, R. N. Macedonia; E-mail: slavko.tasevski@yahoo.com

in pre- and postmenopausal women. BML mostly appears in the lungs and rarely affects organs like the spine, heart, brain, lymph nodes or skin [2]. The diagnosis is usually confirmed with magnetic resonance imaging (MRI) or computed tomography (CT), which findings could mimic metastatic lesions [3]. We present a case report of positron emission tomography/computed tomography (PET/CT) using 18-fluoro-2-deoxy-D-glucose (^{18}F-FDG) in a patient with confirmed BML.

Case report

A 74-year-old patient underwent hysterectomy and bilateral oophorectomy in 2013. The histopathological findings were consistent with uterine leiomyoma. Over the following years, she experienced abdominal pain. The abdominal ultrasound done five years after the leiomyoma surgery showed hypoechoic lesion in the liver. At the follow-up ultrasound, lesion progression was detected. She also underwent a lung CT scan, which revealed formation of bilateral lesions in the lungs. Although a further investigation was recommended, due to unknown reasons, it was not carried out. Eight years after the leiomyoma surgery, she experienced a severe abdominal pain. The CT scan showed a mass in the left lung infiltrating the surrounding ribs, and multiple lung nodules in the right lung. Lesions in the liver and a mass in the epigastric region were also detected. All of these lesions were consistent with metastasis and one of them was biopsied. The immunohistochemistry of the lesion had high positivity of Vimentin, Desmin, focal positivity of Actin, estrogen and progesterone receptors and faint positivity of CD99 and BCL2. There was negative positivity for CD34 and CD117 and low Ki67 index of 3%-5%. These findings were consistent with BML with uterine origin. This patient was referred for ^{18}F-FDG PET/CT scan. The patient was required to fast for at least 4-6 h prior to scanning. The level of glucose in a finger-prick blood sample, before ^{18}F-FDG injection, was 5.4 mmol/L (97.2 mg/dL). The dose of ^{18}F-FDG was 313.47 MBq (8.47 mCi), applied intravenously as per clinical routine. Flouride-18 was synthesized in-house using the GE PET trace cyclotron at our institution. Scan was acquired at 60 minutes post-injection of radiotracer (p.i.). The patient had a skull base to mid-thigh PET scan which was performed on a Biograph mCT PET/CT digital scanner (Siemens, Erlangen, Germany) at 2 minutes per bed position (bp). Fluorine-18-FDG PET/CT findings (Figure 1) were consistent with multiple mildly hypermetabolic lesions in the lungs with a maximum standardized uptake value (SUVmax=3.7) (Figure 2). The largest lesion measured 7.0 cm in its maximum dimension. One of these lesions infiltrated the 5th left rib and thoracic wall (Figure 3). There were also multiple mildly hypermetabolic lesions in the liver (SUV max=2.9) (Figure 4) and in the right supramesocolic space (SUVmax=2.9) (Figure 5), possibly related to the referral diagnosis. The SUVmax of the healthy liver was 3.0. A lymph node near the right iliac external vein had increased ^{18}F-FDG uptake (SUVmax=3.8).

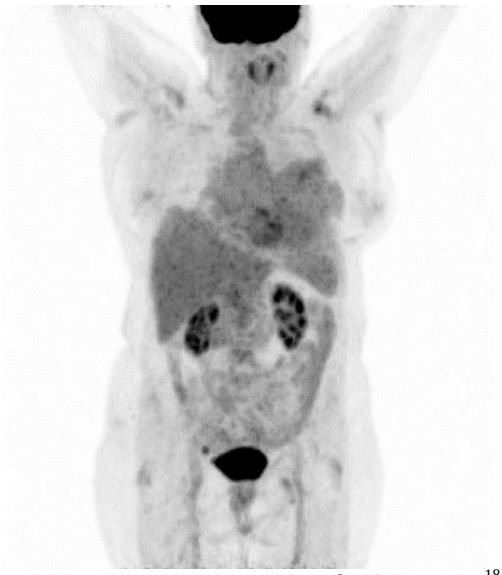

Fig. 1. Positron emission tomography/CT using ^{18}F-FDG. Maximum intensity projection (MIP) image shows lesions in the thorax and abdomen with mild ^{18}F-FDG uptake

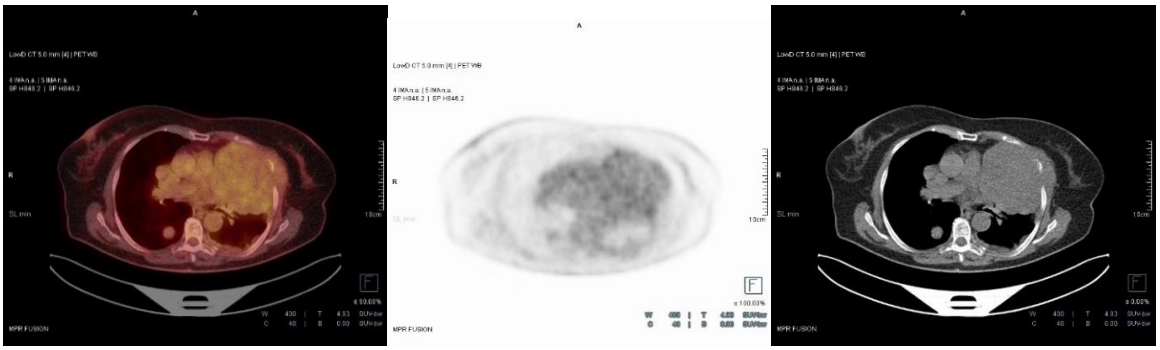

Fig. 2. Fluorine-18-FDG PET/CT fusion, transverse PET and corresponding CT images show mild ^{18}F-FDG uptake in lesion infiltrating the thoracic wall

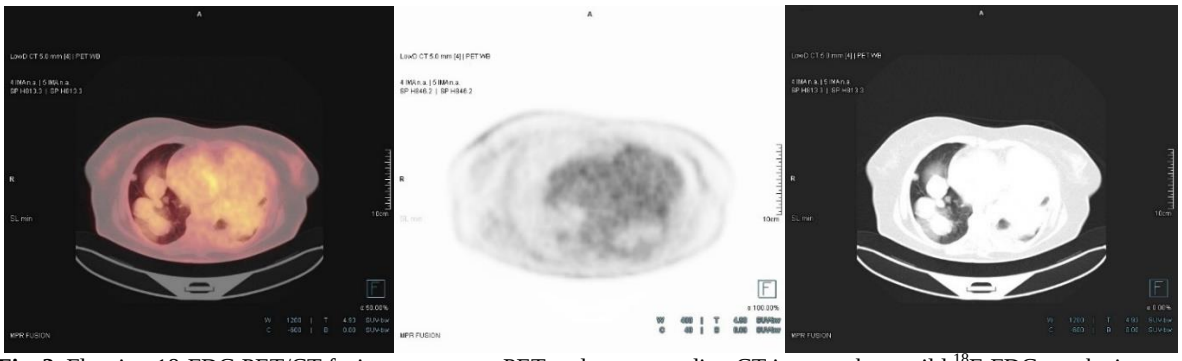

Fig. 3. Fluorine-18-FDG PET/CT fusion, transverse PET and corresponding CT images show mild ^{18}F-FDG uptake in the lung lesions

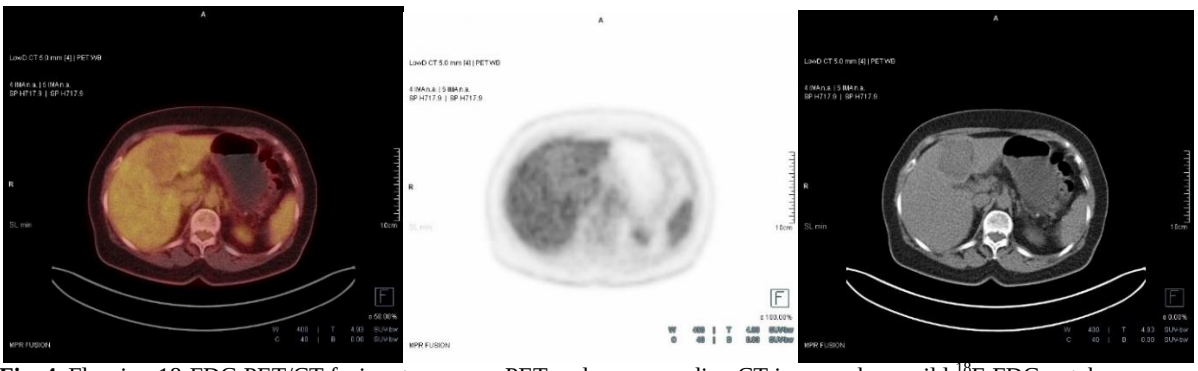

Fig. 4. Fluorine-18-FDG PET/CT fusion, transverse PET and corresponding CT images show mild ^{18}F-FDG uptake in the liver lesion

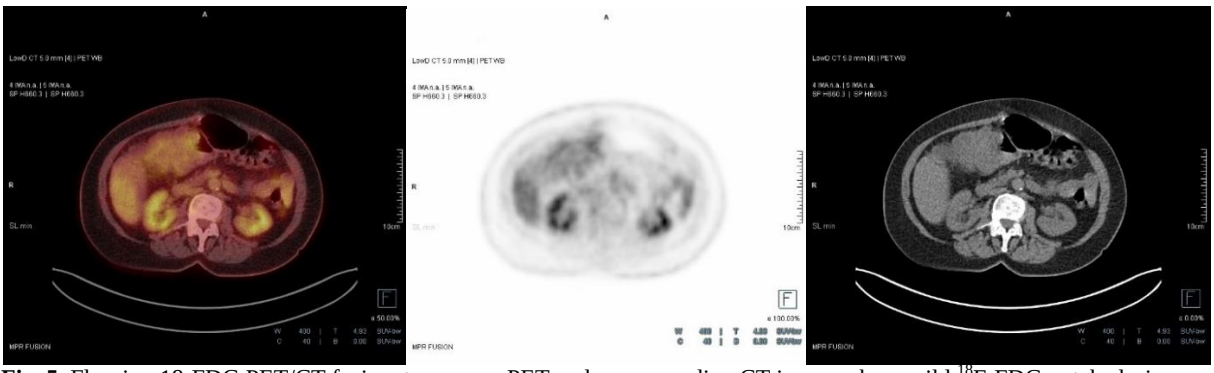

Fig. 5. Fluorine-18-FDG PET/CT fusion, transverse PET and corresponding CT images show mild ^{18}F-FDG uptake lesion in the right supramesocolic space

Discussion

Benign metastasizing leiomyoma is a rare disease with approximately 100 cases in the literature overall [4] and with less than 30 articles which describe the correlation between these lesions and ^{18}F-FDG uptake [5]. There are different theories regarding the metastatic spread of leiomyoma. The most accepted one is the vascular theory. According to this theory, uterine leiomyoma infiltrates the vessels and obstructs the microcirculation, creating nodules in which it can proliferate [6]. These lesions may be asymptomatic and often appear as incidental findings on various diagnostic images, where they could be misinterpreted as malignant lesions. From a pathological point of view, these lesions are composed of proliferative smooth muscle cells arranged in fascicles with no mitoses and without significant atypia. They have low mitotic index and positivity of estrogen and progesterone receptors, desmin and actin. They also have 19q and 22q terminal deletion [7]. Differential diagnosis includes leiomyosarcoma which has miRNA expression, high mitotic index and cellular atypia [8]. We present a case with biopsy con-

firmed BML lung lesions, presenting mild ^{18}F-FDG uptake, similarly to the lesions in the liver and supramesocolic space (ranging from SUVmax=1.1 to 3.7). Up to date, most of the cases found in the literature have had either mild or no ^{18}F-FDG uptake. Only 11 of 33 such cases were reported to have SUVmax values of the lesions. Their values were similar to our case, ranging from 0.2-3.8. There were only three cases with high ^{18}F-FDG uptake lesions, one of them with SUVmax=20.1 [5]. The reason for mild ^{18}F-FDG uptake in BML has not been well-understood, yet. According to one article, there is no significant difference between glycolytic enzymes in patients with leiomyoma and those with leomyosarcoma [9]. Another article reported weaker expression of GLUT-1 and hexokinase activity in leiomyoma in comparison with leiomyosarcoma [10]. One case presented a malignant transformation, where ^{18}F-FDG accumulation highly differentiated from both the benign and malignant patterns of the lesion [11]. Uterine leiomyosarcoma lesions usually do have high ^{18}F-FDG uptake [12]. We should include in the differential diagnosis tumor of uncertain malignant potential, as it is sometimes hyper-metabolic as well. ^{18}F-FDG cannot distinguish whether a hypermetabolic lesion is malignant or benign or of uncertain malignant potential [13]. Uterine leiomyoma lesions generally have lower ^{18}F-FDG uptake or equal to that of the liver [14].

The clinical course of BML is usually indolent and hormone dependent and currently there is no standardized treatment. It is known that it responds well to treatment with Tamoxifen and aromatase inhibitors due to the high estrogen receptor expression. The lack of estrogen hormone results in lesion regression in premenopausal and postmenopausal women. In these cases, wait-and-see strategy could be used with CT or MRI. However, in premenopausal women these lesions can rapidly progress and cause death due to respiratory insufficiency. Therefore, personalized treatment strategy should be implemented depending on individual's hormone receptor status, as well as the size and location of the tumor [15].

Conclusion

Even though the first BML report from Steiner came to light in 1939 [16], it still has remained a rare entity with barely enough data. Regarding ^{18}F-FDG PET/CT, this imaging procedure might be of help in excluding potential malignant transformation of BML to leiomyosarcoma, where high ^{18}F-FDG uptake is expected [17]. However, further investigations are required in order to understand the biological characteristics related to the ^{18}F-FDG uptake in BML and possible implementation of ^{18}F-FDG PET/CT in patients with BML.

Conflict of interest statement. None declared.

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УПАТСТВО ЗА ПРИЈАВА НА ТРУД ОД СОРАБОТНИЦИТЕ НА ММП

"Македонски медицински преглед"(ММП) е стручно списание на Македонското лекарско друштво, првенствено наменето на лекарите од општа практика, специјалистите од одделните медицински дисциплини и истражувачите во областа на базичните медицински и други сродни науки.
Списанието ги има следниве рубрики и категории на трудови:

1. **Изворни трудови**
2. **Соопштувања за клинички и лабораториски искуства**
3. **Прикази на случаи**
4. **Од практика за практика**
5. **Едукативни статии**
6. **Вариае (**писма од редакцијата, општествена хроника**,** прикази на книги, извештаи од конгреси, симпозиуми и други стручни собири, рубриката ,,Во сеќавање,, и др).

Изворните трудови имаат белези на научни трудови, додека трудовите категоризирани во рубриките 2-5 имаат белези на стручни трудови.
Во ММП се објавуваат трудови на членовите на МЛД или на членови на други стручни здруженија. Авторите се одговорни за почитувањето на етичките начела при медицинските истражувања, а изнесените ставови, изведени од анализата на сопствените резултати, не се нужно и ставови на Редакцијата на ММП.
Редакцијата ги испраќа ракописите на стручна рецензија; рецензентот (ите) и Редакцијата ја определуваат дефинитивната категоризација на ракописот кој е прифатен за печатење. Редакцијата го задржува правото ракописите да ги печати според рецензираниот приоритет.
Упатството за соработниците на ММП е во согласност со Ванкуверските правила за изедначени барања за ракописите кои се праќаат до биомедицинските списанија.

1. ТЕКСТ НА РАКОПИСОТ

Сите ракописи се испраќаат во електронска форма на електронската адреса (е-маил) на МЛД-ММП, со двоен проред и најмногу 28 редови на страница. Трудот се поднесува на англиски јазик латиничен фонт Times New Roman големина 12 и апстракт на македонски јазик. Лево, горе и долу треба да се остави слободна маргина од најмалку 3 см, а десно од 2,5 см.. Редниот број на страниците се пишува во десниот горен агол.
Ракописот на трудот треба да е придружен со писмо на првиот автор, со изјава дека истиот текст не е веќе објавен или поднесен/прифатен за печатење во друго списание или стручна публикација и со потврда дека ракописот е прегледан и одобрен од сите коавтори, односно со придружна декларација за евентуален конфликт на интереси со некој од авторите.

Насловната страна треба да има: наслов на македонски и англиски, имиња и презимиња на авторите, како и институциите на кои им припаѓаат, имињата на авторите и насловот на установата се поврзуваат со арапски бројки; автор за кореспондеција со сите детали (тел. е-маил); категорија на трудот; краток наслов (до 65 карактери заедно со празниот простор); како и информација за придонесот за трудот на секој коавтор (идеја, дизајн, собирање на податоци, статистистичка обработка, пишување на трудот).

Насловот треба концизно да ја изрази содржината на трудот. Се препорачува да се избегнува употреба на кратенки во насловот.

Изворните трудови и **соопштувањата** го имаат следниов формален редослед: насловна страна, извадок на македонски јазик (вовед, методи, резултати, заклучок) со клучни зборови, извадок на македонски јазик со клучни зборови, вовед, материјал и методи, резултати, дискусија и заклучоци, литература и прилози (табели, графици и слики) и легенди за прилозите во еден фајл.

Приказите на случаи треба да содржат вовед, детален приказ на случајот, дискусија со заклучок и литература со прилози.

Извадокот на македонски јазик треба да содржи најмногу 250 зборови и да биде структуриран со сите битни чинители изнесени во трудот: вовед со целта на трудот, методот, резултати (со нумерички податоци) и заклучоци. Заедно со извадокот, треба да се достават и до 5 клучни, индексни зборови.

Извадокот на англиски јазик мора да е со содржина идентична со содржината на извадокот на македонски јазик. Клучните зборови треба да се во согласност со MeSH (Medical Sibject Headings) listata na Index Medicus.

Воведот треба да претставува краток и јасен приказ на испитуваниот проблем и целите на истражувањето, со наведување на етичкиот комитет односно институцијата која го одобрила испитувањето (клиничка студија која се работи според принципите на Хелсиншката декларација за пациентите и нивните права).

Методите треба да бидат точно назначени, за да се овозможи повторување на прикажаното истражување. Особено е важно да се прецизираат критериумите за селекција на опсервираните случаи, воведените модификации на веќе познатите методи, како и идентификација на употребените лекови според генеричното име, дозите и начинот на администрација.

Резултатите треба да се прикажат јасно, по логичен редослед. Резултатите се изнесуваат во стандардните SI единици. Во текстот треба да се назначи оптималното место каде ќе се вметнат табелите и илустрациите, за да се избегне непотребното повторување на изнесените податоци. Значајноста на резултатите треба да се обработи статистички, со детален опис на употребените статистички методи на крајот на делот *методи*.

Дискусијата треба да ги истакне импликациите од добиените резултати, споредени со постојните сознанија за испитуваниот проблем.

Заклучоците треба да не бидат подолги од 150 зборови.

2. ПРИЛОЗИ

Како прилог-документација на трудовите предложени за печатење, може да се достават до 5 прилога (табели, фигури,/слики - илустрации).

Табелите се доставуваат на крајот на трудот во истиот фајл. Секоја табела треба да има свој наслов и реден број кој ја поврзува со текстот. Хоризонтални и вертикални линии на табелата не се дозволени; ознаките на колоните во табелата се пишуваат скратено или со симбол, а нивното објаснување се пишува на дното на табелата, во вид на легенда.

Илустрациите се доставуваат со реден број како слика во црно-бела техника, а секоја слика треба да е придружена со легенда (опис).

Микрофотографиите може да содржат посебни ознаки во вид на стрелки или симболи. Покрај описот на сликата, мора да се наведе и зголемувањето и видот на боењето на препаратот (ако тоа веќе не е направено во секцијата *материјал и методи*).
Сите ознаки на фотографиите мора да бидат доволно големи, за да може јасно да се распознаат и по смалувањето во печатницата, при нивното вклучување во печатената страница на списанието.

3. ЛИТЕРАТУРА

Цитираната литература се пишува на крајот на трудот по заклучоците, со редни броеви според редоследот на појавувањето на цитатот на текстот на трудот ставени во средни загради и без простор меѓу нив (ако се последователни треба да се поврзани со цртичка, на пр. [3-6]).
Литературата се цитира на следниов начин (кратенките за насловите на списанијата треба да се според листата прифатени во Индекс Медицус):
а) ***статија во списание*** (се наведуваат сите автори, ако ги има до 4 или помалку; ако ги има повеќе од 4 се наведуваат првите 3 автори и се додава: *и сор*.) Neglia JP Meadows AT, Robison LL *et al*. Second neoplasms after acute lymphoblastic leukemia in childhood. N Engl J Med 1991; 325:1330-6.
б) заеднички автор
GIVIO (Interdisciplinary group for cancer care evaluation). Reducing diagnostic delay in breast cancer. Possible therapeutic implications. *Cancer* 1986; 58: 1756-61.
в) ***без автор*** **-** анонимно. Breast screening: new evidence. (*Editoriall Lancet* 1984; i :1217-8).
г) ***поглавје во книга или монографија***
Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. Vo: Sodeman WA Jr, Sodeman WA, Ed. Pathogenic physiology: mechanisms of disease. Philadelphia; W B Saunders, 1974: 457-72.

Првите отпечатоци на трудовите им се праќаат на авторите за корекција: авторите се должни коригираниот отпечаток да и го вратат на Редакцијата на ММП во рок од 2 дена.

Уплата за испечатен труд во списанието ММП изнесува 3.000, 00 денари и се уплаќаат на жиро сметката на: Македонско лекарско друштво
300000000211884 - Комерцијална банка
со цел на дознака : уплата за стучен труд

Адресата на Редакцијата
Даме Груев бр. 3
Градски ѕид блок II,

1000 Скопје,
Тел.: ++ 389 02 3162 577
Електронска адреса (Е-маил): mld@unet.com.mk

Авторите што сакаат да објавуваат трудови во списанието треба да ја имаат уплатено членарината за тековната година во висина од 1440 денари и за тоа да ја информираат стручната служба на Македонско лекарско друштво, писмено или преку телефон.

Детални информации можете да добиете на телефонот на Друштвото 02 3 162 557.

Известување за рецензентите за ММП

Во склад со правилникот на УКИМ рецензентите што навремено и одговорно ќе ја одработат рецензијата ќе добијат 0.4 бода кои се собираат за унапредување во академските звања. Бодовите можат да се добијат и ретроградно преку побарување во МЛД - 3162 557.