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Original article

ASSOCIATION BETWEEN SERUM VITAMIN D LEVELS AND HUMAN PAPILLOMAVIRUS (HPV) CLEARANCE: A PROSPECTIVE CASE-CONTROL STUDY

АСОЦИЈАЦИЈА ПОМЕЃУ СЕРУМСКИТЕ НИВОА НА ВИТАМИН Д И ЕЛИМИНАЦИЈАТА НА ХУМАН ПАПИЛОМА ВИРУСОТ (ХПВ)

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Abstract

Introduction. Human papillomavirus (HPV) is a leading cause of sexually transmitted infections and cervical cancer worldwide. Emerging evidence suggests a possible role of serum vitamin D levels in influencing HPV infection and clearance.
Aim. To investigate the relationship between serum 25-hydroxyvitamin D [25(OH)D] concentrations and HPV status in women with and without cervical intraepithelial lesions.
Methods. This prospective case-control study included 188 women. Serum vitamin D levels were compared among HPV-positive and HPV-negative groups. HPV genotyping and 25(OH)D concentrations were determined using standardized procedures.
Results. A significant difference in serum vitamin D levels was found between HPV-negative and HPV-positive individuals ($p<0.0001$). No significant differentces were observed among different HPV-positive subgroups.
Conclusion. Vitamin D sufficiency may be associated with a reduced risk of HPV infection or persistence. Further studies are required to elucidate underlying immunological mechanisms.

Keywords: vitamin D, HPV, cervical cancer

Апстракт

Вовед. Хуман папилома вирусот (HPV) претставува една од водечките причини за рак на грлото на матката на глобално ниво, и најчеста сексуално преносliва инфекција. Новите истражувања сугерираат на можно влијание на нивото на витамин Д во крвта врз инфекцијата со HPV и нејзиното елиминирање.
Цел. Да се испита врската помеѓу концентрациите на серумскиот 25-хидроксивитамин Д [25(OH)D] и HPV статусот кај жени со и без цервикални интраепителни лезии.
Методи. Оваа проспективна студија на случаи и контроли опфати 188 жени. Нивото на серумскиот витамин Д беше споредено помеѓу групите позитивни и негативни на HPV. HPV генотипизацијата и концентрациите на 25(OH)D беа утврдени со стандардизирани процедури.
Резултати. Се откри значајна разлика во нивото на серумскиот витамин Д помеѓу HPV-негативните и HPV-позитивните испитанички ($p<0.0001$). Не беа забележани значајни разлики помеѓу различните подгрупи на HPV-позитивни.
Заклучок. Доволното ниво на витамин Д може да биде поврзано со намален ризик од инфекција со HPV или нејзина персистенција. Потребни се дополнителни истражувања за разјаснување на поконкретните имунолошки механизми.

Клучни зборови: витамин Д, HPV, рак на грлото на матката

Introduction

HPV is one of the most prevalent sexually transmitted viruses globally and in the United States. Persistent HPV infection is a known cause of cervical dysplasia and cervical cancer. While HPV infection is not a reportable disease, it is estimated that 1-5.5 million new cases occur annually in the U.S.A, with a prevalence reaching 20 million [1]. Increasing attention has been given to the immunomodulatory effects of vitamin D, particularly its role in viral infection clearance.
Vitamin D is involved in both innate and adaptive immunity, enhancing the body's ability to fight infections like HPV [2].

Association Between Vitamin D Serum Levels and HPV Infection

Research has shown mixed but suggestive findings linking serum 25-hydroxyvitamin D [25(OH)D] con-

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centrations with HPV prevalence, incidence, and clearance. For instance, El-Zein *et al.* observed that higher serum vitamin D levels were associated with reduced HPV prevalence and enhanced clearance in young women [2].
However, other studies have reported more nuanced results. Some show no significant link between vitamin D levels and initial HPV acquisition but suggest a role in promoting clearance among those already infected [3].

Vitamin D and Immune Function

Vitamin D exerts its immunological effects via both innate and adaptive immune pathways [4].
Vitamin D regulates genes involved in immune modulation. It enhances innate immunity and shapes adaptive responses by creating an anti-inflammatory, tolerogenic environment, thus limiting excessive immune activation [5,6]. Vitamin D promotes the production of antimicrobial peptides like cathelicidin and β-defensin 2, enhancing the ability of macrophages to combat infections [7].
Cumulative reports have shown that low Vit-D status is associated with a broad range of disorders, particularly autoimmune diseases such as multiple sclerosis (MS), type 1 diabetes (T1D), inflammatory bowel disease (IBD), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) [1-8]. Indeed, Vit-D is involved in the maintenance of immune system homeostasis and dampens autoimmune responses via modulation of T cells, B cells, monocyte/macrophage, dendritic cells, etc. [9]. Vit-D plays a crucial role in the regulation of Th1/Th2 cell ratios and modification of Th17/Treg ratios by increasing the frequency of Treg cells and reducing Th17 differentiation [9].
Of note, pre-clinical and clinical studies have demonstrated that Vit-D in physiologic concentrations could induce a tolerogenic and/or anti-inflammatory microenvironment [10,11]. It is now accepted that changes in Vit-D levels can substantially affect several aspects of immune cells such as gene expression, DNA methylation, and signaling pathways. Such changes might lead to the disturbance of immune homeostasis and induction of autoimmune disease. Several systematic reviews have investigated the correlations between Vit-D levels and the incidence of autoimmune diseases [1-3].

Host Defence Mechanism Against HPV

Although most people contract HPV during their lives, only 10-15% develop persistent infections, and an even smaller number progress to cancer, indicating effective host defenses in most cases [8].
HPV must bypass multiple physical defenses to infect basal keratinocytes. Entry is aided by microabrasions that disrupt the mucosal barrier, which is normally protected by mucus and antimicrobial peptides. After reaching the extracellular matrix, host proteases modify the viral capsid to enable cell entry. Inside the cell, HPV follows the endocytic pathway and retrograde transport through the Golgi to the nucleus, but many particles are degraded via autophagy [9-13]. Nuclear entry depends on mitotic breakdown of the nuclear envelope [12,13]. Human α-defensins, especially α-defensin 5, block infection by inhibiting capsid processing [14,15].
HPV DNA is detected by innate sensors such as AIM2, IFI16, and cGAS, which initiate inflammatory responses. AIM2 activation leads to caspase-1 and IL-1β production [16], while IFI16 suppresses HPV gene expression by promoting heterochromatin formation [17].
HPV evades systemic immunity due to its restricted intraepithelial lifecycle and absence of viremia. However, strong T cell responses, particularly cytotoxic T cells, are crucial for lesion regression [18-22]. LCs from infected individuals can activate HPV-specific CD8+ T cells [23], and mouse models confirm the necessity of both CD4+ and CD8+ T cells for infection control [24]. UV-induced immunosuppression enhances susceptibility [25]. While natural infection rarely induces protective antibodies, vaccines provide effective immune memory [26-37].
Although DNA viruses mutate slowly compared to RNA viruses, host immune pressure shapes HPV genome evolution [38-42].

Materials and Methods

Study Design and Participants

This prospective case-control study included 188 women, divided into two groups: 107 women with histologically confirmed cervical intraepithelial lesions (case group) and 81 women with normal Pap test results (control group). Inclusion criteria were: age over 18, non-pregnancy status, and no history of certain hematologic or hepatic conditions. Blood samples were collected for vitamin D analysis.

Determination of Vitamin D

Serum 25(OH)D levels were measured using a competitive chemiluminescence enzyme immunoassay on a TOSOH AIA-900 analyzer. The assay involved releasing 25(OH)D from its binding proteins, capturing it on magnetic beads coated with monoclonal antibodies, and quantifying the bound enzyme-labeled 25(OH)D using a fluorogenic substrate.

HPV Testing

Endocervical samples were collected using cytobru-

shes and stored for HPV DNA detection via multiplex PCR. HPV genotyping included high-risk types such as 16, 18, 31, 33, and others. Real-time PCR with specific primers and fluorescent probes identified HPV presence and type.

Statistical Analysis

Data were analyzed using GraphPad Prism 9.0. One-way ANOVA and t-tests were applied to compare vitamin D levels between groups. Significance was set at $p<0.05$.

Results

Serum vitamin D levels significantly differed between HPV-negative and HPV-positive groups ($p < 0.0001$). Post-hoc analysis revealed:

- HPV-negative individuals had significantly higher vitamin D levels compared to:
  - HPV-16 positive: $p < 0.0001$
  - HPV-18 positive: $p < 0.0001$
  - Other HPV types: $p < 0.0001$
- No significant differences in vitamin D levels were found among HPV-16, HPV-18, and other HPV types ($p>0.05$).

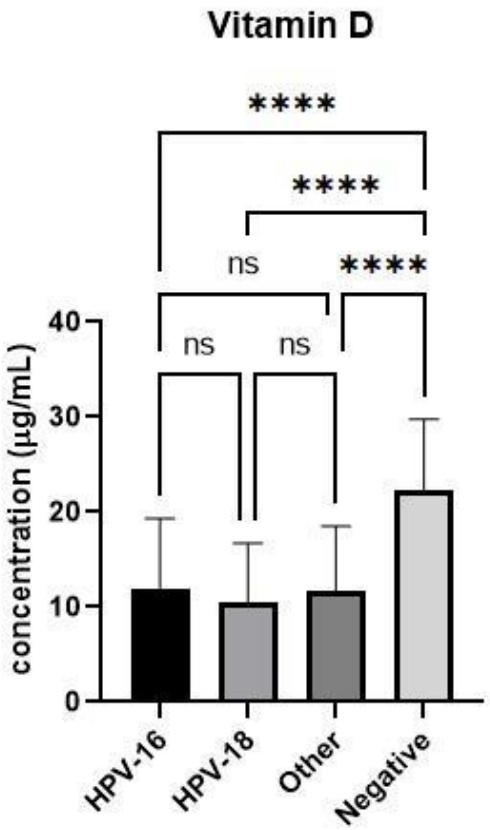

Fig. 1. Serum Vitamin D Concentrations in HPV-Positive and HPV-Negative Women

Mean 25(OH)D levels are shown for women with HPV-16, HPV-18, other HPV types, and those who tested negative for HPV. Error bars represent standard deviations. Statistically significant differences were indicated: **** $p<0.0001$; ns: not significant. Women who tested negative for HPV had significantly higher Vitamin D levels compared to all HPV-positive groups (Figure 1).

The group of patients who tested positive for HPV type 16 had a mean serum vitamin D level of 11.97 µg/mL. Those positive for HPV type 18 had a mean level of 10.4 µg/mL, while patients positive for other HPV types had an average vitamin D concentration of 11.6 µg/mL. HPV-negative patients had a mean vitamin D concentration of 22.27 µg/mL.

Among the patients who tested positive for HPV type 16 (n=39), 30.77% (n=13) had CIN 1, 33.33% (n=12) had CIN 2, and 35.9% (n=14) had CIN 3.

Out of the 14 patients who were positive for HPV type 18, 50% (n=7) had CIN 1, 21.43% (n=3) had CIN 2, and 28.57% (n=4) had CIN 3.

A total of 54 patients tested positive for other high-risk HPV subtypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), among whom 48.15% (n=26) had CIN 1, 33.33% (n=18) had CIN 2, and 18.52% (n=10) had CIN 3.

Table 1. Disposition of patients according to the cervical intraepithelial lesion and the concentration levels of vitamin D. Results are shown as Mean±SEM

Cervical intraepithelial lesion	Vitamin D (ng/ml) ± SEM	p-value(t-test, Mann-Whitney)
CIN1 (n=47)	11.79±0.97	<0.0001
CIN2 (n=33)	11.31±1.08	<0.0001
CIN3 (n=27)	11.34±1.35	<0.0001
Control (n=81)	22.40±0.86	

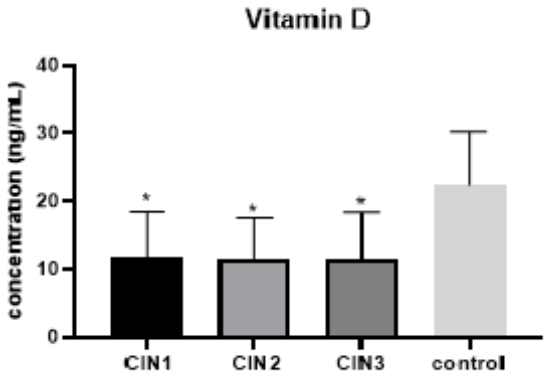

Fig. 2. Mean serum concentration of vitamin D (ng/mL) in women with cervical intraepithelial lesions and women with normal Pap (control group)

Among all participants in the study, 25% were diagnosed with CIN 1 based on histopathological examina-

tion, with a mean serum vitamin D level of 11.79±0.97 ng/mL. In 17.55% of the patients (n=33) diagnosed with CIN 2, the average vitamin D level was 11.31±1.08 ng/mL. For 14.36% of the patients (n=27) with CIN 3, the mean value was 11.34 ± 1.35 ng/mL.

In contrast, the control group, consisting of patients with normal PAP smear results, had a significantly higher mean vitamin D level of 22.40 ± 0.86 ng/mL (see Table 1).

Among patients with cervical intraepithelial neoplasia, 66.35% (n=71) exhibited severe vitamin D deficiency, 17.75% (n=19) had vitamin D deficiency, 14.95% (n=16) had vitamin D insufficiency, and only 0.93% (n=1) had sufficient vitamin D levels.

In comparison, within the control group, 1.23% (n=1) had severe vitamin D deficiency, 40.74% (n=36) had a deficiency, 43.2% (n=38) showed insufficiency, and 14.81% (n=13) had adequate vitamin D levels.

A statistically significant difference ($p<0.05$) was found in serum vitamin D levels between the control group and those with cervical intraepithelial lesions (CIN1, CIN2, CIN3) (Figure 2).

Discussion

The present study demonstrates a significant association between serum vitamin D levels and the presence of cervical intraepithelial neoplasia (CIN), as well as with HPV infection status. Women with HPV infection had notably lower serum vitamin D levels compared to those who tested negative, with a highly significant difference ($p<0.0001$). Notably, no signifycant difference in vitamin D levels was observed among patients infected with HPV-16, HPV-18, or other high-risk HPV subtypes, suggesting that vitamin D deficiency may be a general feature of HPV-related pathology rather than specific to certain HPV genotypes.

Patients with high-grade cervical lesions (CIN2 and CIN3) exhibited markedly lower vitamin D levels compared to the control group with normal cytology. Specifically, the mean vitamin D concentration in the CIN groups ranged between 11.31-11.79 ng/mL, while the control group had a significantly higher mean value of 22.40 ng/mL. These findings are in agreement with previous studies suggesting an inverse relationship between vitamin D levels and the severity of cervical lesions. This may be attributed to the immunomodulatory and anti-inflammatory effects of vitamin D, which can influence HPV clearance and prevent progression to higher-grade lesions.

When examining the distribution of vitamin D status, the majority (66.35%) of patients with CIN presented with severe deficiency, whereas most women in the control group had either insufficiency (43.2%) or sufficiency (14.81%). Only 1.23% of the control group had severe deficiency. This pattern further underscores the potential role of vitamin D deficiency in the pathogenesis and persistence of HPV infection and CIN development.

Regarding HPV subtypes, the data show that both HPV-16 and HPV-18 positive women had similarly low vitamin D levels (11.97 ng/mL and 10.4 ng/mL, respectively), as did women infected with other high-risk HPV types (11.6 ng/mL). In contrast, HPV-negative women had a significantly higher mean level (22.27 ng/mL), reinforcing the notion that adequate vitamin D levels may offer a protective effect against HPV infection.

The stratification of lesion grades among HPV subtypes revealed that women with HPV-16 had a more balanced distribution across CIN1 (30.77%), CIN2 (33.33%), and CIN3 (35.9%), suggesting its strong oncogenic potential. Interestingly, HPV-18 was more common in CIN1 (50%) but was still present in higher-grade lesions. Other high-risk HPV types were predominantly found in CIN1 (48.15%), though a substantial number of patients also had CIN2 and CIN3, indicating their contribution to disease progression.

Contrasting Findings

Some studies report a paradoxical relationship where lower vitamin D levels are associated with increased HPV clearance, possibly reflecting immune activation during ongoing infection [2].

Micronutrients and Viral Outcomes

Research by Shvetsov *et al.* [43] and Troja *et al.* [44, 45] further emphasizes the role of micronutrients-including vitamin D-in the natural history of HPV infection. Their findings indicate that optimal nutritional status might improve immune surveillance and clearance rates.

Clinical Implications

Given its role in immune regulation, vitamin D status should be monitored in populations at risk for persistent HPV infection. Supplementation may be particularly beneficial for individuals with low baseline levels or in postmenopausal women, where immunological decline may impair HPV clearance [43-48].

In summary, this study adds to the growing body of evidence suggesting that low serum vitamin D levels are significantly associated with HPV infection and the development of cervical intraepithelial lesions. While causality cannot be established in this observational design, these findings raise the possibility that vitamin D supplementation could be considered as an adjunct preventive or therapeutic measure, especially in populations at risk for persistent HPV infection and cervical dysplasia. Further longitudinal studies and randomized controlled trials are needed to explore the potential benefits of vitamin D supplementation in

reducing HPV persistence and progression to cervical cancer.

Conclusion

This study demonstrates a clear and statistically signficant association between low serum vitamin D levels and both HPV infection and the presence of cervical intraepithelial lesions. Women with HPV infection-particularly those with high-grade lesions (CIN2 and CIN3)-exhibited markedly lower vitamin D concentrations compared to HPV-negative individuals and those with normal cervical cytology. Moreover, the prevalence of severe vitamin D deficiency was substantially higher in the CIN group than in the control group.

These findings suggest that vitamin D may play a protective role in the pathogenesis of HPV-related cervical lesions, possibly through its immunomodulatory and antiviral effects. Although causality cannot be established from this cross-sectional study, the results support further investigation into the potential of vitamin D as a preventive or therapeutic adjunct in women at risk for persistent HPV infection and cervical neoplasia.

Future longitudinal and interventional studies are needed to determine whether vitamin D supplementation could contribute to reducing HPV persistence, slowing lesion progression, and ultimately preventing cervical cancer.

Conflict of interests: None declared.

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ММР

Original article

ASSOCIATION BETWEEN CYSTATIN C AND HYPERTENSION AS ONE OF THE MAIN CAUSES OF CHRONIC KIDNEY DISEASE

РЕЛАЦИЈА НА ЦИСТАТИН Ц КАЈ ПАЦИЕНТИ СО ХИПЕРТЕНЗИЈА КАКО ЕДНА ОД ГЛАВНИТЕ ПРИЧИНИ ЗА ХРОНИЧНА БУБРЕЖНА БОЛЕСТ

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Abstract

Introduction. Hypertension, as a public health issue, is currently the leading factor in the global burden of disease. It is the primary modifiable risk factor for heart disease, stroke, and kidney failure. Chronic kidney disease (CKD) is both a cause and a consequence of uncontrolled hypertension. Accurate blood pressure measurements are crucial for diagnosing and controlling hypertension.
Aim. This study aimed to explore the association between arterial hypertension and elevated serum cystatin C levels so that to evaluate whether cystatin C can help in early diagnosis of chronic kidney disease.
Methods. The study was designed as an observational prospective study, which involved 28 participants, aged between 18 and 60. Participants were divided into two groups. The first group included patients with blood hypertension, while the second group consisted of healthy participants (the control group). In all participants, blood pressure was measured, serum cystatin C levels were analyzed, and glomerular filtration rate (GFR) was estimated using the CKD-EPI Equations for Glomerular Filtration Rate (Chronic Kidney Disease Epidemiology Collaboration-Cystatin C). The study was conducted at the University Clinic for Nephrology in Skopje.
Results. The first group exhibited significantly higher cystatin C levels than the control group (8.98 mg/L *vs*. 0.54 mg/L). Additionally, the first group showed lower GFR, suggesting a possible decline in kidney function.
Conclusion. There is a strong association between arterial hypertension and elevated serum cystatin C levels. Therefore, chronic kidney disease can be early detected using the CKD-EPI Equations for Glomerular Filtration Rate (GFR).

Keywords: chronic kidney disease, cystatin C, hypertension

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Абстракт

Вовед. Хипертензијата, како јавно здравствен проблем, моментално е водечки фактор во глобалниот товар на болести. Тоа е главниот модифициран фактор на ризик за срцеви заболувања, мозочен удар и бубрежна инсуфициенција.
Хронична бубрежна болест (ХББ) е честа причина за хипертензија и исто така претставува компликација на неконтролирана хипертензија. Стандардизираното мерење на крвниот притисок е од суштинско значење за утврдување на дијагнозата и управувањето со хипертензијата, која е една од главните причини за ХББ.
Цел. Цел на истражувањето е да се евалуира можноста при постоење на артериска хипертензија да се добие покачена серумската вредност на цистатин Ц, во една опсервациска студија.
Методи. Дизајн на студија е опсервациона проспективна студија. Во студијата беа вклучени 28 пациенти на возраст од 18-60 годишна возраст.
Пациентите беа групирани во 2 групи: пациенти со артериска хипертензија и здрави испитаници- контролна група.
Кај пациентите беше изведено мерење на артерискиот крвен притисок, земање крв за пресметување серумска вредност на цистатин Ц и пресметување гломеруларна филтрацијска рата-ГФР со помош на ГФР CKD-EPI-Cystatin C (Chronic Kidney Disease Epidemiology Collaboration-CystatinC, https://www.mdcalc.

com/calc/3939/ckd-epi-equations-glomerular-filtration-rate-gfrweb) равенката. Студијата се изведуваше на ЈЗУ Универзитетска клиника за Нефрологија-Скопје.
Резултати. Серумските нивоа на цистатин Ц кај пациенти со артериска хипертензија беа повисоки од оние кај контролната група здрави испитаници.
Средната вредност на цистатин Ц кај пациентите со артериска хипертензија изнесуваше 8.98mg/L, додека кај здравите испитаници- контролна група изнесуваше 0,54 mg/L. Серумските нивоа на цистатин Ц ја потврдија поврзаноста со постоење на повисока вредност за цистатин Ц кај пациентите со артериска хипертензија. Воедно се добиваат пониски вредности при пресметувањето на ГФР со истите серумските вредности на цистатин Ц, измерени кај пациентите со веќе постоечката артериска хипертензија. Ова укажува дека цистатинот Ц е можен предиктор за развој на ХББ.
Заклучок. Постои поврзаност при постоење на артериска хипертензија кај пациентите со покачената серумска вредност на цистатин Ц.
Воедно, бубрежна инсуфициенција може да се открие со пресметување на серумски концентрации на цистатин Ц за одредување на ГФР CKD-EPI-Cystatin C (Chronic Kidney Disease Epidemiology Collaboration-Cystatin C), кај пациенти со веќе постоечка артериска хипертензија. (https://www.mdcalc.com/calc/3939/ckd-epi-equations-glomerular-filtration-rate-gfrweb).

Клучни зборови: хронична бубрежна болест, цистатин Ц, артериска хипертензија.

Introduction

Hypertension, as a public health problem, is currently a leading factor in the global burden of disease. It is the main modifiable risk factor for heart disease, stroke, and kidney failure [1].
Chronic kidney disease (CKD) is a common cause of hypertension and is also a complication of uncontrolled hypertension. Standardized blood pressure measurement is essential for establishing the diagnosis and management of hypertension, which is one of the main causes of CKD [2]. Table 1 we got values for arterial tension.
The use of ambulatory blood pressure monitoring provides additional assessment of diurnal blood pressure variations. The optimal target blood pressure value in the treatment of hypertension in the general and CKD population remains a matter of debate and is controversial despite recent guidelines and clinical trial data [3].
Medical therapy of patients with hypertension can be difficult and challenging. Further evaluation by a hypertension specialist may be required in the setting of treatment-resistant hypertension to exclude pseudoresistance as well as treatable secondary causes [4].
Treatment with a combination of antihypertensive drugs, including appropriate choice of diuretics based on estimated glomerular filtration rate, is a key component of hypertension management.
In addition to drug treatment, non-pharmacological approaches, including lifestyle modification, the most important of which is dietary salt restriction, should be included in the management of hypertension in patients with CKD.
Serum cystatin C levels are a measure of renal function that appears to be independent of age, sex, and lean muscle mass [5]. There are studies examining the association between serum cystatin C levels and hypertension, in which a positive association was found [6]. However, more studies in humans are needed to confirm the consistency of this finding.

Table 1. Values for arterial tension

Blood Pressure Category	Systolic Blood Pressure in mmHg		Diastolic Blood Pressure in mmHg
Normal Value	Less than 120	and	Less than 80
Elevated Value	120-129	and	Less than 80
Elevated Value (Hypertension Stage 1)	130-139	or	80-89
Elevated Value (Hypertension Stage 2)	140 or higher	or	90 or higher
Hypertensive Crisis (when you should immediately consult a doctor)	Higher than 180	and/or	higher than 120

Cystatin C is a low molecular weight protein that functions as an extracellular inhibitor of cysteine proteases [7]. The kidney plays a major role in the metabolism of low molecular weight plasma proteins and it is assumed that their serum concentrations may reflect changes in glomerular filtration rate (GFR).
Produced at a steady rate by all nucleated cells and released into the bloodstream, cystatin C is freely filtered by the renal glomeruli and subsequently metabolized in the proximal tubule. Given these characteristics, cystatin C has been proposed as a sensitive endogenous serum marker for changes in GFR [8-10].
In a meta-analysis [8], serum cystatin C concentration was clearly superior to serum creatinine concentration as a marker of GFR. Interestingly, cystatin C concentration offers improved clinical sensitivity as a screening test for early renal damage [10,11].
Chronic kidney disease (CKD) is an increasingly prevalent condition globally and is strongly associated with incident cardiovascular disease (CVD) [12]. Con-

trol of hypertension is important in CKD patients, as it leads to slowing of disease progression [13,14]. Patients with CKD and hypertension often require a combination of antihypertensive drugs to achieve target BP [15]. Table 2 we got stages of CKD.

Certain pharmacological therapies provide an additional renoprotective and/or cardioprotective effect independent of BP and this must be taken into account when initiating therapy [16].

Table 2. Stages of kidney damage

Stages of Chronic Kidney Disease (CKD)	Description	GFR	Kidney Function in Percentage (%)
Stage 1	Kidney damage with preserved kidney function	90 or higher	90-100%
Stage 2	Kidney damage with mild dysfunction	80-60	89-60%
Stage 3a	Kidney damage with mild to moderate dysfunction	59-45	59-45%
Stage 3b	Kidney damage with moderate to severe dysfunction	44-30	44-30%
Stage 4	Kidney damage with severe dysfunction	29-15	29-15%
Stage 5	End-stage kidney damage	>15	>15%

Aim. The aim of this study was to evaluate the possibility of obtaining an elevated serum cystatin C level in the presence of arterial hypertension.

Material and methods

Study design: observational prospective study.
The study included 28 patients, aged 18-60 years.
Patients were grouped into 2 groups: patients with arterial hypertension and healthy subjects. Each group consisted of 14 patients.
Blood pressure was measured in patients from both groups using a mercury sphygmomanometer in the sitting position after a 5-minute rest. Patients' legs were uncrossed and their feet were on the floor. The hands were placed at the level of the heart.
Patients had not previously consumed caffeine or tobacco within the last 30 minutes prior to measurement. The sphygmomanometer cuff was rapidly inflated until the first Korotkoff sound, which indicated the patient's systolic blood pressure.
The point at which the sounds disappeared was recorded as the diastolic blood pressure. Systolic and diastolic blood pressure were calculated as the mean of two measurements taken from the left and right arms obtained at 5-minute intervals. The higher-than-the-already-obtained blood pressure values measured from both arms was used for analysis. Blood pressure was measured in the morning, after which subjects took their medications for arterial hypertension.
Controlled hypertension was defined as a systolic blood pressure <140 mm Hg and a normal diastolic blood pressure <90 mm Hg under treatment with antihypertensive drugs.
Uncontrolled hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg under treatment with antihypertensive drugs.
Serum cystatin C levels were measured from venous blood samples. All analyses were performed in a central laboratory.

Results

Patients were divided into two main groups: patients with arterial hypertension and a control group of healthy individuals (Table 3). (Table 3 obtained results).

- In the group with arterial hypertension, aged 18-29 years, there were 5 male and 2 female patients. In comparison, the control group had an equal distribution by gender in the same age group. Both groups, patients with arterial hypertension and control group of healthy subjects, had an equal number of female and male subjects.
- In patients with arterial hypertension, the mean systolic blood pressure for males was 157.5 mm Hg, and the mean diastolic blood pressure was 99.28 mm Hg. The mean GFR in this groupwas 85.4 ml/min/1.73 m^2.
- In healthy subjects, the mean systolic blood pressure for males was 121.7 mm Hg, and the mean diastolic blood pressure was 82.5 mm Hg. The mean GFR in this group was 129.071 ml/min/1.73m^2.

The mean cystatin C level in patients with arterial hypertension was 1.6 mg/L, while in healthy subjects, the control group, it was 0.54 mg/L.

Table 3. Obtained results (abbreviations: M-male; F-female)

	Gender	Blood Pressure	Cystatin C	GFR	CKD Stage	Blood Pressure	Cystatin C	GFR	CKD Stage
1	M	147/95	1.12	79	stage 2	125/82	0.55	148	stage 1
2	M	145/97	1.13	77	stage 2	120/80	0.57	145	stage 1
3	M	150/97	0.99	92	stage 1	127/83	0.55	147	stage 1
4	M	148/100	0.92	100	stage 1	120/80	0.58	142	stage 1
5	M	160/100	1.20	70	stage 2	126/80	0.52	149	stage 1
6	F	145/105	1.10	76	stage 2	125/85	0.52	143	stage 1
7	F	155/97	0.90	94	stage 1	120/80	0.53	136	stage 1
8	F	160/95	1.12	70	stage 2	125/86	0.56	131	stage 1
9	F	155/97	1.12	68	stage 2	120/87	0.55	128	stage 1
10	F	180/100	1.20	60	stage 2	115/80	0.56	124	stage 1
11	F	175/105	0.90	87	stage 1	114/85	0.53	126	stage 1
12	M	167/102	1.13	69	stage 2	123/83	0.57	129	stage 1
13	M	168/105	1.14	67	stage 2	120/85	0.55	129	stage 1
14	M	150/95	0.92	87	stage 2	125/80	0,52	130	stage 1

Discussion

Hypertension and chronic kidney disease are emerging as a global health problem, contributing significantly to high mortality [17]. Hypertension is one of the main causes of chronic kidney disease, and conversely, chronic kidney disease can contribute to the development of hypertension. Cystatin C is an important marker, which can indicate the presence of hypertension, as well as can help in early detection of chronic kidney disease [18-20].

The study aimed to demonstrate the association of uncontrolled arterial hypertension and higher serum cystatin C values. At the same time, in patients with arterial hypertension, as a risk for CKD, monitoring GFR alongside cystatin C levels may help predict the development of CKD-which requires further investigations. Therefore, blood pressure control has been established as a primary strategy for the secondary prevention of CKD [21].

This study showed that serum cystatin C levels in patients with uncontrolled arterial hypertension were higher than those in the control group of healthy individuals. A few previous studies have reported an association between serum cystatin C levels and hypertension [22-24].

A high serum cystatin C level and uncontrolled hypertension can be suggested as a manifestation of microvascular damage to the kidneys, and can lead to development of CKD.

Screening should be focused on high-risk patients, such as those with uncontrolled hypertension. The results of this study indicate the need to increase awareness of subclinical renal dysfunction in hypertensive patients [28].

Conclusion

There is an association between arterial hypertension in patients with an elevated serum cystatin C level.

Renal failure can be detected by calculating serum concentrations of cystatin C to determine the GFR CKD-EPI-Cystatin C in patients with pre-existing arterial hypertension.

Reduced renal function based on serum cystatin C in the presence of uncontrolled hypertension indicates the need to intensify antihypertensive treatment with agents that show a preventive effect.

Conflict of interests: None declared.

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Original article

BULLOUS PEMPHIGOID IN NORTH MACEDONIA: ANALYSIS OF EPIDEMIOLOGICAL CHARACTERISTICS AND COMORBIDITIES

БУЛОЗЕН ПЕМФИГОИД ВО СЕВЕРНА МАКЕДОНИЈА: АНАЛИЗА НА ЕПИДЕМИОЛОШКИТЕ КАРАКТЕРИСТИКИ И КОМОРБИДИТЕТИ

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Abstract

Introduction. Bullous pemphigoid (BP) is the most common autoimmune subepidermal bullous dermatosis, primarily affecting the elderly population. The prevalence of bullous pemphigoid increases significantly with age, and its mortality rate is higher compared to the general population in the same age group. Despite its significance, the global prevalence and comorbidities associated with BP are not fully understood.
Methods. This retrospective study analyzed BP patient data from North Macedonia, focusing on age at diagnosis, comorbidities, and the female-to-male ratio. The study found that the mean age at BP onset in patients was 77.09, with a female-to-male ratio of 1.02.
Results. As for the comorbidities, the most commonly observed ones, in decreasing order of frequency, were hypertension, neurological disease, cataracts, psychiatric disorders, cerebrovascular accidents, and diabetes mellitus.
Conclusion. These findings reflect global trends, suggesting that BP treatment should consider both age and comorbidity profiles to improve patient outcomes.

Keywords: bullous, comorbidities, epidemiology, pemphigoid

Апстракт

Вовед. Булозен пемфигоид (БП) е најчестата автоимуна субепидермална булозна дерматоза, која првенствено ја погодува постарата популација. Преваленцата на булозниот пемфигоид значително се зголемува со возраста и стапката на смртност е повисока во споредба со општата популација од истата возрасна група. И покрај неговото значење, глобалната преваленца и коморбидитетите поврзани со БП не се целосно разбрани.
Методи. Оваа ретроспективна студија ги анализираше податоците за пациентите со БП од Северна Македонија, фокусирајќи се на возраста при дијагностицирање, коморбидитети и односот помеѓу жени и мажи.
Резултати. Студијата покажа дека средната возраст на појава на БП е 77,09 години, со сооднос помеѓу жени и мажи од 1,02. Што се однесува до коморбидитетите, најчесто забележани, по редослед на намалување, беа хипертензија, невролошки заболувања, катаракта, психијатриски нарушувања, цереброваскуларни несреќи и дијабетес мелитус.
Заклучок. Овие наоди ги одразуваат глобалните трендови, сугерирајќи дека третманот со БП треба да ги земе предвид и возраста и профилите на коморбидитет за да ги подобри исходите на пациентот.

Клучни зборови: булозен, коморбидитети, епидемиологија, пемфигоид

Introduction

Bullous pemphigoid (BP) is the most common autoimmune subepidermal bullous dermatosis, primarily affecting the elderly population. BP is caused by autoantibodies targeting the hemidesmosomes, which leads to the formation of subepidermal blisters. It is characterized by the development of large, tense blisters. The prevalence of bullous pemphigoid increases significantly with age, and its mortality rate is higher compared to the general population in the same age group. The prevalence of BP is estimated to range from 1.46 to 47.99 cases per 100,000 people, with differences depending on geographical location and age group. The highest prevalence was reported in the UK, with 47.99 cases per 100,000 people annually [1], while the lowest prevalence was observed in Romania, with 1.46 cases per 100,000 people per year [2]. The

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global average prevalence was 21.84 cases per 100.000 people [3]. The reported incidence of BP ranged from 0.21 to 7.63 cases per 100,000 people. Kuwait had the lowest incidence, with 0.21 cases per 100,000 people per year [4], while the highest incidence was observed in the UK, with 7.63 cases per 100,000 people annually [1]. The global average incidence was 2 cases per 100,000 people per year [3]. The mean age at BP onset in patients ranged from 64 [5] to 82.6 years [6], with an average of 73.4 years [3]. The female-to-male ratios in BP ranged from 1.01 to 5.1. Finland had the lowest ratio at 1.01 [7], while Kuwait reported the highest at 5.75 [4]. The global average female-to-male ratio was 1.87 [3].

As the incidence of BP increases with age, patients often experience a range of comorbidities. Patients with BP often present with various comorbidities that can complicate disease management. The most frequently observed comorbidities include hypertension, neurological disorders, and psychiatric conditions, which are commonly seen in elderly populations [8,9]. Hypertension, for instance, is highly prevalent among BP patients, likely exacerbated by the use of corticosteroid therapy, which is a cornerstone of BP treatment [10]. Comorbidities such as diabetes mellitus, osteoporosis, and malignancies have been documented in BP patients, with these conditions further complicating both diagnosis and treatment of the disease [11].

The aim of this study was to determine the mean age at diagnosis, the female-to-male ratio, and the comorbidities in all bullous patients, as well as separately for male and female patient groups, in individuals with bullous pemphigoid in North Macedonia.

Materials and methods

A cross-sectional, observational, descriptive study was conducted based on the analysis of patients' medical records with confirmed BP. The study included 105 patients consecutively referred for the diagnosis and treatment of BP at the University Clinic for Dermatology in Skopje between January 1, 2015, and December 31, 2020. The diagnosis of BP was established based on typical clinical findings, as well as histopathological and immunopathological criteria. Data about comorbidities of patients with confirmed BP were extracted from their electronic healthcare records (EHR) in the electronic database of the National System for Electronic Health Records [12]. Demographic and clinical data on BP and comorbidities were collected for all patients. The study was approved by the Ethics Committee of the Faculty of Medicine, Ss. Cyril and Methodius University in Skopje and the University Clinic for Dermatology, Skopje.

Statistical Analysis

Data analysis was performed with the SPSS program. The female-to-male ratio and the mean age at diagnoses and comorbidities, both overall and separately in the female and male patient groups, were determined. The comorbidities were analyzed separately for both genders.

Results

A total of 105 patients were included in this study. There were 53 female patients and 52 male patients. The female-to-male ratio was 1.02. The average age at diagnosis was 77.09 years (range, 39-98 years). As for the comorbidities, the most commonly observed ones, in decreasing order of frequency, were hypertension (HTA), neurological disease, cataracts, psychiatric disorders, cerebrovascular accidents, and diabetes mellitus. The other comorbidities, also in decreasing order but at much lower percentages, were thyroiditis, benign prostatic hyperplasia (BPH), osteoporosis, chronic obstructive pulmonary disease (COPD), malignancies, renal insufficiency and hematological diseases (Table 1).

Table 1. Comorbidities among patients with bullous pemphigoid

Variables	N	N (%)
Hypertension	71	67.62
Neurological diseases	48	45.71
Cataracts	43	40.95
Psychiatric disorders	32	30.48
Cerebrovascular accidents (strokes)	30	28.57
Type 2 Diabetes Mellitus	29	27.62
Thyroid disease	9	8.57
Benign prostatic hyperplasia	6	5.71
Osteoporosis	5	4.76
Chronic obstructive pulmonary disease (COPD)	5	4.76
Glaucoma	3	2.86
Malignancies (cancers)	2	1.90
Renal insufficiency	1	0.95
Hematological diseases	1	0.95

The most frequent neurological comorbidity was dementia, with 28(26.67%) cases, followed by Alzheimer's disease with 12(11.43%) cases, Parkinson's disease with 5(4.76%) cases, epilepsy with 2(1.90%) cases and myasthenia gravis with 1(0.95%) case.

In addition, 32 patients (30.48%) were diagnosed with a psychiatric condition, including 31(29.52%) with anxiety-depressive disorder and 1(0.95%) with schizophrenia.

All 9 patients with thyroid disease had hypothyroidism. Malignancy as a comorbid condition was present in 2 patients, specifically lung cancer and bladder cancer. The hematological disease diagnosed in 1 patient was idiopathic thrombocytopenia.

Fifty-three female patients were included in this study. The average age at diagnosis was 76.36 years; the oldest patient was 98 years old and the youngest was 39 years old. The most commonly observed comorbidities

in female patients, in decreasing order of frequency, were hypertension, neurological diseases, psychiatric disorders, cataracts, diabetes mellitus, cerebrovascular accidents, hypothyroidism, osteoporosis, and malignancy. Nine of the female patients had no comorbid conditions. Fifty-two of the patients were male. The average age at diagnosis for male patients was 75.19 years; the oldest was 91 years old and the youngest was 55 years old. The most common comorbid diseases, in decreasing order of frequency, were hypertension, cataracts, cerebrovascular accidents (CVA), neurological disorders, psychiatric disorders, diabetes mellitus, benign prostatic hyperplasia (BPH), chronic obstructive pulmonary disease (COPD), chronic kidney disease, hypothyroidism, osteoporosis, and hematological conditions such as idiopathic thrombocytopenia. Eleven of the male patients had no comorbid conditions.
Table 2 and Table 3 show the comorbid diseases observed in the female and male patient groups,respectively.

Table 2. Comorbidities among female patients with bullous pemphigoid

Comorbidities		N (%)
Hypertension (HTA)		38 (20.14)
Neurological disorders	Dementia – 16 (8.48) Alzheimer's – 10 (5.3) Parkinson's – 2 (1.06) Epilepsy - 1 (0.53)	29 (15.37)
Psychiatric disorders	Anxiety-depressive disorder	21 (11.13)
Cataracts		19 (10.07)
Type 2 Diabetes Mellitus (DM2)		18 (9.54)
Cerebrovascular accidents (CVA)		10 (5.3)
Thyroid disease		8 (4.24)
Osteoporosis		4 (2.12)
Chronic obstructive pulmonary disease (COPD)		3 (1.59)
Cancer (Ca)	Bladder cancer – 1 (0.53) Lung cancer -1 (0.53)	2 (1.06)
Glaucoma		1 (0.53)

Table 3. Comorbidities among male patients with bullous pemphigoid

Comorbidities		N (%)
Hypertension (HTA)		33 (17,16)
Cataracts		24 (12.48)
Cerebrovascular accidents (CVA)		20 (10.4)
Neurological disorders	Dementia –12 (6.24) Parkinson's – 3 (1.56) Alzheimer's – 2 (1.04) Epilepsy - 1 (0.52) Myasthenia gravis -1 (0.52)	19 (9.88)
Psychiatric disorders	Anxiety-depressive disorder-10(5.2) Schizophrenia -1 (0.52)	11(5.72)
Type 2 Diabetes Mellitus (DM2)		11 (5.72)
Benign prostatic hyperplasia (BPH)		6 (3.12)
Chronic obstructive pulmonary disease (COPD)		2 (1.04)
Glaucoma		2 (1.04)
Thyroid disease		1 (0. 52)
Osteoporosis		1 (0. 52)
Renal insufficiency		1 (0.52)
Hematological diseases		1 (0.52)

Discussion

A total of 105 patients were included in this study. The female-to-male ratio was 1.02. This study revealed similar results to most previous studies conducted in different countries worldwide. In the extensive study on the epidemiological characteristics of BP in different countries worldwide that conducted such research between 1992 and 2021, the countries were divided into 3 categories: countries with a female-to-male ratio >1-1.49, 1.5-2.49, and >2.5.
Our results are similar to female-to-male ratios in the USA [13], Finland [7], Turkey [14], Italy [15], Switzerland [16] and Poland [17]. They all have a female-to-male ratio between >1 and 1.49. Countries with a female-to-male ratio between 1.5 and 2.49 are: England [18], Germany [19], Romania [2] and France [6]. Kuwait is

a country with a female-to-male ratio of ≥ 2.5[4,20]. The mean female-to-male ratio worldwide is 1.87 [3].
Finland had the lowest female to male gender ratio of 1.01 [7], while two separate studies in Kuwait reported the highest gender ratios of 5.1 [20] and 5.75 [4]. Overall, BP, like the majority of autoimmune diseases, show a consistent bias towards females. There are several theories that attempt to explain this gender bias, including the effects of the menstrual cycle, hormonal influences on the microbiome, and immune-related genes located on the X-chromosome [21]. However, the exact mechanisms behind the higher incidence of autoimmune blistering diseases in females, and autoimmunity in general, remain unclear.
The average age of patients at diagnosis in our study was found to be 77.09 years (range, 39-98 years) for both genders. Globally, the age of onset ranges from 64 [5] to 82.6 [6] years, with an average of 73.4 years [3]. The majority of countries in the world where extensive research on the age of onset of BP has been conducted fall into the category of over 75 years, which aligns with the results of our study. The remaining countries in this age group are: UK [18], France [6], Finland [7], Switzerland [16] and Israel [22]. Countries with an estimated age of onset of BP between 70-75 years are: the USA [13], Germany [19], Romania [2], Turkey [5]. The average age of onset of BP before 70 years has been reported in: Kuwait [20], India [23] and Tunisia [24]. Potential explanations for the generally late onset of the majority of autoimmune conditions, particularly BP, include the erosion of immune tolerance over time, and an accumulating environmental burden that evolve a patient's "umwelt" in later years.
Serwin *et al.* [17] reported an average age at diagnosis of 68.9 years for female patients and 67.3 years for male patients, while Aşkın Ö *et al.* [14] found it to be 74.94 years for females and 72.17 years for males. Findings from these studies are similar to our study, where the average age at diagnosis was lower in male patients. Jung *et al.* [25], reported an average age at diagnosis of 73.7 years for females and 76.1 years for males. Unlike our results, which showed a younger age at diagnosis in males, the study by Jung *et al*. found females were diagnosed at a younger age, although their age averages were closer to ours.
In our study, the most commonly observed comorbidity in bullous pemphigoid patients was hypertension, found in 71 patients (67.62%). Other comorbidities, excluding neurological disorders (45.71%), psychiatric disorders (30.48%), and cerebrovascular accident/ stroke (28.57%), which were the most frequently observed after hypertension, included: diabetes mellitus in 29 patients (27.62%), thyroid disease in 9 patients (8.57%), benign prostatic hyperplasia in 6 patients (5.71%), osteoporosis in 5 patients (4.76%), chronic obstructive pulmonary disease (COPD) in 5 patients (4.76%), malignancies in 2 patients (1.90%), renal insufficiency in 1 patient (0.95%), and hematological disease (idiopathic thrombocytopenia) in 1 patient (0.95%).
These comorbidities - arterial hypertension, diabetes mellitus, cardiac diseases, and renal impairment - have been associated with BP in many other studies [26-29]. Cardiovascular diseases, including hypertension and coronary artery diseases, were reported to co-exist with bullous pemphigoid in38% to 70% cases [30].
In a case-control study by Lee *et al.*, BP was significantly associated with hypertension (67.0%), diabetes mellitus (36.3%), chronic kidney disease (16.5%), end-stage renal disease (7.7%), and osteoporosis (12.1%). The results were very similar to our findings [27]. Also, in a Turkish study, the most commonly observed comorbidity in BP was hypertension, present in 26 patients (45%), then diabetes mellitus in 14 patients (24%), chronic kidney disease in 4 patients (0.7%), and osteoporosis in 4 patients (0.7%) [14]. A study that evaluated a Finnish cohort of BP patients with an average age of 77 years also reported that the most commonly observed comorbidities in these patients were hypertension (44%), diabetes mellitus (34%), and ischemic heart disease (26%) [31].
Consistent with our findings, previous studies showed an association between BP and a significantly increased odds of having diabetes [32,33]. On the other hand, antidiabetic medications, particularly dipeptidyl peptidase-4 (DPP-4) inhibitors and metformin, have been shown to increase the risk of developing BP [34]. A Finnish registry-based study confirmed that 19.6% of patients with BP have diabetes, and the use of DPP-4 inhibitors was significantly associated with an increased risk of developing BP, while the association with metformin was not significant [35]. These findings align with a Taiwanese cohort study, which found that the cumulative incidence of BP was significantly higher in patients treated with DPP-4 inhibitors compared to those in the non-DPP-4 inhibitor control group over a 6-year period [34]. Future studies are needed to determine the relationship between treatments of diabetes and the development of BP.
The high prevalence of hypertension and diabetes in patients with bullous pemphigoid can be pathogenetically explained by inflammation.
Next to BP-230-a and BP-230-e, a third isoform named BP-230-b was mainly expressed in the myocardium and skeletal muscle of mice [36]. It seems that, similar to neurologic diseases, cross-reactivity of autoantibodies to exposed structural proteins may promote the development of BP, but evidence is still lacking.
In our study, osteoporosis was found in 5 patients (4.76%). A cross-sectional study from the USA that included 8,864 patients with BP showed an increased OR for osteoporosis and pathological fractures. Patients with long-term systemic steroid treatment were at even higher risk of osteoporosis. Possible pathogenetic links between BP and osteoporosis, next to sys-

temic steroid therapy, are chronic systemic inflammation with enhanced bone resorption, decreased physiccal activity, and hypovitaminosis D [37]. As patients with BP seem to be at higher risk of osteoporosis, pathological fractures, and hypovitaminosis D, they should be encouraged to be physically active and to substitute vitamin D if necessary.

In our study, renal insufficiency was found in 1 patient (0.95%). An association between BP and kidney disease has also been documented in the literature, where the severity of skin lesions was found to correlate with the severity of kidney disease [38,39]. Skin manifesttations of end-stage renal disease include bullous diseases [40]. Autoimmune processes or immune stimulation may be a common underlying mechanism for both skin and renal diseases.

In our study group, the frequency of neurological and psychiatric comorbidities was as follows: neurological diseases in 48 patients (45.71%), psychiatric disorders in 32 patients (30.48%) and cerebrovascular accidents in 30 patients (28.57%). The association of BP with neurologic and psychiatric diseases has been well acknowledged and confirmed by several case-control and cohort studies [11-14,50,51]. The mechanisms underlying this association are not clear, but an autoinflammatory reaction against BP180 or the neuronal isoform of BP230 in the human brain has been suggested [42,43]. Among our study group, the most commonly observed neurological comorbidities were dementia in 28 patients (26.67%), followed by Alzheimer's disease in 12 patients (11.43%), and Parkinson's disease in 5 patients (4.76%). In a literature review, it was documented that 26.4% to 55.8% of patients with BP had at least one neurological disease, compared to 9.1% to 20.5% in the control group, depending on the study criteria and design [44,45]. A Taiwanese population-based study identified stroke (36.8%), dementia (17.7%), Parkinson's disease (11.9%) and epilepsy (5.8%) as the most commonly associated neurologic diseases [46].

A German retrospective population-based study comprising 1,743 patients with BP who were matched with 10,141 controls showed a significant association with Alzheimer's disease. The same study identified a significantly increased life-time prevalence for Parkinson's disease in patients with BP compared to age-and sex-matched controls [47]. Psychiatric comorbidities were also frequently observed in our bullous pemphigoid patients, which is simular to findings in the literature. In our study, 32 patients (30.48%) were diagnosed with a psychiatric condition, including 31(29.52%) with anxiety-depressive disorder and 1(0.95%) with schizophrenia. In a case-control study from Germany, psychiatric disease was found in 20.3% of BP patients compared to 9.3% in controls [41]. A Danish register-based cohort study focusing on psychiatric disorders identified a significantly elevated risk of psychiatric disorders [48]. In patients with a diagnosed psychiatric disorder, the risk of developing BP increased with a mean latency of 14.6 years. In contrast, a recently published meta-analysis of cohort studies calculated that patients with BP exhibited no significantly increased risk for depression and schizophrenia. [49]. Another Taiwanese case-control study reported that the association of BP with schizophrenia occurred predominantly in female patients [46].

In our study, 30(28.57%) patients with bullous pemphigoid experienced cerebrovascular accidents (strokes). A Taiwanese population-based study identified stroke in 36.8% of patients with bullous pemphigoid [46]. The above-mentioned German retrospective population-based study including 1,743 patients with BP identified an increased life time prevalence for stroke [47]. In line with these results, a population-based follow-up study from Taiwan including 390 patients and 1,950 matched controls observed 89 events of stroke (22.8%) in patients with BP compared to 223 events (11.4%) in controls during a 3-year follow-up period [50].

Thyroid disease in our study was found in 9 patients (8.57%): 8 females and 1 male. All 9 patients had hypothyroidism. Several case reports of patients with autoimmune thyroid diseases who later developed BP have been published, suggesting an association between these autoimmune disorders [51,52]. Recently, a significant association between thyroiditis and BP has been reported [47]. An Italian hospital-based study observed significantly higher anti-TPO autoantibody levels in patients with BP [53].

In our study, malignancy as a comorbid condition was present in 2 patients, specifically lung cancer and bladder cancer. The association between BP and malignancy has not been conclusively proven or disproven. In 2021, a Turkish case-control study with 145 BP patients showed higher rates of malignancies compared to controls. The type of malignancy was not reported, but skin cancers were excluded, and subgroup analysis found men to be at higher risk than women [54]. In contrast, many studies and a meta-analysis observed no significantly increased overall risk of malignancy or cancer-related mortality in patients with BP [41,55-58]. A German cohort study observed an association between BP and hematological malignancies but not with non-hematological malignancies [59]. A population-based cohort study with an added case-control design found a 50% increased risk in patients with preexisting melanoma to develop BP [60]. Atzmony *et al*. [55] reported no significant association between bullous pemphigoid and malignancies; but a possible association between hematologic malignancies and bullous pemphigoid might have existed.

Conclusion

At present, available epidemiologic information on BP

in our country is sparse. So far, there is no registry of patients with bullous pemphigoid in our country, and hence, the incidence and prevalence of this disease remain unknown. The results of this retrospective study may contribute to the creation of such a registry. Familiarity with the possible comorbidities in patients with bullous pemphigoid will assist clinicians in managing the disease.

Conflict of interests: None declared.

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ММР

Original article

CELLULAR AND HUMORAL IMMUNITY IN PATIENTS WITH COVID-19 CORRELATED WITH SEVERITY OF CLINICAL PRESENTATION AND SARS COV-2 VACCINATION STATUS

ЦЕЛУЛАРЕН И ХУМОРАЛЕН ИМУНИТЕТ КАЈ ПАЦИЕНТИ СО ПРЕЛЕЖАН COVID-19 ВО КОРЕЛАЦИЈА СО ТЕЖИНА НА КЛИНИЧКА СЛИКА И SARS COV-2 ВАКЦИНАЛЕН СТАТУС

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Abstract

Introduction. The COVID-19 pandemic has changed life across the planet, claiming approximately 7 million lives and more than 776 million cases reported globally, leaving many people with lasting sequelae. Of particular importance is understanding the immune system's defense responses against SARS-CoV-2 infection, with an emphasis on T-lymphocyte cells that modulate both cellular and humoral part.

Aims. To determine epidemiological and clinical features, especially comorbidities in patients who have had severe and mild COVID-19, to determine differences in cellular and humoral immune response and to correlate with vaccination status for SARS CoV-2.

Methods. A 6-month-prospective cohort study was conducted at the University Clinic for Pulmonology and Allergology and the Institute of Immunobiology and Human Genetics at the Faculty of Medicine - Skopje. The study included 88 patients with pre-existing COVID-19 divided into two groups: patients with mild clinical manifestations and patients with severe clinical manifestations requiring hospitalization. The parameters of patients' medical history, number and type of comorbidities, parameters of cellular and humoral immune response, data on vaccination against SARS-CoV-2 were analyzed.

Conclusion. The severity of the clinical presentation correlated directly with the number of comorbidities, and inversely with the vaccination status. Comorbidities were present in 87.7% of patients with a severe clinical course. No correlation was found with the smoking status. The study showed that 97.72% of all patients had positive neutralizing antibodies for SARS-CoV-2. Positive cellular immunity had 54.55% of patients, significantly higher in the group with severe COVID-19 and vaccinated patients. There was a positive correlation between cellular and humoral immunity, but in 2 cases (4.16%) where the humoral response was absent, a positive cellular response was verified.

Keywords: COVID 19, SARS CoV-2, cellular and humoral immunity, vaccination

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Апстракт

Вовед. Пандемијата на COVID-19 го смени животот на целата планета, однесе приближно 7 милиони животи и повеќе од 776 милиони случаи пријавени на глобално ниво, а многу луѓе останаа со трајни секвели. Сеуште постои голем научен интерес како да ја спречиме, контролираме и излечиме оваа болест. Особено е важно разбирањето на одбрамбените одговори од страна на имунолошкиот систем против инфекцијата со SARS-CoV-2, со акцент на Т-лимфоцитните клетки кои го модулираат специфичниот имунолошки одговор, вклучувајќи го целуларниот и хуморалниот дел.

Цели. Да се одредат епидемиолошките и клиничките карактеристики, особено коморбидитетите кај пациентите кои прележале тежок и лесен COVID 19, да се одредат разликите во целуларниот и хуморалниот имунолошки одговор кај различните групи пациентите и да се корелираат со вакциналниот статус за SARS CoV- 2.

Методи. Проспективна кохортна студија во времетраење од 6 месеци реализирана на ЈЗУ Универзитетска клиника за пулмологија и алергологија и Институтот за имунобиологија и хумана генетика при Медицински факултет - Скопје. Во студијата беа вклучени 88 пациенти со наполнети 18 години

и постари, со прележан COVID 19 кои се поделени во две групи: лесна клиничка манифестација и пациенти со тешка клиничка манифестација и хоспитализација. Анализирани се параметрите од медицинската историја на болните, бројот и типот на коморбидитети, параметри за целуларен и хуморален имунолошки одговор, податок за вакцинација против SARS-CoV-2.
Заклучок. Тежината на клиничката слика корелира правопропорционално со бројот на коморбидитети, а обратнопропорционално со вакциналниот статус. Кај 87,7% од пациентите со тешка клиничка слика се застапени коморбидитети. Не се најде корелација со пушачкиот статус и тежината на клиничката слика. Студијата покажа дека 97,72% од сите пациенти имаа позитивни неутрализирачки антитела за SARS-CoV-2, со изразито висок титар над референтните вредности. Позитивен целуларен имунитет имаа 54,55% пациенти, со сигнификантно поголем опфат во групата со тежок COVID-19 и вакцинираните пациенти. Постои позитивна корелација помеѓу целуларниот и хуморалниот имунитет, но во 2 случаи (4,16%) каде изостана хуморалниот одговор верификуван е позитивен целуларен одговор.

Клучни зборови: COVID 19, SARS-CoV-2, целуларен и хуморален имунитет, вакцинација

Introduction

Coronavirus infectious disease 2019 (COVID-19) was declared a global pandemic by the World Health Organization in March 2020 and is still present in human pathology. While symptoms of COVID-19 in the majority of infected children and young adults are mild [1], severe illness occurs in approximately 13% of the population with mortality exceeding 7 million [2]. Serious risk factors for increased mortality rates in adults and immunocompromised individuals include: obesity, chronic lung disease, diabetes, organ transplantation, and cardiovascular comorbidities, as well as male sex and older age [3-8]. Despite being initially described as a respiratory disease, COVID-19 has been observed to affect other organs and systems over time, such as the cardiovascular system, central nervous system, kidneys, liver, thyroid, etc. [3-5].
In scientific aspect, the enigma of diversity in the course of disease still remains. Understanding the immune system's defensive responses against SARS-CoV-2 infection is of particular importance. The immune response involves the pro- and anti-inflammatory cells of the immune system, particularly T lymphocyte cells that modulate the specific immune response, including cellular and humoral immunity, the activity of natural killer cells, the antiviral action of interferon, and the proliferation of T and B lymphocytes [9,10]. In healthy subjects, there is a correlation and coordination in the cascading immune response that occurs with viral infection. Antiviral immunity generally consists of neutralizing antibodies that block viral infection and cytotoxic CD8+ T cells, which eliminate cells infected with the virus. There is compelling evidence regarding the role of neutralizing antibodies in the protective immune response to SARS-CoV-2 infection [11]. However, the role of CD4+ and CD8+ T cells after viral entry is complex and requires comprehensive analysis. SARS-CoV-2 induces unrestricted generation and release of various mediators and cytokines in the bloodstream. A consequence of the action of inflammatory mediators is systemic inflammation, dysregulation of innate and acquired immunity responses, and further infiltration of various immune effector cells into various tissues. Numerous scientific publications have shown that the overexpression of cytokines and an exaggerated immune response ultimately cause organ dysfunction and cytotoxicity [9,12]. Jouan and Wang, and numerous other authors have suggested that an inadequate immune response lies behind the pathogenesis of ARDS [13,14]. According to Zhang *et al*., both genetic and acquired factors clearly demonstrate the critical role of effective interferon signaling during acute infection. Severe clinical outcomes are characterized by a slow decline in viral load and early and persistent inflammation with elevated interferon (IFN)-α, TNF, and IFN-γ [15]. T cell responses develop early and are correlated with protection, but in severe disease, they are relatively impaired and are associated with intense activation and lymphopenia [16]. Current evidence suggests that SARS-CoV-2-specific T cell responses are essential for viral clearance, can prevent infection without seroconversion, provide strong memory, and mediate the identification of viral variants. They also increase after vaccination, providing excellent protection against severe infection and death [17]. Antibody responses are highly effective in clinical care, and their analysis is facilitated by relatively easy detection and evaluation [18,19]. The immune response of an organism exposed to SARS-CoV-2 infection remains a topic of considerable scientific and professional interest.

Aims of the study

1. To determine the epidemiological, clinical, and pathological features of COVID-19 patients with severe clinical manifestations requiring hospitalization.
2. To determine epidemiological, clinical, and pathological features in patients who have had COVID-19 with mild clinical manifestations, or asymptomatic patients who have tested positive for SARS-CoV-2.

3. To determine differences in cellular immune response in patients with severe COVID-19 and those with mild or asymptomatic clinical manifestations.
4. To determine differences in humoral immune response in patients with severe COVID-19 and those with mild or asymptomatic clinical manifestations.
5. To correlate clinical, pathological and immunological characteristics with the vaccination status of patients for SARS CoV-2 virus.

Material and methods

Material

The study was performed at the University Clinic for Pulmonology and Allergology and the Institute of Immunobiology and Human Genetics at the Faculty of Medicine-Skopje. Eighty-eight patients were enrolled, aged 18 years or older, with previous COVID-19, examined and/or treated at the University Clinic for Pulmonology and Allergology-Skopje. All patients provided voluntarily and handwritten informed consent to participate in the study. Patients were divided into groups according to the following criteria: Group A: COVID-19 patients with severe clinical manifestations for which hospitalization was required; Group B: Patients who have had COVID-19 with mild clinical manifestations, or asymptomatic patients who have tested positive for SARS-CoV-2. Exclusion criteria in the study were: patients with malignant diseases, psychiatric patients, prisoners, pregnant women, individuals under 18 years of age, patients who are not legally capable of giving informed consent.

Methods

This was a prospective cohort study conducted over a period of 6 months. The parameters of patients' medical history, epidemiological data - gender, age, smoking status, data on COVID-19 recoveries and severity of illness, number and type of comorbidities, data on SARS-CoV-2 vaccination - number of doses and type of vaccine, parameters of cellular and humoral immune response to SARS-CoV-2 were analyzed.
Each patient's blood was taken for standard biochemical and immunological analyses, as follows:

- For cellular immunity using the *QuantiFERON* SARS-CoV-2 method - enzyme immune method ELISA (Enzyme-linked immunosorbent assay), which allows measuring the generated IFN-γ of CD4+ and CD8+ T cells in plasma samples stimulated by SARS-CoV-2 antigens - antigen 1 (CD4+ T cell epitopes from the S1 subunit RBD of the S protein from SARS-CoV-2), antigen 2 (CD4+ and CD8+ T cell epitopes from the S1 and S2 units of the S protein from SARS-CoV-2).
- For humoral immunity (IgG for SARS CoV-2) using an automatic chemiluminescent analytical method (CLIA).

A data collection instrument is a specially designed database, prepared according to the needs of the study, to collect sufficient data for verification of the assumeptions made. A standard descriptive statistical analysis, with determination of frequencies, was made with Microsoft Excel, 2016 and IBM SPSS Statistics, 2022.

Results

Our study included 88 patients with an equal gender distribution, 44(50%) men and 44(50%) women. Patients' age ranged from 31 to 83 years, with a mean age of 53.43 years, predominantly between 44 and 61.75 years. According to the average age, the majority belonged to the group of working population. Predominantly, or half, were non-smokers, a total of 44(50%), while 29 (32.95%) were smokers, and 15(17.05%) were former smokers. According to the severity of clinical presentation, more patients had a severe clinical course with hospitalization (group A), 49 patients (55.68%), while 39 patients (44.32%) had a mild illness or were asymptomatic (group B). Among

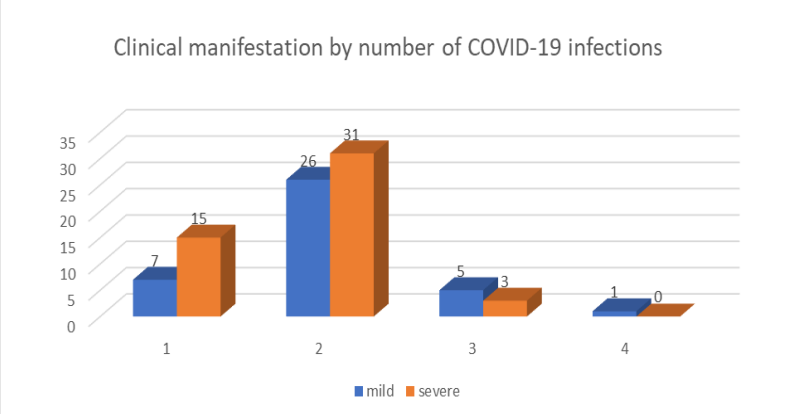

Fig. 1. Severity of clinical presentation, according to number of confirmed COVID-19 infections

those with one COVID-19 infection, 7(7.95%) pts had a mild clinical course, while 14(17.05%) had a severe illness; with two COVID-19 infections, 26(29.55%) pts had a mild, while 31(35.23%) pts had a severe clinical presentation; with three COVID-19 infections, 5 (5.68%) pts had a mild, and 3(3.41%) had a severe illness; and with four COVID-19 infections, 1(1.14%) patient had a mild illness, and there were no patients requiring hospitalization (Figure 1).

Out of the total 88 pts, 71(80.68%) were vaccinated, while 17(19.32%) were unvaccinated. Patients with mild clinical presentation had a slightly lower vaccination coverage 33(37.5%), compared to 38(43.18%) unvaccinated pts with severe clinical presentation, while 6(6.82%) unvaccinated pts had mild clinical presentation, compared to 11(12.5%) unvaccinated pts with severe clinical presentation. Of the 29 smokers, 15(51.72%) pts had a severe illness, compared to 14(48.28%) with a mild course. Of the 44 non-smokers, 26(59.09%) had a severe illness, while 18(40.91%) had a mild presentation or were asymptomatic. Of the 15 former smokers, 8(53.33%) had a severe illness with hospitalization, while 7(46.67%) had a mild clinical course.

There were 26 (29.55%) pts without comorbidities, while a total of 62(71.45%) had comorbidities, of which 14(15.91%) pts had 1 comorbidity, 20(22.73%) had 2, and 28(31.81%) had 3 or more comorbidities. Correlation of comorbidities with smoking status: among those with comorbidities, 16 (18.18%) patients were smokers, 32(36.36%) were non-smokers, and 14(15.91%) were former smokers, while among those without comorbidities, 13(14.77%) pts were smokers, 12(13.64%) were non-smokers, and 1(1.14%) was former smoker. Of the total of 26 pts without comorbidities, 19(73.07%) were in group B (mild illness), while 7(26.92%) pts had a severe clinical picture (group A). Of the total of 14 pts with 1 comorbidity, 9(64.28%) were in group A, while 5(35.71%) were in group B. Of the total of 20 pts with 2 comorbidities, 14(70%) were in group A, and 6(30%) were in group B. Of the total of 28 pts with 3 or more comorbidities, 20(71.43%) had a severe illness while 8 (28.57%) had a mild clinical course. Comorbidities were present in 4 out of the total of 49(87.7%) pts in group A (Figure 2).

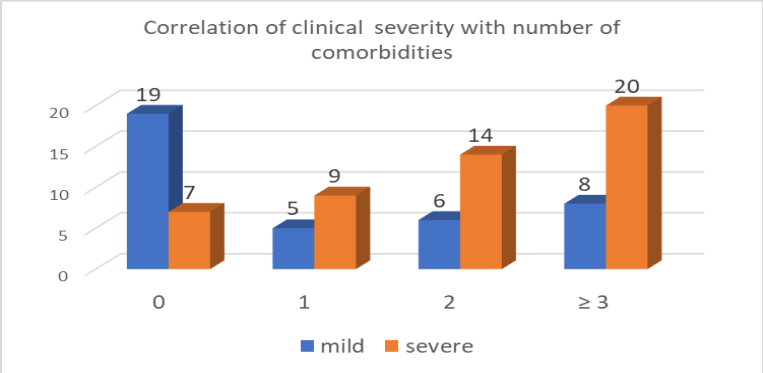

Fig. 2. Comorbidities correlated with severity of clinical presentation

Cellular immunity was determined by measuring IFN-γ produced by CD4+ and CD8+ T cells in plasma samples stimulated with SARS-CoV-2 antigens-antigen 1 (CD4+ T cell epitopes from the S1 subunit RBD of the S protein of SARS-CoV-2), antigen 2 (CD4+ and CD8+ T cell epitopes from the S1 and S2 subunits of the S protein of SARS-CoV-2) using the QuantiFERON SARS-CoV-2 method. In our study, 38(43.18%) pts

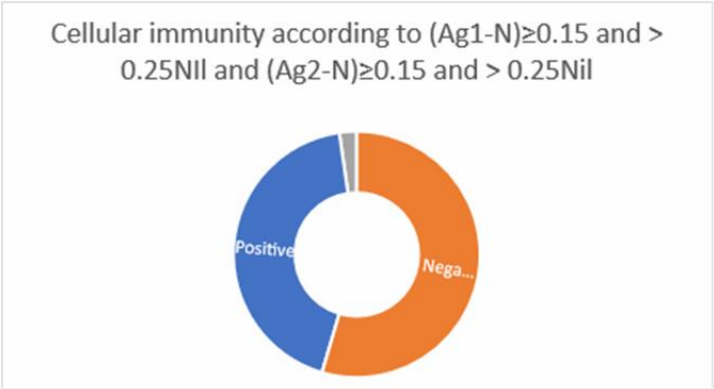

Fig. 3. Cellular immunity according to (Ag1-N) ≥0.15 and > 0.25NIl and (Ag2-N) ≥0.15 and > 0.25Nil

had negative cellular immunity, 48(54.55%) had positive cellular immunity, and 2(2.27%) had undetermined cellular immunity (Figure 3).
Correlation of cellular immunity with severity of clinical course: out of a total of 49 pts in group A (severe illness), 30(61.22%) had positive cellular immunity, 18(36.73%) had negative cellular immunity, and 1(2.04%) patient had undetermined cellular immunity. Out of a total of 39 pts in group B (mild illness), 18(46.15%) had positive cellular immunity, 20(51.28%) had negative cellular immunity, and 1(2.56%) patient had undetermined cellular immunity (Figure 4).

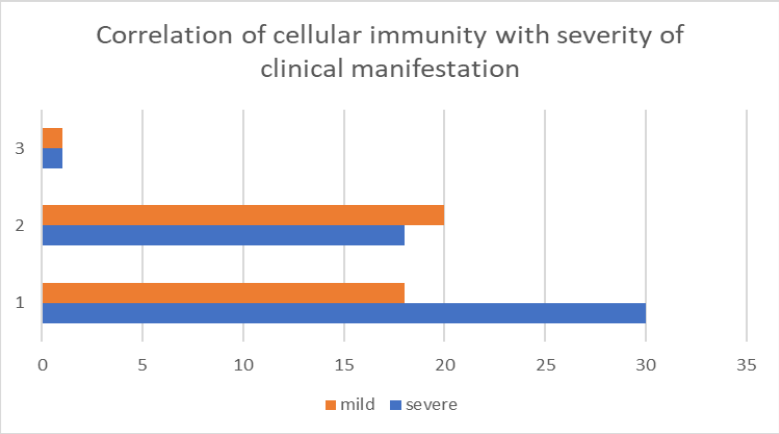

Fig. 4. Correlation of cellular immunity with severity of clinical course

Out of a total of 71 vaccinated pts, 37(52.11%) had positive cellular immunity, 33(46.48%) had negative cellular immunity, and 1(1.41%) vaccinated patient had undetermined cellular immunity. Out of 17 unvaccinated pts, 11 (64.71%) had positive cellular immunity, 5(29.41%) had negative cellular immunity, and 1(5.88%) patient had undetermined cellular immunity.
According to the titer of IgG antibodies (IU/mL) using the CLIA chemiluminescent method- reference value >1 IU/ml, antibodies were absent in only 2 pts, while the remaining 86(97.72%) pts had a positive titer. The distribution ranged from 2.28 to 2756 IU/mL, with a mean antibody value of 307.92 IU/mL. Patients with severe COVID-19 had a higher IgG antibody titer, an average of 384.06 IU/mL, while pts with mild COVID-19 had a lower average IgG antibody titer, of 228.9 IU/mL. Out of 71 vaccinated patients, 68(95.77%) had a positive IgG titer, 1(1.41%) patient had a borderline IgG titer, and 2(2.82%) pts had a negative IgG titer, while out of 17 unvaccinated patients, 14(82.35%) had a positive IgG titer, 1(5.88%) had a borderline IgG titer, and 2(2.27%) had a negative IgG titer.
Among patients with positive cellular immunity, 46(95.83%) had a positive IgG titer, 2(4.17%) had a negative IgG titer, and there were no patients with a borderline IgG titer. Among patients with negative cellular immunity, 34(89.47%) had a positive IgG titer, 2(5.26%) had a negative IgG titer, and 2(5.26%) had a borderline IgG titer. Among patients with indeterminate cellular immunity, 2(100%) had a positive IgG titer, while there were no patients with a negative and borderline IgG titer (Figure 5).

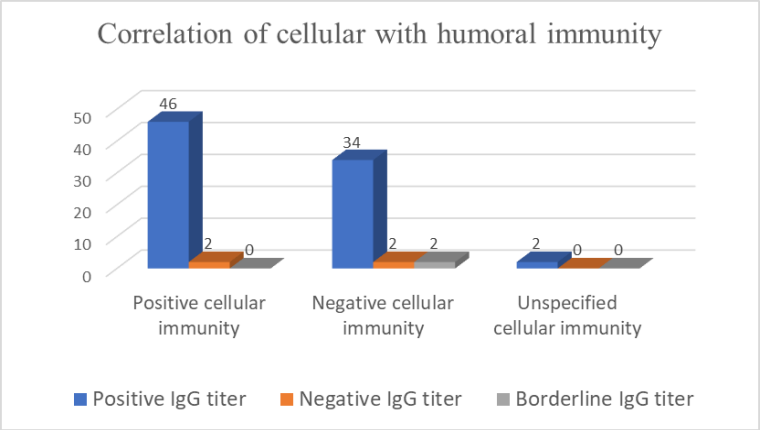

Fig. 5. Correlation of cellular with humoral immunity

The average IgG titer in pts with positive cellular immunity was 389.47 IU/mL, in pts with negative cellular immunity, it was 218.98 IU/mL, and in pts with indeterminate cellular immunity, it was 40.75 IU/mL.

Discussion

Although 5 years have passed since the first known case of COVID-19 was detected in China in November 2019, and in our country the first case was reported on February 26, 2020, the disease is still present in everyday clinical practice. Epidemiological predictions are that the disease will remain among us as a predominantly seasonal infection, with a relatively lower risk of severe clinical picture globally, but with an individually variable risk, particularly in immunocompromised individuals [20,21]. The virus has mutated over time, but the human immune system, including both humoral and cellular immunity, has enabled a more appropriate defensive response, hence, the severe clinical pictures of the past that were due to an inadequate immune response, including cytokine storms, fluid extravasation, interstitial pneumonias and ARDS [6], are now rare. According to our results and according to the available clinical studies, there is a strong relationship between comorbidities and severity of the illness; patients who were not vaccinated had a more severe clinical outcome [21,22]. Our data coincide with data from the world literature, confirming the relationship between the levels of neutralizing IgG antibodies after a severe infection, but also their protective role in mild forms of the disease [23,24]. Infection and vaccination with SARS-CoV-2 induce immune responses of both T-cells and B-cells in immunocompetent individuals. However, the mechanisms of antiviral effects mediated by CD 4+ T cells are not fully elucidated. The study by Shimizu J *et al*. showed that the inhibitory effect on viral replication was mostly attributed to interferon-γ (IFN-γ) present in the supernatant of polyclonally stimulated human CD4+ T cells. These results highlight the potential role of IFN-γ as a mediator against SARS-CoV-2 derived from CD 4+ T cells and suggest that understanding the IFN-γ-dependent susceptibility of SARS-CoV-2 is necessary in controlling clinical outcomes [25]. In addition, the characterization of new SARS-CoV-2 variants in terms of IFN-γ susceptibility will have important implications on the selection of therapeutic strategies. In our study, more than half of the patients (54.55%) had positive cellular immunity, with evidence of IFN-γ from CD4+ and CD8+ T cells in plasma samples stimulated with SARS-CoV-2 antigens. Most of the positive subjects had previously had a severe clinical picture (30 out of 48 patients, or 62.5%), significantly more than patients with a mild clinical picture where a higher percentage had negative cellular immunity (20 out of 38, or 52.63%). In two patients from the group with positive cellular immunity (4.16%), a negative IgG titer was determined, which confirms that even in the absence of a humoral response, cellular immunity has its own independent role in the human defense system. T-cell memory encompasses a broad recognition of viral proteins, estimated at about 30 epitopes in each individual. This may limit the impact of individual viral mutations and will probably underpin protection against severe disease from viral variants [26]. We were surprised that almost all subjects had exceptionally high titers of IgG neutralizing antibodies, which can be due to an immune response to the disease, to vaccination, but probably also to numerous reinfections with SARS CoV2, which were with milder and/or asymptomatic clinical manifestations, leading to a booster immune response.

The study by Simard *et al.* as well as numerous other studies support the importance of promoting vaccination in all individuals, particularly in those with pre-existing medical conditions, to reduce severe complications, even during the Omicron wave [27].

Conclusion

The severity of the clinical presentation correlates directly with the number of comorbidities, and inversely with the vaccination status. The majority of severe COVID-19 pts (87.7%) had comorbidities, 40.8% had 3 or more comorbidities, the most common being hypertension, obesity, cardiomyopathy, COPD, and diabetes mellitus. There was no significant difference in the severity of the clinical course in the vaccinated cohort, but unvaccinated patients had a greater number of severe clinical manifestations that required hospitalization. No correlation was found between smoking status and severity of the clinical picture. Patients with severe COVID-19 had a significantly higher IgG antibody titer, averaging 384.06 IU/mL, compared to 228.9 IU/mL in patients with mild COVID-19. The study showed that 97.72% of all patients had positive neutralizing antibodies for SARS-CoV-2, with a significantly high titer above the reference values. Positive cellular immunity was found in 54.55% of patients, with a significantly higher presence in the group with severe COVID-19. No significant difference was found in the response to cellular immunity in vaccinated patients with different clinical manifestations, while unvaccinated patients had a higher percentage of positive findings (11 out of 17 patients - 64.71%). There was a positive correlation between cellular and humoral immunity, but in 2 cases (4.16%) where the humoral response was absent, a positive cellular response was verified.

Conflict of interests: None declared.

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Original article

CONGENITAL DIAPHRAGMATIC HERNIA - DIAGNOSIS, TREATMENT AND FAMILY PLANNING

КОНГЕНИТАЛНА ДИЈАФРАГМАЛНА ХЕРНИЈА – ДИЈАГНОЗА, ТРЕТМАН И ПЛАНИРАЊЕ НА СЕМЕЈСТВО

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Abstract

Introduction. Congenital diaphragmatic hernia is a rare structural and genetic disorder. Early diagnosis, proper treatment and expectance of the outcome in terms of future family planning are needed.
Aim. To increase the awareness of early diagnosis and to present the current protocols for diagnosis, treatment and family planning if congenital diaphragmatic hernia occurs, particularly in early childhood.
Methods. For the purpose of this retrospective study, 20 mothers who gave birth to children with congenital diaphragmatic hernia were enrolled. They had been given questionnaires regarding the timing of their fetus's diagnosis, the treatment that followed, and the frequency of this condition in their family. Before the treatment, parents of the patients signed a consent form. Their data were collected and analyzed in the period of two years.
Results. Early diagnosis can be life saving because the baby needs to be intubated as soon as the mother gives birth. Some mothers had to plan their deliveries ahead in institutions with highly developed intensive care, with
Discussion. Early diagnosis is of crucial importance for timely preparation and treatment of both mothers and babies. ECMO treatment, to provide the best treatment protocol for better quality of life for both mother and child. In the literature the need for sterilized conditions during the treatment is described because of the possible complication such as sepsis caused by healthcare-associated infections.
Conclusion. Congenital diaphragmatic hernia is a condition for which awareness must be raised for proper diagnosis, treatment and family planning.

Keywords: congenital diaphragmatic hernia, awareness, diagnosis, treatment

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Апстракт

Вовед. Конгениталната дијафрагмална хернија е ретко структурно и генетско нарушување. Потребна е рана дијагноза, соодветен третман и очекување на исходот во однос на идното планирање на семејството.
Цел. Да се зголеми свеста за рана дијагноза и да се претстават тековните протоколи за дијагноза, третман и планирање на семејството доколку се појави конгенитална дијафрагмална хернија, особено во раното детство.
Методи. За целите на оваа ретроспективна студија беа вклучени 20 мајки кои родиле деца со конгенитална дијафрагмална хернија. Ним им беа дадени прашалници во врска со времето на дијагноза на нивниот фетус, третманот што следел и зачестеноста на оваа состојба во нивното семејство. Пред третманот, родителите на пациентите потпишале согласност. Нивните податоци беа собрани и анализирани во период од две години.
Резултати. Раната дијагноза може да спаси живот бидејќи бебето треба да се интубира веднаш штом мајката ќе се породи. Некои мајки мораа однапред да ги планираат своите породувања во институции со високо развиена интензивна нега, со ECMO третман, за да обезбедат најдобар протокол за третман за подобар квалитет на живот и на мајката и на детето.
Дискусија. Раната дијагноза е од клучно значење за навремена подготовка и третман и на мајките и на бебињата. Во литературата, потребата од стерилизирани услови за време на третманот е опишана поради можните компликации како што е сепсата предизвикана од инфекции поврзани со здравствената заштита.
Заклучок. Конгениталната дијафрагмална хернија е состојба за која мора да се подигне свеста за правилна дијагноза, третман и планирање на семејството.

Клучни зборови: конгенитална дијафрагмална хернија, свесност, дијагноза, третман

Introduction

Congenital diaphragmatic hernia (CDH) is a rare genetic medical condition that develops in the early stages of intrauterine development resulting in abnormal opening of the diaphragm, which enables the abdominal organs to enter in the thoracic cavity. The severity of the opening is highly correlated with the extent to which abdominal organs press against the lungs, thus resulting in collapse, pulmonary hypertension, dextroposition of the heart and various other changes [1].

Estimation and early diagnosis with ultrasonographic fetal screening in the first and second trimester play a significant role in planning ahead the birth of these children that will be operated in a matter of hours. According to specialized centers for fetal medicine and intrauterine treatment, fetal operations performed between 24-26 weeks of gestations have shown satisfactory prognosis before and after delivery. The planning of the delivery should be made in a highly equipped institution with developed intensive care, including Extracorporeal Membrane Oxygenation (ECMO) treatment and postsurgical monitoring. All institutions worldwide are not equipped with the necessary machines, protocols and knowledge to provide care for these patients. Parents are forced to go to institutions capable to provide the best protocol needed for their future offspring [2].

The health of the mother must be observed as a diagnostic tool as well, because some pregnant patients have difficulty breathing, decreased oxygen saturation and tachypnea due to polyhydramnios [3].

When a mother has a child with CDH, especially during her first pregnancy, she sometimes does not want future pregnancies due to the fear of facing the same risks with her other children. A pediatric geneticist is consulted to give advice to parents on family planning [4].

Aim

The aim of this study was to present congenital diaphragmatic hernia, its diagnosis, treatment and family planning and to increase the awareness about this condition. Some experiences have been presented about the occurrence and treatment of CDH, emphasizing the importance of broader education and timely treatment of pregnant patients by gynecologists, obstetricians, pediatrics, cardiologists, surgeons and other medical staff.

Material and methods

In this retrospective study 20 mothers were enrolled and questioned about their condition and the condition of their children in a two-year period. All included patients signed an informed consent for participation in the study. The mothers had given birth to 20 babies with congenital diaphragmatic hernia. They were asked about the timing of their babies' diagnosis with CDH. Also, they were questioned about the presence of increased amniotic fluid. Furthermore, an analysis was made regarding the need for incorporating ECMO as the adjuvant treatment of these children. Genetic counselling was also included into the treatment and rehabilitation protocol, which was carried out in the follow-up period when parents were considering expanding their family.

All 20 pregnant patients were diagnosed with this fetal condition and had been treated in the Clinical Center of Ljubljana. Patients did not experience any symptoms during the first two trimesters when they went to work, did their daily activities, and did not experience any medical issues. All patients had performed prenatal genetic tests, which showed that their future child was genetically normal. In the second and third trimester, pregnant women had a rapid weight increase which correlated with the increase of their amniotic fluid.

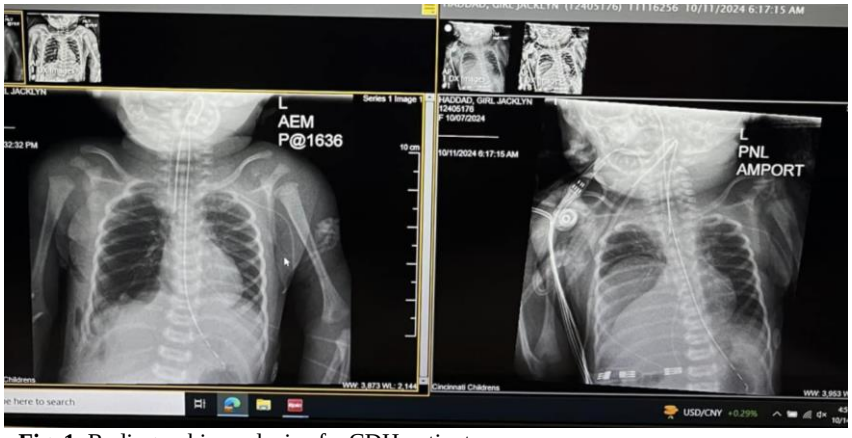

Fig. 1. Radiographic analysis of a CDH patient

This led to difficulties with their diaphragm, difficulty in breathing, sitting, sleeping during every normal physiological activity, which led to the assumption that something was wrong and made them seek medical help. During a screening exam in the beginning of the third trimester, their fetuses were diagnosed with CHD. The ultrasonographic prognostic calculator is used measuring the biparietal diameter and lung volume was promising for fetal survival.
A team of specialists comprising obstetricians, fetal cardiologists, pediatricians, surgeons and other medical staff was composed to help in the treatment protocol for the mothers and their children.
Due to the complexity of the surgery that is to be performed, the mothers must be hospitalized, a delivery plan must be established, since seconds after delivery the baby must be stabilized, intubated if necessary, and prepared for the next step-surgery as early as 24-48 hours.
After delivery besides clinical examinations of a baby, radiographic images were performed to observe the lungs before and after the surgery (Figure 1).
Five of the examined babies had a severe condition and were in need of ECMO (Figure 2).

Fig. 2. A baby with CDH attached to ECMO

After overcoming the need for ECMO, 14 babies were transferred to the semi-intensive unit postoperatively, and one baby required a longer stay in an intensive unit due to pulmonary hypertension. Each baby underwent a cardiological examination, which showed normal results. Blood and urine tests were also performed and all showed normal results.
The babies received additional care and were prepared for discharge with instructions on home disinfection, frequent appointments, and, if necessary, control radiographs of the lungs.
One of the mothers had congenital diaphragmatic hernia herself and had dominantly transmitted the condition to her daughter, but the genetic test did not show any syndromes, mutations or deletions in either of them.
After a two-year follow up, the children developed normally, with slight sensitivity regarding their lungs, with frequent respiratory infections in comparison to other children of the same age. The child with pulmonary hypertension was in need of oxygen when he was ill. Besides the scars, no other differences were observed as these children aged.
Advices from the pediatrician geneticist were welcomed throughout the entire period, before, during and after surgery.
On regular postpartum visits, the mothers did not have any significant problems related to the previous presence of polyhydramnios.

Results

This retrospective study analyzed the diagnosis of CDH, its therapy, the condition of the mothers, and the two-year outcome of the treatment protocol.
Timely diagnosis of CDH is of great importance for both the mothers and their babies. Sometimes increased levels of amniotic fluid help for therapists to suspect that there is a hidden problem.
Due to the need for precise treatment protocols, these babies must be referred to highly equipped institutions with very developed equipment and skilled medical staff.
Patients needed to be informed about the risks of prolonging that pregnancy and risks after delivery regarding survival and future of their offspring. Since the pandemic, these patients communicate more through social media, exchanging valuable information about their condition, expectations and outcomes. Clinical examinations, such as radiographs of the thorax, showed successful shift of the abdominal organs where they belong, and placement of the heart to the left. All 100% of the babies had normal hearts on their cardiological exam. All 100% babies with CDH survived the surgery, and 25% of the examined babies were in need for extracorporeal membrane oxygenation (ECMO). Due to surgery and due to the time needed for lung expansion, it must be taken in consideration that these patients have a lower lung capacity than normal babies, which makes them more vulnerable. After being discharged home, these children do not differ from their peers as they age.
Genetic counselling is a huge predictor in making a decision whether to give birth to a child with CDH, because some genetic mutations and syndromes can be associated with CDH.

Discussion

Mothers must be monitored with high precision and genetically analyzed to determine the presence of congenital anomalies. Prenatally, increased amniotic fluid levels are an early diagnostic sign and can serve as a predictor seen by ultrasonographic screening, which should be done regularly and meticulously in every trimester. This was proven by Korkmaz *et al*. and

shown in this study, where increased amniotic fluid level was found in 100% of the examined mothers [5].
Because some patients cannot afford the financial costs of the procedures, they seek help through social media or look for other options, because some of the treatments must be highy paid. Poley *et al*. showed that cost-effectiveness of treatment is one of the most important factors in decision-making, and many patients are willing to accept the challenges of carrying, giving birth to, coping with surgery, and taking care of a child with CDH [6].
Extracorporeal membrane oxygenation is a life saving machine helping with the function of the lungs and brain of more critical patients. Kays says that ECMO improves survival in those CDH patients who are most severely affected, which was proven in this study [7].
Parents must be advised to take disinfection and sterilization measures seriously, because the lungs can be very sensitive through the entire lives of children with CDH. Bonfis *et al*. emphasize the role of the lung volume as a predictor to the rate survival of these children [8].
If the child is proven to be genetically healthy, there is no hesitation in supporting the potential for growth. However, awareness must be raised for future generations to develop more accurate genetic tests, as reported by Gofin *et. al.* [9].

Conclusion

Children with congenital diaphragmatic hernia can have a better quality of life if their condition is detected early intrauterine with the help of ultrasonographic screening and treated properly. Depending on the severity of the condition, ECMO has been proven to be effective in highly equipped tertiary clinical centers with trained medical staff. A small percentage of patients can have problems with pulmonary hypertension that must be taken seriously. Genetic counselling should be available throughout the entire period, from planning conception, during pregnancy and after giving birth.
Of great importance is timely follow-up pregnant women in medical institutions that have the most contemporary equipment so that timely and accurate diagnosis is made, allowing for planning future procedures and treatments of this condition.

Conflict of interests: None declared.

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Original article

ПРОЦЕНКА НА ЕФЕКТИВНОСТА НА ПЛИКАТУРИРАНА КОЛПОСУСПЕНЗИЈА КАЈ ПАЦИЕНТКИ СО СТРЕС УРИНАРНА ИНКОНТИНЕНЦИЈА

ASSESSMENT OF THE EFFECTIVENESS OF THE PLEATED COLPOSUSPENSION IN STRESS URINARY INCONTINENCE

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Abstract

Introduction. Urinary incontinence (UI) is a common medical issue that can affect women of all ages. UI is rarely life-threatening, but impacts patients' mental, physical and social well-being. Ultrasound in urogynecology enables accurate evaluation of both static and dynamic relationships in the lower urinary tract.
Methods. This study aimed to demonstrate the use of ultrasound in pre- and postoperative evaluation of patients with SUI. Additionally, it assessed the effectiveness of pleated colposuspension. A total of 40 preoperative SUI patients were included, divided into two subgroups with 20 patients each: the first group was underwent an isolated colposusension, and the second, in addition, underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy. Using transperineal 2D ultrasound, α and β angles were measured at rest and during Valsalva maneuver.
Results. The α and β angles were significantly lower in postoperative state compared to the Preoperative one. A t-test confirmed that these increments were statistically significant ($p \leq 0.05$).
Conclusion. By monitoring α and β angles pre- and postoperatively, clinicians can objectively evaluate the success of surgical interventions for SUI.

Keywords: stress urinary incontinence, pleated colposusopension, transperineal ultrasound, α angle, β angle

Апстракт

Вовед. Уринарна инконтиненција (UI) е честа состојба која може да ги афектира жените во било која возраст и е претставена со широк дијапазон на симптоми. Ретко е животно загрозувачка, но влијае на физичката, менталната и социјалната благосостојба. Ултразвукот во урогинекологијата овозможува евалуација на статички и динамички соодноси на долен уринарен тракт.
Методи. Во оваа студија е прикажана употребата на ултразвукот во пре- и пост- оперативно следење на пациентките со стрес уринарна иноктиненција (SUI). Вкупно 40 жени влегоа во оваа проспективна рандомизирана студија. Беа поделени на две групи од по 20. Со помош на трансперинеален 2D ултразвук, се мереа α и β аглите во мир и во тек на Валсалва маневр. Во првата група беше изведена изолирана колпосуспензија, а во втората група беше направена и тотална абдоминална хистеректомија со билатерална салпингоофоректомија.
Резултати. α и β аглите беа сигнификантно помали во тек на постоперативните контроли во споредба со предоперативните вредности на истите. Т-тестот го потврди овој инкремент со медицински значајна статистичка сигнификантност($p \leq 0.05$).
Заклучок. Со мониторирање на овие два агли пред и постоперативно, може да се добие објективност во клиничката евалуција и проценка на успешноста на хируршкиот третман на SUI.

Клучни зборови: Стрес уринарна инконтиненција, пликатурирана колпосуспензија, трансперинеален ултразвук, α агол, β агол

Introduction

Urinary incontinence (UI) is a common condition, that significantly changes the quality of life and affects over 303 million women and 121 million men all over the world [1]. According to the latest epidemiological

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data, the prevalence in women older than 20 years is 17%, and in women older than 60 years 38% [2]. Despite this high prevalence, urinary incontinence remains insufficiently diagnosed and treated. It is estimated that only 25% of people with incontinence seek help. Untreated incontinence is associated with lower quailty of life, more often hospitalization, depression, social isolation and urinary infections [3]. The prevalence of urinary incontinence is 3 times higher in women than in men in all age groups [4]. The hypermobility of the bladder neck is closely related to the onset of stress urinary incontinence (SUI). Pathophysiology of SUI includes inadequate anatomic support of the blader neck and the proximal urethra, which results in hypermobility and descent because of increased intra-abdominal pressure. One of the noninvasive, but unfortunately not so attractive diagnostic tool is transperineal ultrasound (TPUS) [5]. Previous studies have established that this is a very sensitive method for evaluation of the urethral mobility. A lot of parameters and angles are included enabling a complete evaluation of urethral mobility and research continues in the right direction for determining the most adequate ultrasound technique. In addition, the most commonly used parameters are the front urethral angle (α angle defined with the os that passes through the proximal urethra on one side and os that passes through symphysis pubica on the other side) showing the mobility of the bladder neck, and posterior urethrovesical angle (β angle, located between the proximal urethra and posterior wall of the bladder) showing the mobility of the proximal part of the urethra [6]. Because this is a common condition, the evaluation of the patient should begin with an assessment of the stage of this condition and its impact on her lifestyle. For this purpose, we used a standard questionnaire, detailed anamnesis, obstetric anamnesis, information about urinary infections, surgical or neurological disorders and medications she was taking for other health issues.

Materials and methods

This was a prospective cohort randomized study, conducted at the University Clinic for Gynecology and Obstetrics in Skopje and included 40 patients with diagnosed SUI. The study was approved by the Ethics Committee of the Faculty of Medicine in Skopje and a written consent for participation in the study was obtained.
Patients were divided into two groups:

- First group: 20 patients with isolated SUI without genital prolapse or eventual prolapse of the anterior vaginal wall-urethrocystocellae, in whom pleated colposuspension was performed and
- Second group: 20 patients with intravaginal genital prolapse and SUI, in whom transabdominal hysterectomy with bilateral adnexectomy+pleated colposuspension were performed.

All of the patients were thoroughly investigated (vaginal exam, questionnaire and ultrasound) preoperatively, after 6 weeks and after 6 months of the operation.
Inclusion criteria: SUI treated with pleated colposuspension.
Exclusion criteria: exteriorized genital prolapse, which makes pressure onthe bladder neck and masks SUI, surgical and anesthesiological contraindications, patient's refusal of surgery.The study took place at the University Clinic for Gynecology and Obstetrics, Department of urogynecology and pelvic static, where the patients were recruited. Every patient had:

- A completely filled-out questionnaire for genital prolapse and SUI,
- A clinical exam,
- An ultrasound of the lower urinary tract.

The recruiting was randomized, so the data for the age, BMI and parity were derived from the randomization. The questionnaire consisted of 24 questions regarding everyday signs and symptoms during rest or any activity, both during the day and night, and how incontinence influenced on patients. *Physical exam* was made in lithotomy position, the same position in which the operation for incontinence treatment was made, in order to avoid bias. It included examination for diagnosing a genital prolapse using the POPQ system (Pelvic Organ Prolapse Quantification System) by defining nine point positions on the anterior and posterior vaginal wall, along with the Pozzy maneuver. An examination for the presence of urinary incontinence (Marshall test -the patient should cough while she is in lithotomy position while she is standing) was made, and additionally, *ultrasound of the lower urinary tract*. The bladder was filled with 300-400ml of urine, and the examination was made with the MindrayM5 ultrasound device with convex transducer of 3,5-5MHz. Our Department works in accordance with the recommendations of the German Association for Urogynecology and Functional Sonography, with some modifications.
The application of the transducer can be endosonographic or external. We used an external application with a transperineal transducer with 3.75 MHz. The anatomic orientation is as follows:

- Cranial parts should be presented at the bottom of the image.
- Ventral parts should be presented on the right side of the image.
- It is very important to define the correct position of the bladder neck by using the

coordinate system.

Results

The two investigated groups were treated with different surgical methods. In the first group, an isolated pleated colposuspension was made, and in the second

group, in addition to pleated colposuspension, a total abdominal hysterectomy with bilateral adnexectomy was made. The values of α and β angles were measured before and after the mentioned surgical treatments (Figures 1, 2, 3 and 4) measurments before surgigal treatment). The control of urinary stress incontinence and its dynamic was evaluated using the Marshall test at rest and Valsalva maneuver, and the values of the angles were measured with a transperineal ultrasound at two different time points, at 6 weeks and at 6 post-surgery. Of all the parameters that were included for every single patient, a standard descriptive analysis was made and the results are presented in Table 1 and the results of paired *t* test for changes in α and β angles before and after surgical treatment are presented in Table 2.

Table 1. Results of descriptive statistical analysis for α and β angles in patients

Angle/^{0}	N	Range	Min	Max	Mean	S.E	S.D.	Variance
$\alpha_r{}^0$	40.0	99.0	48.0	147.0	84.9	3.1	19.6	383.2
$d\alpha_v{}^0$	40.0	93.0	34.0	127.0	83.0	3.2	20.1	404.1
$\beta_r{}^0$	40.0	104.0	91.0	195.0	131.9	3.9	24.7	609.7
$\beta_v{}^0$	40.0	141.0	40.0	181.0	126.1	4.6	29.0	843.4
$\alpha_r{}^1$	40.0	72.0	30.0	102.0	63.7	2.7	16.9	285.5
$\alpha_v{}^1$	40.0	59.0	32.0	91.0	60.3	2.9	18.5	341.3
$\beta_r{}^1$	40.0	82.0	63.0	145.0	100.4	3.3	20.9	435.0
$\beta_v{}^1$	40.0	87.0	57.0	144.0	94.1	3.3	21.0	441.5
$\alpha_r{}^3$	40.0	74.0	30.0	104.0	64.6	2.6	16.6	274.7
$\alpha_v{}^3$	40.0	70.0	32.0	102.0	63.8	3.2	20.1	404.3
$\beta_r{}^3$	40.0	92.0	55.0	147.0	101.5	3.4	21.6	465.3
$\beta_v{}^3$	40.0	81.0	64.0	145.0	96.2	3.1	19.6	384.1

$\alpha_r{}^n$ $\beta_r{}^n$; indexes: r (rest); *v* - Valsalva, 0 -pre-surgical (onset), and after surgical treatment

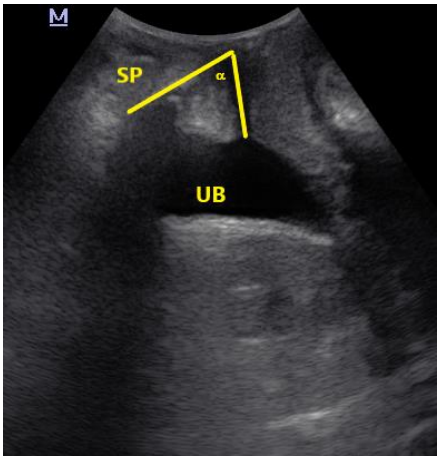

Fig. 1. α angle during rest

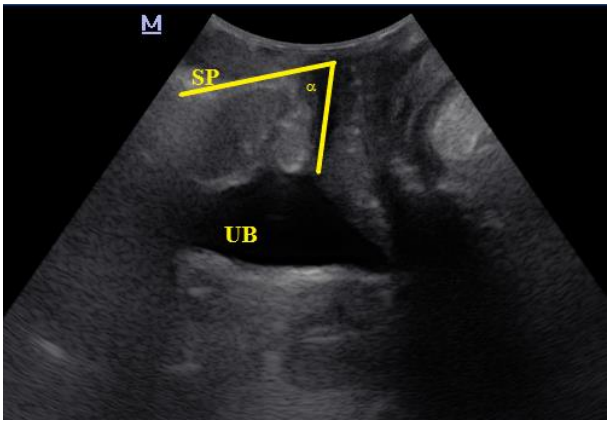

Fig. 3. α angle during Valsalva maneuver

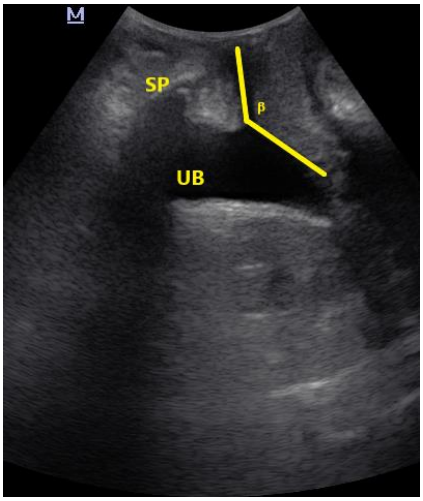

Fig. 2. β angle during rest

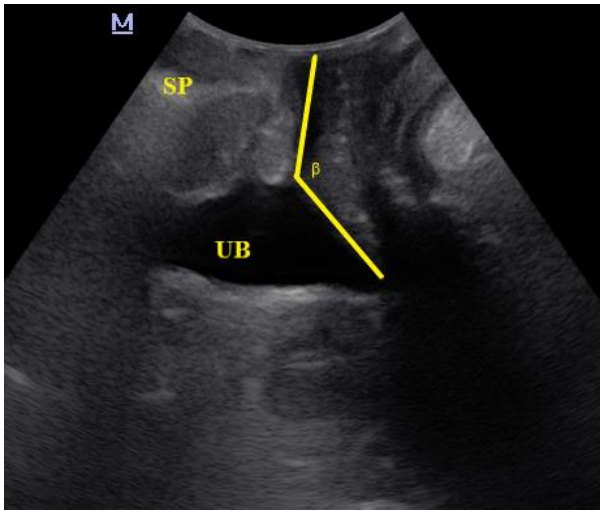

Fig. 4. β angle during Valsalva maneuver

Table 2. Results of paired *t* test for changes in α and β angles before and after surgical treatment

	Mean	S.D	S.E.	Uper and lower limits		t	df	Statistical significance
$\alpha_r^3 - \alpha_r^0$	-16.30	12.55	2.81	-22.17	-10.43	-5.81	19	≤ 0.000
$\alpha_v^3 - \alpha_v^0$	-15.45	18.97	4.24	-24.33	-6.57	-3.64	19	≤ 0.002
$\beta_r^3 - \beta_r^0$	-31.55	25.53	5.71	-43.50	-19.60	-5.53	19	≤ 0.000
$\beta_v^3 - \beta_v^0$	-34.15	21.40	4.79	-44.17	-24.13	-7.14	19	≤ 0.000

$\alpha_r{}^n\beta_r{}^n$; index : *m- r* rest; *v Valsalva*, 0 - preoperative (on onset) and postoperatively

Discussion

Because of the fact that SUI is a significant medical issue, its diagnosis is of exceptional importance. However, even today, the definitive diagnosis is made only with physical examination. Ultrasound (US) is not intended to replace clinical history taking or physical examination, but instead provides a better understanding of the disease entity [7]. Much research regarding diagnosis tends to change the focus towards new methods, such as ultrasound. To be more concrete, transperineal ultrasound can be a standard tool with high repeatability.

Transperineal ultrasound of the lower urinary tract, especially of the bladder neck and its relationships with surrounding structures, like statically during rest and dynamically during Valsalva maneuver,

promise a great step forward in standardization of diagnostics, its objectification and assessment of the success of SUI treatments [6].

In this study, we assessed the success of pleated colposuspension in patients with SUI, who were divided into two groups.

The first group encompassed female patients who underwent isolated pleated colposuspension, and the second group consisted of female patients who, in addition to pleated colposuspension, also underwent total abdominal hysterectomy with bilateral adnexectomy. Measuring α and β angles, before, 6 weeks and 6 months after the operation, showed a significant reduction in these two angles in both groups, especially in the second one, as well as reduced mobility of the bladder neck. This is one step further in finding a definite diagnostic protocol, as well as a predictive factor for follow-up of patients diagnosed with SUI and surgically treated.

There are studies in which comparison of the angles was made, but the control group consisted of healthy individuals, and the other group of patients with SUI [8-10]. The results are consistent with the findings from our study, which makes the applicability of the transperineal ultrasound more convincing as a noninvasive and easily available diagnostic tool.

Conclusion

This study underscores the efficiency of pleated colposuspension in patients with SUI, regardless of whether it is performed isolated or together with total abdominal hysterectomy with bilateral adnexectomy, because it enables repair of the exact physiologic anatomy of the pelvic floor. This helps to correct the symptoms of incontinence, and improves quality of life. We define this improvement not only subjectively by patients' statements, but also objectively by using transperineal ultrasound of the lower urinary tract. To be more concrete, by measuring the α and β angles, we show the mobility of the bladder neck. The results have shown decreased values after surgical intervention, indicating that the mobility of the bladder neck decreased. Clinically, this is manifested by a reduction in symptoms. Based on these results, we can conclude that this noninvasive method, which is easy to be applied, can become a part of the algorithm for diagnosis, evaluation and follow-up of patients with SUI who underwent surgical treatment for incontinence.

Conflict of interests: None declared.

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MMP

Original article

CLINICAL-PATHOLOGICAL EVALUATION AND FOLLOW-UP OUTCOMES IN PRIMARY BLADDER TUMOR PATIENTS TREATED AT UROLOGY CLINIC

КЛИНИЧКО-ПАТОЛОШКА ЕВАЛУАЦИЈА И ИСХОДИ ОД СЛЕДЕЊЕТО КАЈ ПАЦИЕНТИ СО ПРИМАРНИ ТУМОРИ НА МОЧЕН МЕУР ТРЕТИРАНИ ВО УРОЛОШКА КЛИНИКА

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Abstract

Aim. To evaluate the clinical-pathological characteristics, recurrence rate, and progression of primary bladder tumors in patients treated at a tertiary urology center, with a focus on histologic grade, tumor size, and number assessed via cystoscopy.

Methods. This retrospective, observational study included 117 patients with histologically confirmed urothelial carcinoma who underwent initial transurethral resection of bladder tumor (TURBT) at the University Clinic for Urology in Skopje from January 2019 to December 2024. Patients were categorized by gender, tumor invasiveness (pTa, pT1, pT2), histologic grade (PUNLMP, low-grade, high-grade), tumor count, and size. Follow-up was performed using cystoscopy, urine cytology, and ultrasound at 3, 6, 9, and 12 months. Recurrence was defined as tumor relapse at any of these intervals. Exclusion criteria were: non-urothelial histology, missing data, prior bladder cancer treatment, or carcinoma *in situ* (CIS), which was excluded due to its distinct biological behavior.

Results. The mean age of patients was 66.5±9.6 years (range 41-88), with a male-to-female ratio of 3.5:1. Pathological staging showed 43 patients (33.9%) had pTa, 37(29.1%) had pT1, and 37(29.1%) had pT2 tumors. Histologic grading revealed 2 patients (1.6%) with PUNLMP, 37 (29.1%) with low-grade carcinoma, and 78 (64.6%) with high-grade tumors. Non-muscle invasive bladder cancer (NMIBC) was present in 80 patients (63.0%), and muscle-invasive bladder cancer (MIBC) in 37 (29.1%).

Recurrence occurred in 14 of 117 followed patients (12.0%). There was no recurrence in PUNLMP cases: 16.2% of low-grade tumors, and 10.2% of high-grade tumors. The relatively lower recurrence in high-grade cases is attributed to the high proportion of pT2 tumors managed by cystectomy. Tumor multiplicity and size were significant predictors: recurrence was 4.8% in solitary tumors and 50.0% in patients with 3-5 tumors. Tumors >3 cm had a recurrence rate of 24.0%, compared to 5.7% in those <3 cm.

Conclusion. Tumor grade, size, and multiplicity were strongly associated with recurrence following TURBT. These findings emphasize the importance of personalized follow-up based on pathological and cystoscopic features.

Keywords: bladder cancer, urothelial carcinoma, TURBT, recurrence, tumor grade, tumor size, cystoscopy, pT stage

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Апстракт

Цел. Да се процени ризикот за рецидив на примарни тумори на мочен меур кај пациенти третирани во терцијарен уролошки центар, со фокус на хистолошкиот степен, големината и бројот на тумори.

Методи. Ретроспективна студија на 117 пациенти со уротелен карцином, третирани со иницијална TURBT на Универзитетската клиника за урологија - Скопје (јануари 2019 - декември 2024). Пациентите беа категоризирани според пол, туморска инвазивност (pTa, pT1, pT2), хистолошки степен, број и големина на тумори. Следењето се вршеше на 3, 6, 9 и 12 месеци. Исклучени беа пациенти со CIS, неуротелен карцином или претходен третман.

Резултати. Просечна возраст: 66,5 години; мажи: жени=3,5:1. Од нив, 43 пациенти имале pTa, 37 pT1, и 37 pT2 тумори. Хистолошки: 2 PUNLMP, 37 нискостепени, 78 високостепени. NMIBC беше присутен кај 63%, MIBC кај 29%. Рецидив се јави кај 14 пациенти (12%): 16,2% кај нискостепени, 10,2% кај високостепени (главно поради радикална хирургија кај pT2). Рецидив бил поврзан со број и големи-

на на тумори-50% кај пациенти со 3-5 тумори и 24% кај тумори >3 cm.
Заклучок. Хистолошкиот степен, големината и мултиплицитетот значајно влијаат на ризикот за рецидив. Индивидуализиран мониторинг е неопходен.

Клучни зборови: уротелен карцином, рецидив, градус, стадиум, големина на тумор.

Introduction

Bladder cancer (BC) is a significant public health concern due to its high incidence, high recurrence rate, and progression. Its impact extends beyond prognosis to the patient's quality of life, while also imposing a considerable burden on healthcare systems [1]. A central challenge in the management of bladder tumors is their high rate of recurrence and progression. Moreover, bladder cancers exhibit diverse phenotypes with varying responses to surgical, chemotherapeutic, radiotherapeutic, and immunotherapeutic modality. This clinical heterogeneity has prompted the need for deeper insights into tumor genotypes and their correlation with phenotypic expression.
Accordingly, contemporary urology aims to centralize and standardize preoperative diagnostic protocols for all BC patients, with the goal of developing personalized treatment strategies following primary transurethral resection (TURBT). These strategies are tailored based on both phenotypic and genotypic tumor features.
Furthermore, the evaluation and management of recurrence in urothelial carcinoma play a crucial role in disease control, treatment planning, and determining the prognosis for patients.
Globally, bladder cancer accounts for approximately 3% of all newly diagnosed cancers, ranking as the 10th most common cancer and the 7th among males. Incidence rates differ significantly by gender, with men being diagnosed four times more often than women. The global incidence is 9.5/100,000 in men and 2.2/100,000 in women [2]. According to GLOBOCAN 2020, the highest incidence is reported in Southeast Europe (26.6 per 100,000 in men and 5.8 per 100,000 in women) [3]. In North Macedonia, the Cancer Registry reported an incidence rate of 11.52 per 100,000, with 239 new cases and 137 deaths in 2020, of which 101 were males and 36 females [4].
This study evaluated patients diagnosed with primary bladder tumors, focusing on histopathological characteristics, cystoscopy findings, recurrence, and progression during structured follow-up.

Materials and Methods

This was an observational, retrospective study that included patients diagnosed with primary bladder tumors and treated surgically at the University Clinic for Urology in Skopje between January 2019 and December 2024. The primary inclusion criteria were: histologycally confirmed urothelial carcinoma, age between 18 and 88 years, and signed informed consent for participation.
Patients were excluded from the study if they had non-urothelial histology, missing or incomplete clinical-pathological data, underwent prior bladder cancer treatment at another institution, or declined to provide informed consent.
Patients were evaluated using standard diagnostic modalities, including laboratory analysis, ultrasound, and cystoscopy. TURBT was used both for diagnosis and treatment. Postoperative monitoring included cystoscopy, cytology and ultrasonography at 3, 6, 9, and 12 months.
Recurrence was defined as any recurrence recorded at 3, 6, 9, or 12 months. If no recurrence was recorded during these follow-ups, patients were classified as non-recurrent.
Patients were categorized by gender, histologic grade (low-grade or high-grade), tumor configuration, and microscopic tumor extension (pT-stage) according to the European Association of Urology (EAU) guidelines: pTa-noninvasive papillary carcinoma; pT1-invasion into the lamina propria; and pT2-invasion into muscularis propria (detrusor muscle).
Recurrence was defined as any tumor relapse observed at any of the follow-up intervals.
Descriptive statistics was used to summarize demographic and clinical-pathological features. Associations between variables and recurrence were analyzed using chi-square tests.

Results

The mean age of patients was 66.5±9.6 years (range 41 to 88 years). The median age detected in men with urothelial carcinoma was 66.0 years, while in women 68.5 years. The male-to-female ratio was approximately 3.5:1, with 99 male and 28 female patients included in the study.
Pathological staging revealed that pTa was observed in 43 patients (33.9%), pT1 in 37 patients (29.1%), and pT2 in 37 patients (29.1%). One patient (0.8%) had a carcinoma *in situ* (Tis). A small number of patients (7.9%) had tumors of non-urothelial origin or non-malignant diagnoses. Non-muscle invasive bladder cancer (NMIBC), comprising pTa and pT1 stages, was diagnosed in 80 patients (63.0%). Muscle invasive bladder cancer (MIBC), defined as pT2, was present in 37 patients (29.1%). The single patient diagnosed with carcinoma *in situ* (CIS) was excluded from the study due to the unique clinical and biological behavior of CIS, which differs significantly from papillary lesions

and could introduce bias in evaluating recurrence and staging patterns in the main study cohort.
When stratified by tumor invasiveness, 43 cases (33.9%) were classified as noninvasive carcinoma (pTa), and 37 cases (29.1%) were invasive to the lamina propria (pT1). True muscle-invasive carcinoma (pT2) was found in 37 patients (29.1%). There were 33 male and 4 female patients with pT2 disease.
Histological grading revealed that **2 patients (1.71%)** were diagnosed with *Papillary urothelial neoplasm of low malignant potential (PUNLMP)*, **37 patients (31.62%)** had *low-grade papillary urothelial carcinoma*, and **78 patients (66.67%)** had *high-grade papillary urothelial carcinoma* (Table 1). This distribution reflected a predominance of high-grade tumors in the studied cohort.

Table 1. Pathological characteristics

Characteristics	No. of Patients (%)
Pathological Stage	
• pTa	43(33.9%)
• pT1	37(29.1%)
• pT2	37(29.1%)
• Tis	1(0.8%)
• Non-urothelial or non-malignant	10(7.9%)
Histologic Grade	
• PUNLMP	2(1.71%)
• Low-grade	37(29.1%)
• High-grade	78(64.6%)

Tumor recurrence was analyzed in relation to histopathological grade (Table 2). No recurrence was observed (0%) in the two patients diagnosed with papillary urothelial neoplasm of low malignant potential (PUNLMP).. Low-grade papillary urothelial carcinoma showed recurrence in 6 out of 37 patients (16.2%). In contrast, high-grade tumors, although more aggressive in nature, demonstrated recurrence in only 8 out of 78 patients (10.2%).

Table 2. Tumor recurrence in relation to histopathological grade

Tumor Grade	Number of Patients	Recurrence (n)	Recurrence Rate (%)
PUNLMP	2	0	0.0%
Low Grade	37	6	16.22%
High Grade	78	8	10.25%

This apparent paradox-where high-grade tumors show a lower recurrence rate-may be explained by the fact that a significant proportion of these high-grade cases were diagnosed at the pT2 stage and subsequently treated with radical cystectomy. As such, recurrence assessment in these patients was conducted post-cystectomy and primarily focused on distant or upper tract recurrences, rather than local intravesical relapse. On the other hand, patients with low-grade tumors typically underwent bladder-sparing management and were followed up with cystoscopy, which allows for more frequent detection of local recurrences.
These findings highlight the need to interpret recurrence data in light of the treatment modality and tumor stage, and further support the role of individualized follow-up strategies based on both histological grade and pathological staging.

Recurrence Analysis Across Follow-Up Intervals based on tumor count and tumor size

Recurrence data were analyzed from all available follow-up intervals (3, 6, 9, and 12 months). If a recurrence was identified at any time point, the patient was marked as recurrent. A total of 117 patients were included in this analysis.

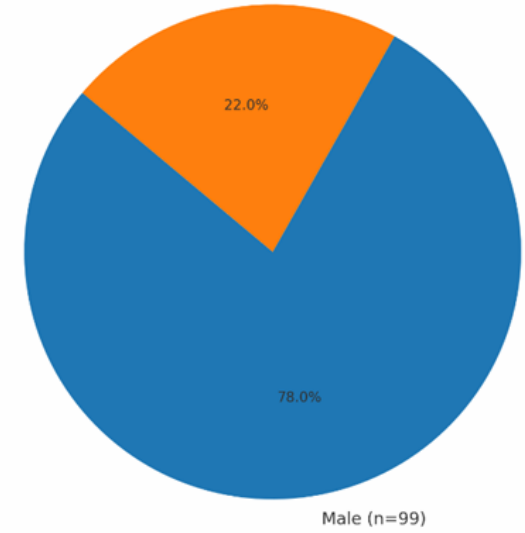

Fig 1. Gender Distribution of Patients with Primary Bladder Tumors

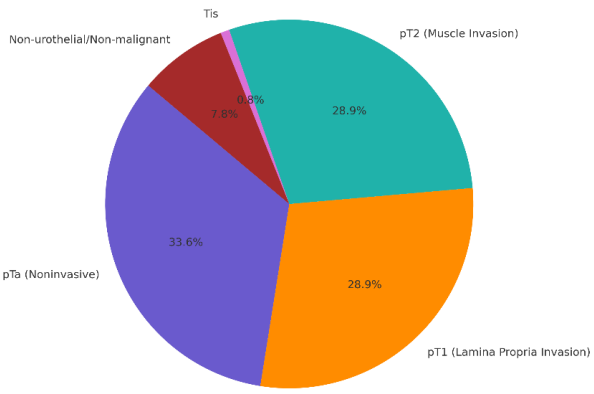

Fig. 2. Distribution according to the pathological stage

Tumor multiplicity (Table 3) assessed via cystoscopy demonstrated a notable correlation with recurrence. Recurrence was observed in 4 patients (4.8%) with solitary tumors. In contrast, higher recurrence rates were recorded in patients with multiple tumors: 21.4%

in those with two tumors, 50.0% in patients with 3-5 tumors, and 22.2% in those with more than five tumors. These results indicate an increasing trend in recurrence with tumor multiplicity, particularly in patients with 3 to 5 lesions.

Table 3. Recurrence analysis based on tumor count

Tumor Count (Cystoscopy)	Number of patients	Recurrence (n)	Recurrence Rate (%)
Solitary (1)	84	4	4.8
2 Tumors (2)	14	3	21.4
3–5 Tumors (3)	10	5	50.0
>5 Tumors (4)	9	2	22.2
Total	117	14	100

Tumor size evaluated during cystoscopy (Table 4) showed a trend toward higher recurrence rates with increasing tumor size. Patients with tumors smaller than 3 cm had a recurrence rate of 5.7%, compared to 12.8% for tumors exactly 3 cm and 24.0% for tumors larger than 3 cm. These findings align with existing literature indicating that larger tumor size is associated with a greater likelihood of recurrence, potentially due to increased tumor burden and more complex resection.

Table 4. Recurrence analysis based on tumor size

Tumor Size	Patients	Recurrence	Recurrence Rate (%)
<3 cm	53	3	5.7%
3 cm	39	5	12.8%
>3 cm	25	6	24.0%
Total	117	14	100

Statistical analyses demonstrated a **significant association** between **tumor grade** and both **tumor multiplicity (≥2 tumors)** and **tumor size (≥3 cm)**. Using the Chi-square test, higher-grade tumors were more likely to be associated with multiple tumors and larger size ($p<0.05$), indicating a strong correlation between aggressive pathological features and tumor burden.

Discussion

This study offers a detailed evaluation of primary bladder tumors treated at our Urology Clinic, emphasizing pathological features that predict recurrence after TURBT. Our results align with recent literature and advocate for individualized risk-based follow-up.

The cohort reflects typical urothelial carcinoma demographics, with a male predominance and a mean age consistent with international reports [1]. Non-muscle invasive bladder cancer (NMIBC) accounted for 63% of cases, a figure aligning with recent urologic epidemiology models [5].

Tumor histologic grade was a significant, though nuanced, predictor. While low-grade tumors recurred at a rate of 16.2%, high-grade tumors recurred at 10.2%. This discrepancy likely reflects the use of radical cystectomy in high-grade/pT2 tumors, eliminating sites of intravesical recurrence [6,7]. Recent multicenter models confirm that recurrence must be interpreted within the context of tumor biology and treatment strategy [8].

Tumor multiplicity was a strong prognostic factor. Patients with 3-5 tumors had a recurrence rate of 50%, compared to 4.8% in solitary tumor. This is in agreement with contemporary findings showing that multiplicity significantly increases recurrence risk and often reflects urothelial field change [11].

Tumor size similarly impacted recurrence: tumors >3 cm had a recurrence rate of 24%. This trend is corroborated by recent studies linking larger tumor burden with more difficult resections and increased residual disease [8,12]. Good quality TURBT and complete detrusor muscle sampling are essential to reduce recurrence in such patients [12].

Our findings strongly support risk-adapted surveillance, consistent with EAU (9,10) and contemporary recommendations. Patients with multiple or large tumors, even if low-grade, benefit from intensified follow-up and possible intravesical therapy [11,13,14].

Conclusion

This study underscores the value of structured follow-up and guideline-driven management in patients with primary bladder tumors. Institutional improvements in postoperative therapy and resection standards can play a crucial role in improving clinical outcomes.

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Conflict of interests: None declared.

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MMP

Case Report

ATYPICAL LOCALIZATION OF DIFFUSE LARGE B-CELL LYMPHOMA – PRIMARY ABDOMINAL MUSCLE LYMPHOMA

АТИПИЧНА ЛОКАЛИЗАЦИЈА НА ДИФУЗЕН КРУПНО-КЛЕТОЧЕН ЛИМФОМ-ПРИМАРЕН АБДОМИНАЛЕН ЛИМФОМ

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Abstract

Lymphomas are a heterogeneous group of malignancies affecting B cells, T cells, and, rarely, natural killer (NK) cells that usually originate in the lymph nodes, but may originate in any organ of the body. Primary muscle lymphoma accounts for just 0.1% of all lymphomas, with a much smaller proportion occurring in the abdominal muscles.

Because skeletal muscle lymphoma is a rare and clinically diverse disease, primary lymphoma is usually not included in the differential diagnosis of muscle mass, making diagnosis challenging.

We present the case of a 57-year-old male who was referred to our hospital with a three-month history of progressive muscular tenderness, lump growth on the front abdominal wall, with no history of trauma to the involved area. The patient reported lymphoma-associated symptoms, such as night sweats, fever, rapid weight loss. Contrast-enhanced computed tomography (CT) of the abdomen was performed and revealed an intramuscular tumor with an inhomogeneous mass (size 74x47 mm). Surgical extirpation of the tumor was performed and histological examination confirmed a final diagnosis of primary diffuse large B-cell lymphoma on the abdominal wall.

Not having much experience with this rare pathology, a review of literature, albeit scarce, showed that the standard chemotherapy regimen with R-CHOP is being utilized for skeletal muscle DLBCL. We started R-CHOP therapy; three cycles were completed uneventfully and complete remission was attained. Currently, the patient is in remission five years after the initial diagnosis.

Keywords: primary skeletal muscle lymphoma, diffuse large B cell lymphoma, R-CHOP

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Апстракт

Лимфомите претставуваат хетерогена група на малигни тумори на Б-клетките, Т-клетки и ретко клетки природно убијци (NK) кои обично потенуваат од лимфните јазли, но тие можат да потекнуваат и од било кој друг орган на телото. Примарниот мускулен лимфом сочинува само 0,1% од сите лимфоми, а само многу мал дел од нив се јавуваат во стомачните мускули.

Бидејќи лимфомот на скелетните мускули е ретка и клинички разновидна болест, примарниот лимфом обично не е вклучен во диференцијалната дијагноза на мускулната маса, што ја прави дијагнозата предизвик.

Ние презентираме случај на 57-годишен маж, упатен во нашата болница со тримесечна историја на прогресивна мускулна осетливост, раст на грутки на предниот абдоминален ѕид, без историја на траума на зафатената област. Пациентот дава податок за симптоми поврзани со лимфом, вклучувајќи ноќно потење, треска, брз губиток на тежината. Реализирана е компиутерска томографија на абдомен со контраст и откриен е интрамускулен тумор со нехомогена маса (големина 74x47 mm). Извршена е хируршко отстранување на туморот и хистолошкиот резултат во прилог на конечна дијагноза на примарно дифузен крупно Б-клеточен лимфом на абдоминалниот зид.

Немајќи големо искуство со оваа ретка патологија, консултација на литература покажа дека стандардниот режим на хемотерапија по R-CHOP се користи за DLBCL на скелетните мускули. Ја започнавме терапијата со R-CHOP, при што успешно завршени три циклуси и постигната целосна ремисија на болеста. Во моментов пациентот е во ремисија пет години од првичната дијагноза.

Клучни зборови: примарен лимфом на скелетните мускули, дифузно крупно клеточен Б-лимфом, R-CHOP

Introduction

Diffuse large B-cell lymphoma (DLBCL), an aggressive high-grade non-Hodgkin lymphoma (NHL), comprises the most common NHL in adults, accounting for 25% of all NHL, typically presenting with rapidly growing lymph nodes in the neck or abdomen [1,2]. Over the past two decades, molecular classification of DLBCL for therapeutic purposes has been the main scientific focus; nonetheless there has been noted considerable heterogeneity in clinical presentation[1]. Approximately 40% of cases present with extranodal disease, albeit not usually as a primary focus of disease, most commonly involving the gastrointestinal tract, as well as the head and neck (Waldeyer's ring) [1,3].
Skeletal muscle involvement is rare, and isolated skeletal muscle is even rarer. Some data suggest that skeletal muscle or intramuscular lymphomas represent less than 2% of B-cell lymphomas [4,5]. When it occurs, thighs, upper extremities, calves and pelvis are most frequently affected [6]. To demonstrate the rarity of this phenomena, we made a systematic literature review with data stating that as of 2022, there were eight cases of extranodal NHL involving the masticator muscles and only one case of intramuscular lymphoma in the orofacial area [5,7]. Skeletal muscle lymphoma manifests simply as a soft tissue mass with swelling and pain, often imitating a soft tissue infection and initially treated as such, which can lead to a diagnostic challenge resulting in a delayed diagnosis [8,9]. That was the case with our patient, whose lymphoma presented as an isolated involvement of the *rectus abdominis* muscle, thus gaining merit for a thorough review and publication.

Case Presentation

A 57-year-old man presented to our hospital with a six-month history of progressive muscular tenderness, with lump growth formation on the front abdominal wall, located on the left side over the *m. rectus abdominis*, above the umbilicus. Except for localized pain and soft-tissue swelling in his left hemiabdomen, the patient reported lymphoma-associated symptoms, such as night sweats, fever and steep weight loss, which receded after surgical intervention. His past medical history was otherwise unremarkable, with no history of trauma to the involved area, no comorbidities and chronic use of medications.
Physical examination showed no abnormalities; peripheral lymph nodes were not palpable, and the liver and spleen were also not palpated. Blood workup was unremarkable. Initial laboratory testing showed that only lactate dehydrogenase levels were elevated 950 U/L (normal range, <248 U/L); other blood test results were normal. Tumor markers were negative. A blood marrow biopsy was performed and showed no infiltration. The initial PET/CT scan did not show active metabolic disease.

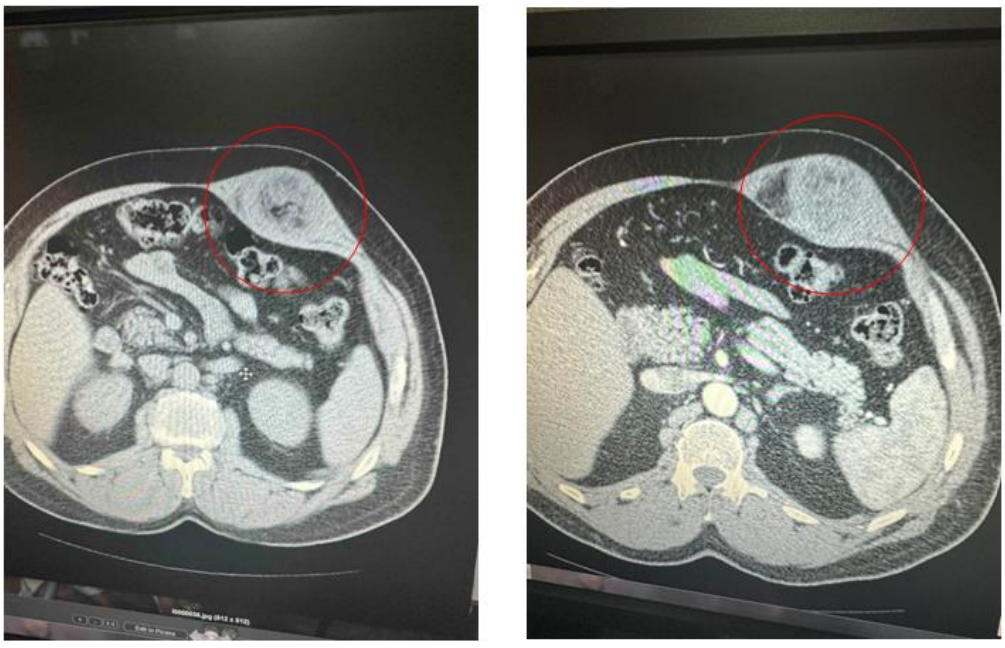

Fig.1. Abdominal contrast enhanced CT, the mass is outlined in red

Initially thought to be a ventral hernia, an ultrasonographic evaluation of the abdomen was made showing a mass that was suspected to be a lipoma, leading to a consultation with a digestive surgeon. Contrast-enhan-

ced CT of the abdomen was performed in July 2019, revealing an inhomogeneous mass measuring 74x47 mm, arising from the anterolateral abdominal muscles, suspected for a desmoid tumor, with no concomitant lymphadenopathy (Figure 1).

A fine-needle aspiration biopsy indicated chronic inflammation, for which antibiotic therapy was prescribed, noting that a surgical biopsy or total excision would be mandatory if no abatement occurred.

Soft-tissue infection, desmoid tumor, and lipoma had constituted the gamut of possible diagnosis. Two months prior to the first hematological evaluation, on 27 July 2019, the patient underwent surgical extirpation of the mass after the unsuccessful course of the antibiotic treatment. Histopathological examination of the lesion revealed a tumor, weighing 97 grams, polymorphic cell infiltration of nodal Ly aggregates, with surrounding histiocytoid and band fibroblastoid prolixferation, along with deposits of “young” collagen. Immunohistochemisty confirmed the diagnosis of diffuse large B-cell lymphoma. So, based on the initial findings and staging work-up of the patient, finally a diagnosis of primary diffuse large B-cell lymphoma on the abdominal wall - stage IVB was established.

The dilemma was whether chemotherapy should be initiated at all, due to the local nature of the process. All initial treatment options were discussed at our expert collegium; however, owing to the aggressiveness (high proliferative index), we agreed to start with the R-CHOP regimen (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone). Three cycles in total were administered, which were well tolerated, with no significant side effects and without treatment delays. The patient was then placed under regular follow-up, initially every three months during the first year and every six months in the subsequent years. The patient did not experience any relapse. This is the fifth year that the patient is in remission with no signs of disease and follow-up continues.

Discussion

Clinical differential diagnosis between muscle lymphoma and other malignant tumors occurring in soft tissue can be challenging. The differential diagnosis includes muscle hematoma, infectious and inflammatory myositis, sarcoidosis, rhabdomyolysis, sarcoma, denervation muscle edema, and metastatic disease among other etiologies.

Most patients are initially being treated with antibiotics because the most common diagnosis associated with skin swelling and pain is cellulitis. Some patients report trauma to the site of tumor growth prior to the onset of symptoms; they undergo imaging methods and are scheduled for follow-up.

Although primary skeletal muscle DLBCL is rare, our literature review found 9 case reports on this topic. We excluded all other primary muscular lymphomas that were not DLBCL. We also excluded DLBCLs that were disseminated to other organs or were with lymph node involvement. We included only cases where DLBCLs extended to one or more muscles. All cases had histopathological confirmation.

The literature review showed that primary muscle DLBCL appearing in the lower extremities is more common. The mean age of onset is 72 years, with predominance in males (66%); the most common type of growth is nodular (90%), without B symptoms. Laboratory findings in most cases reveal elevated LDH, without other abnormalities. MRI was the first most commonly used imaging method, but the most accurate was FDG-PET. Of the cases reviewed, majority had disease extended to multiple muscles, and multiple intramuscular nodules. Only one of the cases had diffuse mass growth intramuscularly. Of the cases reviewed for which treatment details were available, all initially began with R-CHOP protocol, although varying in number of cycles. Only one case showed disease progression with the initial R-CHOP protocol, so the treatment was escalated [10-15].

Our case was the only one presenting with B symptoms. Although there is another case of primary site in *m. rectus abdominis* reported in the literature, our case presented with single intramuscular mass with dimensions of 7 cm. Our patient was somewhat younger than the median age reported in the literature. Our patient had laboratory findings of elevated LDH, similar to other cases. In contrast to other cases, our patient was treated with only 3 cycles of R-CHOP and showed complete remission for nearly 5 years after treatment.

The mechanism by which lymphomas appear primarily in muscles is still not clear. Skeletal muscles do not have lymph nodes, but they have a lymphatic drainage system [16]. There are multiple theories regarding origin: dissemination via hematogenous or lymphatic pathway, extension from adjacent organs [12], *de novo* primary extranodal disease, or an aberrant lymph node in the skeletal muscle that cannot be detected at the time of diagnosis [17,18]. A few of the reviewed cases were associated with trauma to the location some period before the onset of symptoms.

There are other questions unanswered and further research is needed.

Conclusion

Primary lymphoma is not usually included in the differential diagnosis of a muscle mass. Due to its unusual clinical manifestation, an accurate diagnosis could be delayed. This case report reminds both physicians and pathologists that maintaining an open mind is the cornerstone of any diagnosis. Due to the extreme rarity of the muscle localization of DLBCL,

this diagnosis should be considered as a part of differential diagnosis of masses in cases that do not respond to antibiotic treatment. Furthermore, early diagnosis can yield a prompt initiation of treatment, which is essential in these time-sensitive pathologies. Although imaging cannot definitively establish the diagnosis of muscle lymphoma, since the disease presents with characteristic findings on CT and MRI, the radiologist may be the first provider to suggest this diagnosis. It is crucial that only biopsy can ensure definitive diagnosis, despite the many noninvasive imaging techniques that can be and were in this case utilized. An early initiation of treatment is a *sine qua non* in all types of malignancies. In our case, 6 months elapsed between the patient's first doctor visit and the first chemotherapy cycle.

There are no internationally prognostic indexes or scoring systems defined for this rare subgroup of lymphomas, and only a modest number of cases have been published. However, due to the high proliferative index, we classified this patient as having a localized but high-risk lymphoma and we started the R-CHOP therapy, the backbone regimen for all NHL. The patient completed three cycles uneventfully and complete remission was attained. Currently, the patient is in remission five years after the initial diagnosis.

Conflict of interests: None declared.

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Case report

DELAYED-INTERVAL DELIVERY IN TWIN PREGNANCIES: A CASE REPORT OF 54 LATENCY DAYS

ПРИКАЗ НА СЛУЧАЈ НА ОДЛОЖЕН ИНТЕРВАЛ НА ПОРОДУВАЊЕ КАЈ БЛИЗНАЧКА БРЕМЕНОСТ: ПРИКАЗ НА СЛУЧАЈ СО 54 ДЕНА ЛАТЕНТНОСТ

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Abstract

Introduction. In multiple pregnancies with threatened premature delivery or preterm premature rupture of membranes of a single sac, prolonging the pregnancy after the delivery of the first baby may improve the chances of survival of the second baby.
Case report. We report a case of quadrigeminy pregnancy after controlled ovarian stimulation and artificial insemination. After embryo reduction of two embryos at 8 gestational weeks, a diamniotic, dichorionic pregnancy continued. At 22 gestational weeks, the patient was hospitalized due to premature rupture of the first amniotic sac and premature labor that resulted in the delivery of the first twin. The second twin was left *in utero* and management included a combination of antibiotics, tocolytics and cervical cerclage. The pregnancy was continued for 54 days, with monitoring of maternal and fetal parameters, which enabled the spontaneous delivery of the second baby with improved neonatal outcomes. This case supports the benefits of prolonging the pregnancy of the second twin.

Keywords: multiple pregnancy, delayed delivery, selective delivery

Апстракт

Вовед. Кај повеќеплодова бременост со закана за предвремено породување или предвремено пукање на плодовите обвивки кај едниот плод, продолжувањето на бременоста по раѓањето на првиот плод може да ги подобри шансите за преживување на вториот плод.
Приказ на случај. Прикажуваме случај на квадригемина бременост по контролирана стимулација на јајниците и артефицијална инсеминација. После реализирана ембриоредукцијата на два ембриона во 8 гестациски недели, диамниотската, дихорионска бременост продолжи да се развива. Во 22 гестациска недела, пациентката беше хоспитализирана со предвремена руптура на водењакот на првиот плод, по што следеше прподување на првиот близнак. Вториот близнак беше задржан во матката со вклучување на комбинација од антибиотици, токолитици, кортикостероиди и цервикален серклаж. Бременоста продолжи 54 дена, со следење на параметрите на мајката и фетусот, што на крајот резултираше со спонтано породување на вториот гемел со добар неонатален исход. Овој случај е во прилог на поддршка на продолжувањето на бременоста на вториот близнак кај предвремено продување на првиот плод.

Клучни зборови: повеќеплодова бременост, одложено породување, селективно породување

Introduction

The implementation of assisted reproduction during the last decades has increased the incidence of multiple pregnancies. Preterm delivery is the most common complication of multiple gestations and is associated with high perinatal mortality and morbidity. Frequently, when preterm labor occurs, delivery of the first fetus is inevitable. Traditionally, this situation is managed by delivery of both fetuses, either vaginally or by cesarean section. However, in selected cases, the preterm birth of one sibling may not require delivery of the remaining fetus(es), who may remain *in utero* for an extended period. This event is defined as a delayed delivery of the second twin, and has been reported as a management strategy to decrease morbidity and optimize the survival of the remaining fetuses after the spontaneous preterm birth of one fetus during a multifetal gestation [1-3]. There is no clear definition of delayed-interval delivery as gestational age and the interval has varied widely based on numerous studies. Nevertheless, a delayed-interval delivery is generally defined as an active attempt (antibiotics, tocolysis and/or cerclage placement) to

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increase latency between the delivery of the first fetus and subsequent fetuses in the second trimester [4].
Despite the interest in this subject, in the literature there is still a lack of a universally-accepted protocol for the optimal management of these cases [5-8].
Here, we report the results of one dichorionic pregnancy with a delayed delivery of 54 days assisted at our center.

Case report

The patient A.L., a 34-year-old woman, was admitted to our hospital for treatment of primary infertility. Following a detailed examination, it was confirmed that the patient had anovulatory cycles, and her husband's spermogram finding was in favor of asthenoteratozoospermia.
The patient was indicated for and underwent controlled ovarian stimulation with AIH (Artificial Insemination Husband), after which she became pregnant. A control ultrasound examination at 7 weeks of gestation confirmed a quadrigeminy pregnancy: four gestational sacs with viable embryos and positive heart action. After consulting the couple and explaining all the potential risks of a multiple pregnancy, an embryo reduction of two embryos was performed. Following the intervention and during subsequent control ultrasound examinations, normal growth and development of two viable fetuses was confirmed. At 12 weeks of gestation, ultrasound examination confirmed two viable fetuses with positive heart activity, bichoriata biamniata, including normal laboratory parameters and negative microbiological swabs. The control ultrasound examination at 20 weeks of gestation was normal as well, with normal laboratory parameters and negative microbiological swabs.
The patient came to our hospital at 22 weeks of gestation with pain and contractions, and a finding of prolapsed amnion. Laboratory results on admission did not indicate infection; microbiological swabs were negative. A spontaneous abortion and expulsion of a female fetus soon occurred. After consultation and a detailed explanation to the couple about global experiences and potential risks for both mother and fetus, with their consent it was decided to continue the pregnancy. A high ligation of the umbilical cord was performed with absorbable suture. The patient was treated with bed rest and antibiotic therapy. After 6 days, due to the local finding, a cervical cerclage was placed, followed by antibiotic therapy and preventive tocolysis.
At 28 weeks of gestation, the patient was hospitalized due to contractions. On admission, she had normal laboratory results and negative microbiological swabs. One maturation dose (Flosteron/ Betamethazone) and tocolysis were administered (Atosiban- a subsequent infusion of 100 micrograms/min for up to 45 hours). At 29.6 weeks of gestation, premature rupture of membranes (PROM) was confirmed, with regular contractions on CTG. Ultrasound examination indicated that the fetus was in cephalic presentation. According to some studies, in such cases, delivery primarily ends with a Caesarean section, while other studies prefer vagi-

Table 1. Medical History of the patient

Date	Gestational week	Le	CRP (<0.50)	Vaginal smears	Medical History
20.09.2018					KOS et AIH
26.10.2018	8 g.w.				Quadrigemini Embryo reduction
26.11.2018	12 g.w.	9.1	0.12	Negative Normal flora	
22.01.2019	20 g.w.				
06.02.2019	22+ g.w.	9.6	0.25	Negative Normal flora	PROM AB SPONTANUS/ G1
08.02.2019	23 g.w.	9.88	1.73		antibiotic
09.02.2019	23 g.w.	8.08	1.08		antibiotic
10.02.2019	23 g.w.	9.08	0.50		antibiotic
12.02.2019	23+ g.w.				CERCLAGAE
14.02.2019	24 g.w.	8.0	0.64		Antibiotic/ Tocolysis
21.03.2024	28 g.w.	9.73	0.84	Negative Normal Flora	Antibiotic Betamethasone
25.03.2019	29 g.w.	14.6	0.14		Oligohydramnios Contractions Hospitalization Tocolysis
30.03.2019	29.6 g.w.	13.17	0.17		CERCLAGAE EX PARTUS IMMATURUS SPONTANEUS 1210 gr/ 40 cm Apgar score: 7/8
09.05.2019					Baby Home

nal delivery. Our decision was for the patient to give birth naturally. The cerclage suture was removed, and 54 days after the delivery of the first twin, a spontaneous delivery occurred of a live female neonate weighing 1,210 g, 40 cm long and Apgar score = 7/8. The baby was cared for at the Neonatal Intensive Care Unit (NICU) and was discharged home 40 days after the delivery.

Discussion

According to the literature, intentional delayed delivery of the second embryo in twin pregnancies is a very rare occurrence. The increasing use of assisted reproduction techniques during the last ten years has resulted in a parallel increase of its incidence [9].

Since Carlson's pioneering case report in 1880, there have been only two prospective studies of delayed-interval twin delivery [10]. Arias reported 8 cases managed with cerclage, tocolysis and antibiotics, with a mean interval to the second birth of 48 days [2]. Arabin *et al*. reported 50 cases, 38 twins and 12 triplets, with a mean interval of 19 days for twins and 18 days for triplets [6]. Most publications are case reports or retrospective cohort studies.

Gestational age at delivery is an important factor in neonatal survival as in most cases it is directly related to fetal weight. Prolongation of gestational age and subsequent increase in fetal weight can lead to significant improvement in fetal outcome [11,12]. This implies that the rate of survival after delayed delivery is higher compared to delivery occurring at the time of initial delivery [13-16]. A delay of two or more days in the delivery of babies born before 30 weeks of gestation is associated with improved infant survival and higher infant birth weight [17]. Problems with early deliveries include prolonged duration of respiretory support, an increased number of surgical procedures, and longer hospital stay. There is also increase in the frequency of necrotizing enterocolitis, patent ductus arteriosus, and retinopathy of prematurity [18,19].

It is generally accepted that ligation of the umbilical cord after delivery should be performed high up in the cervix using an absorbable suture. This helps in reducing the risk of infection [6].

The primary maternal risks associated with attempting delayed-interval delivery are intrauterine infection and maternal sepsis. Before attempting this procedure, the following conditions should be excluded: no reassuring fetal status, congenital abnormalities, rupture of the membranes of the remaining fetus, severe hemorrhage, and maternal infections or diseases. Monochorionicity is not a contraindication. Antibiotic therapy is routinely administered in cases of asynchronous delivery [6]. Our case was not complicated by signs of infection. Our management consisted of antibiotic therapy after the first delivery, and a repeated course of antibiotics when elevated white blood cell and C-reactive protein levels were documented in blood tests.

The use of prophylactic cerclage is the most controversial issues among the recommended procedures [7,8,20-22]. For some authors, it is a routine procedure, while for others it is recommended only if the etiology of the spontaneous delivery is cervical insufficiency [7,8,20]. Some studies report that cerclage leads to a longer delivery interval [14,23] however, cerclage is also considered to increase the risk of infection and membrane rupture in twin pregnancies [6,16], and is therefore contraindicated in such pregnancies to prevent preterm birth [24]. Reinhard *et al*. placed cerclage in all cases of delayed-interval delivery and observed a shorter delay compared to Arabin and van Eyck who did not employ cerclage. Reinhard *et al*. did not report the occurrence of infection in their patients [25]. In our case, a cervical cerclage was performed, and no intrauterine infection was detected.

Tocolysis was an integral part of the management in most documented reports. This is mainly because patients presented with some form of contractions. Routine tocolysis is however debatable. Suppression of premature contractions can be achieved with tocolytics such as b-mimetics, magnesium sulfate, oxytocin-receptors inhibitors or nonsteroidal anti-inflammatory drugs. Tocolysis may be used precautionary after the first twin's birth, or only later during uterine contractions, but never in the presence of a well-established chorioamnionitis [26].

Steroid use in delayed-interval delivery is universally practiced because of the risk of preterm delivery of the remaining fetus. Some believe that the delivery of the subsequent fetus should be delayed till 28-32 weeks [1,2,27-29]. Another study concluded that continuation beyond 32 weeks is not necessary [30]. However, one study delayed delivery till 36 weeks [5]. The author believes that the timing of delivery should be guided by the neonatal performance of the institution's newborn unit and the perceived risk of infection. For the delivery of twin 2, labor was usually spontaneous, with vaginal delivery in 76% of cases [6,31]. The indications for labor induction or cesarean section are not specific to this situation, mainly abnormal fetal heart rate tracings, symptoms or signs of infection, DIC, preeclampsia.

In our patient, gestation contractions started at 29.6 gestational weeks; membranes of the second twin ruptured spontaneously, and the baby was delivered vaginally. The interval between the delivery of the first and the second fetus was 54 days. No maternal morbidity occurred before or after delivery.

There are several unresolved issues in clinical practice, in addition to the original decision to attempt delayed second twin delivery: how to manage the placenta, how to prevent infection, whether to use tocolysis,

whether to perform cerclage, the place of outpatient management, and when to induce the delivery of twin 2.
Modern management procedures, including tocolytics, corticosteroids, antibiotics and cervical cerclage appear to be important in the overall success of treatment in a retained twin. Although both tocolytics and cerclage appear to prolong the mean delivery interval, no statistically significant difference has been found [32]. There are no clear indications for the use of prophylactic cervical cerclage and it does not appear to improve the survival of the second twin. The use of antibiotics is widely practiced, but only after isolation of a specific pathogen in cervicovaginal secretions. Vaginal examinations should be avoided; however, the length and dilatation of the cervix should be monitored ultrasonographically. Monitoring should be carried out and limited to a weekly full blood count, prothrombin time and fibrin degradation product (FDP). Even a large placental mass can remain in the uterus and produce no demonstrable clinical symptoms [30,32].
Delayed 2nd-twin delivery raises several ethical issues. Pre-viable delivery of the first twin is a dramatic and often unpredictable event. The perspective of successful delayed delivery can only be estimated after 24 hours, but the initial decision must be made on an emergency basis regarding whether to expedite delivery of the second twin or attempt expectant management. Furthermore, there is a conflict of interest between the potential benefits for the fetus and the potential risk for the mother. This type of conflict of interest is similar to the one more commonly addersed in cases of preterm premature rupture of membranes before 24 weeks. Thus, couples should receive complete information as early as possible, at the beginning of labor or PROM. They should be informed that the most likely outcome in case of delivery of twin 1 will be the rapid delivery of twin 2, even in case of expectant management. Parents should also be advised that delayed-interval deliveries carry a risk of maternal complications such as infections and hemorrhage [33].

Conclusion

In conclusion, delayed-interval delivery is a useful and possible therapeutic option for managing the remaining fetus, enabling the improvement of neonatal survival and decreasing morbidity. In carefully selected twin pregnancies, the survival of the second-born twin may be improved with delayed-interval delivery, particularly if the first twin was born at or after 22-24 weeks. Selecting optimal candidates for delayed-interval delivery is fundamental, and parents should always be counseled about the potential risks and benefits of the procedure. Management protocols in the studies vary, making it difficult to propose a uniform strategy for delayed-interval delivery. Further research in this field is needed to generate standardized management guidelines for the deferred delivery. In the case reported herein, prophylactic antibiotics enabled the delivery of the second fetus at 29 weeks, corresponding to 54 days of latency. This case supports the benefit of prolonging the pregnancy of the second twin.
Large multicenter studies should be performed investtigating the best management strategy as well as the neonatal and maternal morbidity.

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MMP

Case report

SUCCESSFUL MANAGEMENT OF ISOLATED OLIGOHYDRAMNIOS IN THIRD TRIMESTER OF PREGNANCY – A CASE REPORT

ТРЕТМАН НА ИЗОЛИРАН ОЛИГОХИДРАМНИОН ВО ТРЕТ ТРИМЕСТАР – ПРИКАЗ НА СЛУЧАЈ

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Abstract

The amniotic fluid (AF) is the fluid that surrounds the fetus in the amniotic cavity during intrauterine development, and is fundamental for proper fetal development and growth in a nonrestricted, sterile, and thermally controlled environment.
Oligohydramnios is defined as a decreased amniotic fluid volume (AFV) for gestational age. The volume of amniotic fluid changes over gestation, increasing linearly until 34 to 36 weeks of gestation, at which point the AFV levels off (approximately 400 mL) and remains constant until term.
Sonographically, oligohydramnios is diagnosed when the maximum vertical pocket of liquor is <2 cm or the amniotic fluid index is <5 cm (less than 5 percentile).
Isolated oligohydramnios refers to the presence of oligohydramnios in an otherwise uncomplicated pregnancy, without evidence of fetal structural or chromosomal abnormalities, fetal growth restriction, or infection, and in the absence of maternal hypertensive disorders or renal disease.

Keywords: oligohydramnios, preterm birth, preterm rupture of membranes

Апстракт

Амнионската течност е течноста којашто го опкружува фетусот за време на интраутериниот развој и игра голема улога за правилен раст и развој на плодот.
Олигохидрамнион се дефинира како намалена количина на околуплодова течност за гестациската возраст. Волуменот на амнионската течност се менува во тек на гестацијата, со линеарно зголемување до 34-36г.н. и останува константна до раѓање.
Сонографски олигохидрамнион се дијагностицира кога максималниот вертикален слободен џеб е <2cm или индексот на амнионска течност (AFI) <5cm.
Изолираните олигохидрамниони укажуваат на намалена количина на амнионска течност во некомплицирана бременост без доказ за фетални структурни или хромозомски абнормалности, рестрикција во растот или инфекција, како и отсуство на болести на мајката-хипертензија и ренална болест.

Клучни зборови: олигохидрамнион, предвремено породување, предвремена руптура на мембрани

Introduction

The amniotic fluid is the fluid that surrounds the fetus in the amniotic cavity during intrauterine development, and is fundamental for proper fetal development and growth in a nonrestricted, sterile, and thermally controlled environment.
The amniotic fluid volume is the sum of fluid flowing into and out of the amniotic space. It is the result of a complex interaction of fetal, placental and maternal factors, and appears to be conserved and maintained in a dynamic equilibrium. The volume of amniotic fluid changes over gestation, increasing linearly until 34 to 36 weeks of gestation, at which point the AFV levels off (approximately 400 mL) and remains constant until term [1].
Oligohydramnios is a condition characterized by decreased amniotic fluid volume for gestational age, often arising from maternal, fetal, or placental complications. Sonographically, oligohydramnios is diagnosed when the maximum vertical pocket of liquor is <2 cm or the amniotic fluid index is <5 cm (less than 5 percentile) [2,3]. This condition can be associated with poor fetal outcomes, including impaired lung development, growth restriction, and an increased risk of labor complications. Causes may include premature rupture

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of membranes, placental insufficiency, and fetal renal abnormalities.

Oligohydramnios has traditionally been considered to be a sign of potential adverse perinatal outcome, as well as a possible indicator of placental insufficiency and fetal compromise. Thus, the identification of oligohydramnios usually mandates close fetal surveillance. Furthermore, the diagnosis of oligohydramnios at term is often considered an indication for induction of labor, even in otherwise uncomplicated pregnancies.

During the late second or third trimester, a reduction in AFV may also cause umbilical cord compression, resulting in fetal heart rate decelerations and operative deliveries. Oligohydramnios presents several other challenges: ultrasound visualization is impaired due to lack of contrast and limited fetal mobility, and amniocentesis, when indicated, may be very difficult to perform.

Isolated oligohydramnios refers to the presence of oligohydramnios in an otherwise uncomplicated pregnancy without evidence of fetal structural or chromosomal abnormalities, fetal growth restriction, or infection, and in the absence of maternal hypertensive disorders or renal disease.

Case report

A 35-year-old primigravida at 32 weeks of gestation presented with oligohydramnios. The pregnancy was well controlled. Prenatal genetic test revealed low risk. The first and second trimester screenings showed normal results. Microbiological samples were negative as well as OGTT.

Valsalva maneuver was negative, vaginal pH -4. Ultrasonographic examination revealed fetus with positive heart activity, pelvic presentation, fetal biometry in correlation with gestational age, reduced amniotic fluid (AFI-5 cm, LVP-2,4cm) (Figure 1) normal function of placenta, dopler of umbilical artery normal range of

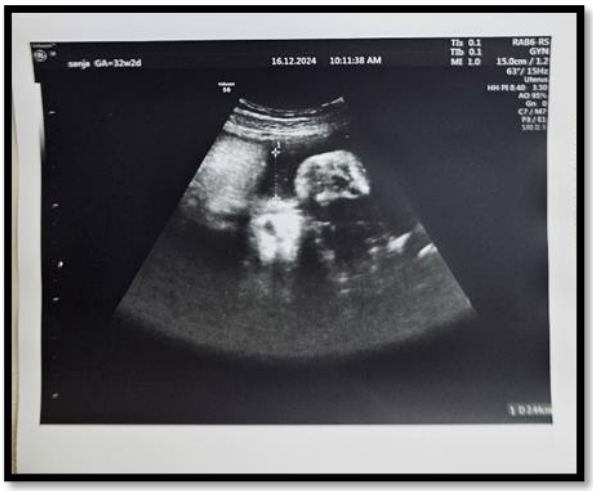

Fig. 1. Ultrasound evaluation of amniotic fluid on admission to hospital - LVP-2,4cm

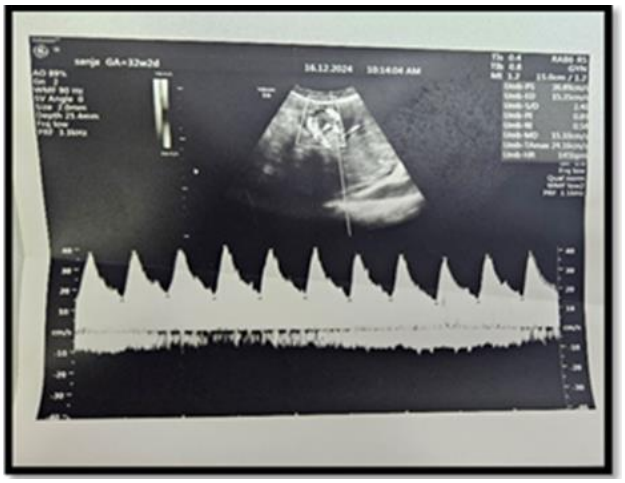

Fig 2. Dopler of umbilical artery -– normal range

values for gestational age (Figure 2). Laboratory findings: WBC-11.6 x10^19/L, RBC-3.86 x 10^12/L, HGB-116 g/L, CRP-2.6 mg/L.The patient was hospitably treated with rehydration therapy, corticosteroid therapy for fetal lung maturation, antibiotic and thromboprophylactic therapy [4].

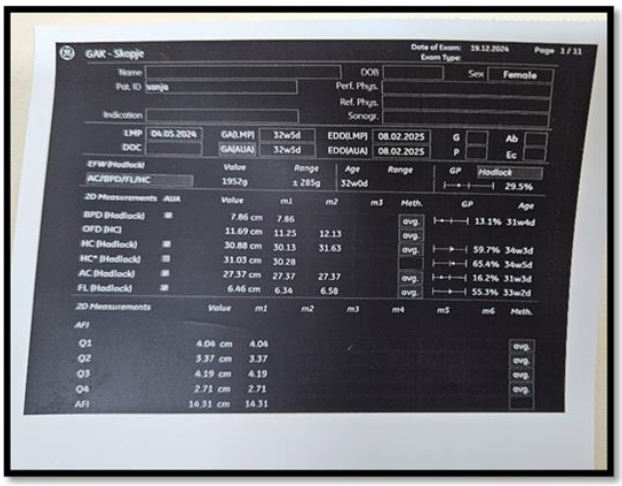

Fig. 3. Fetal biometry and AFI on discharge of hospital

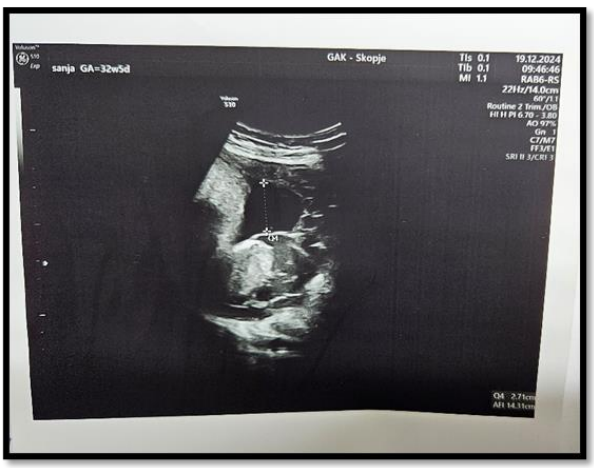

Fig. 4. Amniotic fluid index within normal range – AFI-14cm

At the time of hospitalization, ultrasound examinations revealed an increase in amniotic fluid index. The patient was discharged after 7 days with AFI - 14 cm (Figures 3 and 4), fetal biometry corresponding to gestational age (Figure 3), dopler parameter of fetoplacental unit – normal range (Figure 5).

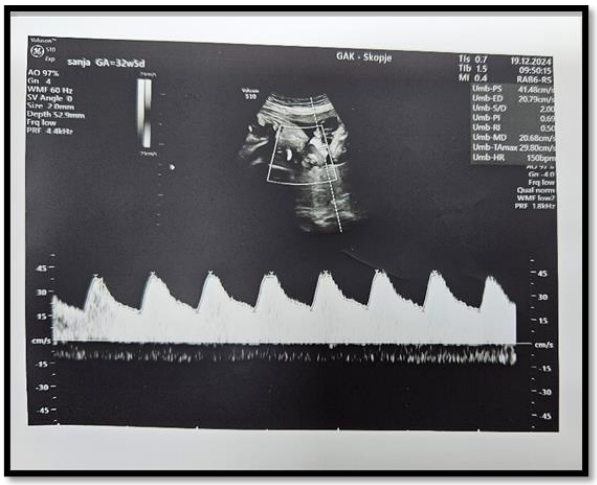

Fig. 5. Dopler of fetoplacental circulation witin normal range

By the time of giving birth, the fetus was ambulatory monitored every 5 days, by non-stress test [5] and ultrasonographical evaluation [6]. After two weeks of monitoring, the patient was admitted to the hospital at 34 weeks of gestation because of the premature rupture of membranes. She gave birth via cesarean section to a live female baby, with 2750 g/34 cm and Apgar score of 8/9.

Discussion

This case underscores the significance of a proactive and individualized approach in managing isolated oligohydramnios during the third trimester. The successful increase in AFI following targeted intervenetions demonstrates that isolated oligohydramnios, while a condition of concern, can be effectively managed with prompt and appropriate care.

A multidisciplinary approach, including continuous fetal monitoring and timely medical interventions, played a pivotal role in safeguarding both maternal and fetal health. Diagnostic tests, including the Valsalva maneuver and vaginal pH assessments, were crucial in ruling out premature rupture of membranes and infections, allowing for better management of the condition.

The eventual decision for cesarean delivery, necessityated by fetal presentation and ongoing evaluations, highlights the importance of flexibility and readiness to adapt management strategies in response to evolving clinical circumstances.

Conclusion

In summary, isolated oligohydramnios in the third trimester of pregnancy requires vigilant monitoring and tailored management strategies to optimize outcomes for both mother and child. This case illustrates that with an evidence-based and proactive approach, positive results are achievable, even when complications arise.

The successful delivery of a healthy infant reinforces the need for continued research and development of best practices regarding oligohydramnios management. It serves as a testimony to the importance of a collaborative and informed healthcare team, dedicated to navigating the complexities of obstetric care. Ongoing education and support for healthcare providers are essential to ensure the best possible care for pregnant individuals facing similar challenges in the future.

Conflict of interests: None declared.

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MMP

Case report

MANAGEMENT OF POLYTRAUMA (DILEMMAS IN COMPLEX POLYTRAUMA) – CASE REPORT

МЕНАЏМЕНТ НА ПОЛИТРАУМА (ДИЛЕМИ КАЈ КОМПЛЕКСНИ ПОЛИТРАУМИ)- ПРИКАЗ НА СЛУЧАЈ

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Abstract

Polytrauma, a patient's condition with multiple injuries that involves multiple organs or systems, is the leading cause of mortality in young adults. Trauma-related injuries are a major public health concern due to their associated morbidity, high disability, mortality, and socioeconomic consequences. This case report explores the clinical dilemmas encountered in the management of a complex polytrauma patient resulting from a fall from height. We report the case of a 60-year old man, presented with multiple injuries, including traumatic hemorrhagic shock, bilateral pneumothorax, bilateral haemothorax, serial bilateral fractures of the ribs, sternal fracture, compressive fracture of lumbar vertebra at l1 level, fracture of *processes transversus* of vertebrae Th9,11, L1, 3, 5, fracture of the right pubic bone, multiple fractures of the sacral body, fracture of the right acetabulum and acetabular protrusion, multiple open fractures of the left tibia, fibula fractures, fracture of the right patella, and multiple fractures of the right tibia.This case underscores the challenges faced by trauma teams in balancing aggressive intervention with the potential for adverse outcomes. It highlights the importance of a multidisciplinary approach, involving trauma surgeons, thoracic surgeons, plastic and reconstructive surgeons and intensive care specialists to navigate the intricacies of polytrauma care. Ultimately, this case emphasizes the necessity of individualized treatment plans that address both immediate survival and long-term functional recovery.

Keywords: polytrauma, management, dilemmas, multidisciplinary

Апстракт

Политраума претставува состојба при која пациентот има повреда на повеќе органи и системи, и му е загрозен животот. Овие повреди се проблем за јавното здравје поради високата стапка на попреченост, смрт и социоекономските последици. Овој приказ на случај ги прикажува клиничките дилеми со кои се среќаваме во менаџментот на комплексен политрауматичен пациент при пад од висина. 60-годишен маж, со повеќе повреди, вклучувајќи трауматски хеморагичен шок, билатерален пневмоторакс, билатерален хемоторакс, сериска билатерална фрактура на ребра, фрактура на градната коска, компресивна фрактура на лумбален пршлен l1 ниво, фрактура на пршленските попречни продолжетоци Th9,11,L1,3,5, фрактура на десната срамна коска, повеќекратни фрактури на сакрално тело, фрактура на десниот ацетабулум и ацетабуларна испакнување, повеќекратни отворени фрактури на левата тибија, фрактури на фибула, фрактура на десната патела и повеќекратни фрактури на десната тибија. Овој случај ги нагласува предизвиците со кои се соочуваат тимовите за траума во балансирањето на агресивната интервенција со потенцијалот за негативни исходи. Ја нагласува важноста на мултидисциплинарниот пристап, кој вклучува траума хирурзи, торакален хирург, пластичен и реконструктивен хирург и специјалист за интензивна нега за да се движите низ сложеноста на негата за политраума. На крајот на краиштата, случајот ја илустрира потребата од индивидуализирани планови за лекување кои го земаат предвид и моментното преживување и долгорочното функционално закрепнување.

Клучни зборови: политраума, менаџмент, дилеми, мултидисциплинарен

Introduction

Polytrauma, involving injury to multiple organ or systems, poses tremendous challenges in emergency management and long-term recovery. In many cases, patients die at the scene. Patients who manage to reach

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hospital alive have a good chance of surviving the traumatic event [1]. Immediate and early trauma deaths are determined by severe primary brain injuries, or significant blood loss (hemorrhagic shock) after blunt, or penetrating trauma. Direct or indirect mechanical forces induce organ and soft tissue injuries, or fractures. This case report explores the clinical dilemmas encountered in the management of a complex polytrauma patient resulting from a fall from height (paragliding) (Figure 1).

Case report

We report the case of a 60-year-old man, presented with multiple injuries resulting from a fall from height, including traumatic hemorrhagic shock, bilateral pneumothorax (Figure 2), bilateral hemothorax (right 38 mm, left 22 mm), serial bilateral rib fractures (right 3, 4, 5, 6, 8, and left 6, 7, 8, 9, 11, 12). sternal fracture, compressive fracture of the lumbar vertebra at l1 level, fracture of *processes styloid* of vertebrae Th9, 11, L1, 3, 5, fracture of the right pubic bone (Figure 5), multiple fractures of the sacral body, fracture of the right acetabulum and acetabular protrusion (Figure 5), multiple open left tibia fractures (Figure 4), fibula fractures, fracture of the right patella, and multiple right tibia fractures (Figure 3). The initial assessment revealed a significant hemodynamic instability, prompting immediate resuscitation, hemodynamic stabilization in intensive care unit and prioritization of life-threatening injuries. The complexities in decision-making involve which surgical intervention should be initially performed *versus* opting for conservative management, along with addressing the potential complications from multiple surgeries. The serial bilateral fracture of ribs and sternal fracture resulted with pneumothorax and hemothorax. Bilateral video-assisted thoracoscopic surgery (VATS) was performed, no active bleeding was detected and bilateral drainage was initially performed. The open multiple fracture of the right tibia and fibula with contaminated open wound in anterior-medial part was complexities in decision-making regarding the type and the position of the external fixator. Surgical stabilization with modular external AO fixation on the lateral side and drainage with VAC dressing was performed. The VAC dressing was performed on 2 days and the wound was closing step by step. One week after the accident, the patient underwent a surgery of the right knee, osteosynthesis of the right tibia with blocking screw and spongioplasty-ceraform with Nano gel. (CORTEX SCREW SELF TAPING 4.5MM, L=28MM PURE TITCORTEX SCREW 4.5/36MM. PURE TIT-SYNERGYCORTEX SCREW 4.5/34MM. PURE TIT-SYNERGYCORTEX SCREW 4.5/70MM. PURE TIT-SYNERGYCORTEX SCREW SELF TAPING 4.5MM, L=24MM-PURE TITCORTEX SCREW SELF TAPING 4.5MM, L=28MM PURE TITCORTEX SCREW SELF TAPING 4.5MM, L=30MM PURE TITCORTEX SCREW SELF TAPING 4.5MM, L=42MM-PURE TITLOCK. SCREW TAPING 5MM, L=75MM-PURE TITLOCK. SCREW TAPING 5MM, L=70MM-PURE TITLCP-PLT 4.5/5.0 LEFT 7H, L=180MMLOCK. SCREW TAPING 5MM, L=75MM-PURE TITCORTEX SCREW SELF TAPING 4.5MM, L=30MM PURE TITCORTEX SCREW 4.5/38MM PURE TIT-SYNERGY).

Fig. 1. Picture taken right after the injury

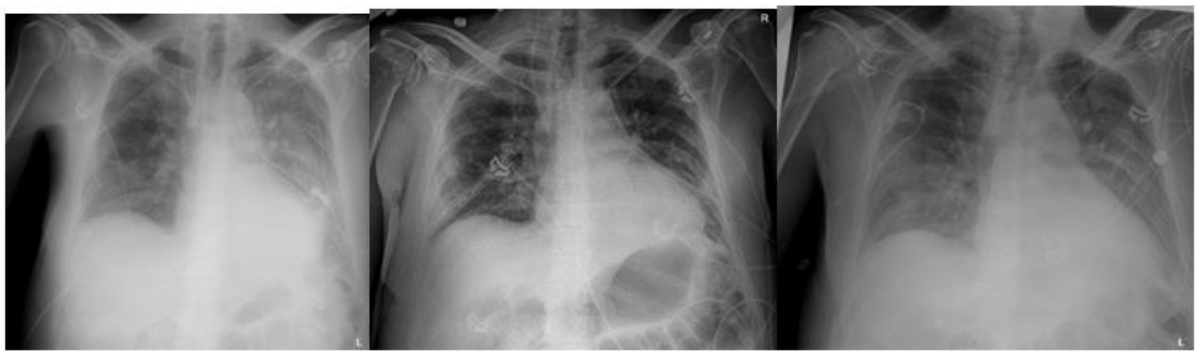

Fig. 2. X-rays on the day of injury, after VATS, and control X-ray

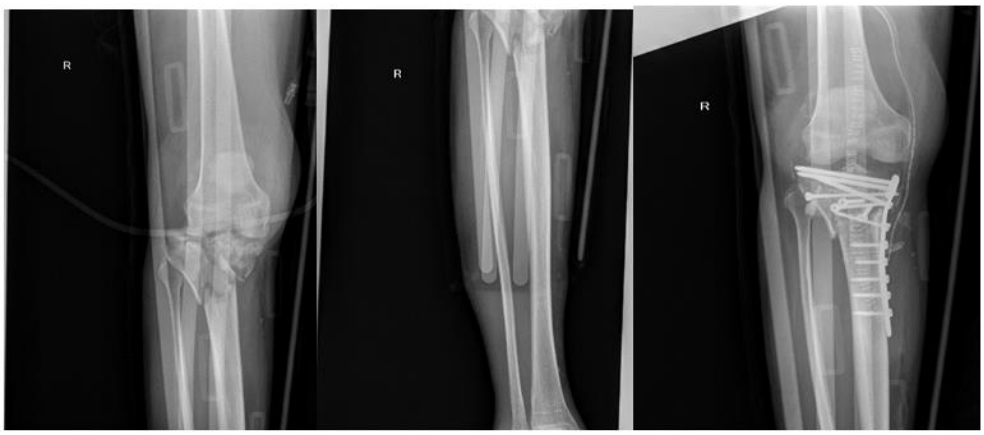

Fig. 3. CT scan, X-rays right leg on the day of the injury and after surgery

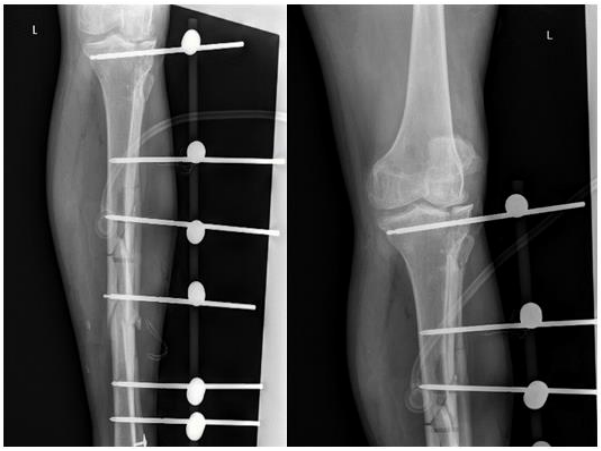

Fig. 4. CT scan, X-rays - left leg external fixation

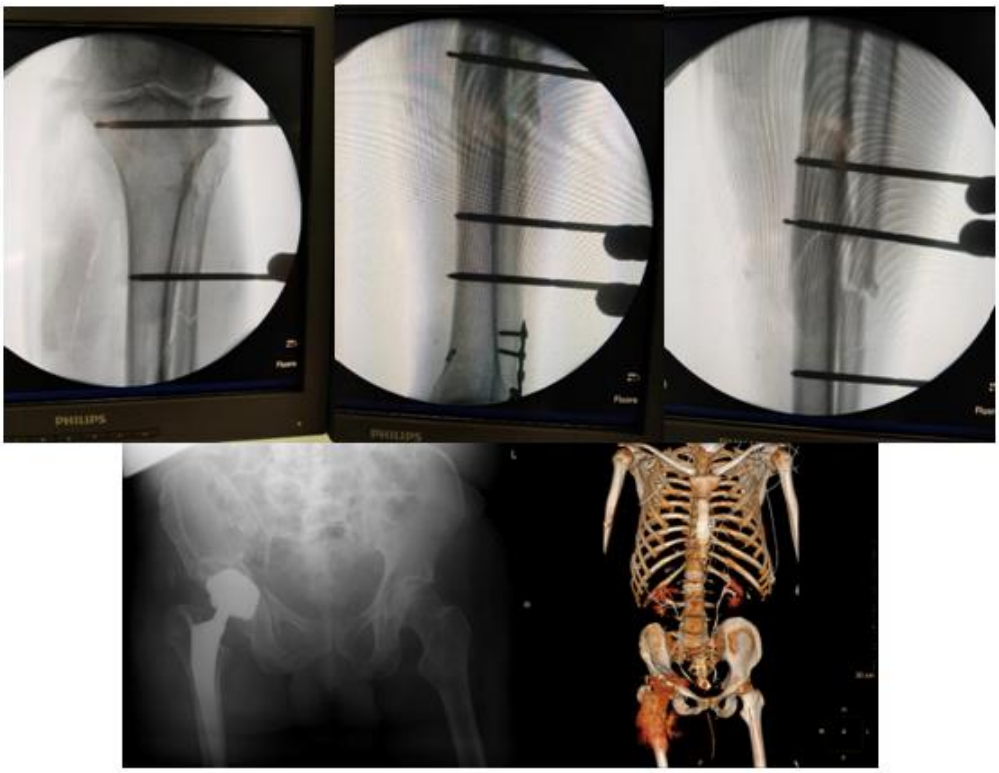

Fig. 5. X-rays, CT scan - pelvis on the day of injury

Discussion

The objectives of the primary survey are to identify and treat immediately life-threatening conditions, using the ABCDE approach for each patient. Airway assessment and management are the first priorities in the ATLS protocol. It should be cleared or secured before proceeding with the rest of the ABCDE assessment [1-3]. An appropriate verbal response from the patient indicates a patent airway with adequate oxygen delivery to the lungs, and cerebral perfusion pressure to supply blood to the brain. Simple maneuvers, including chin lift/jaw thrust, may assist airway patency. If there is evidence of neurological deficit, neck pain, or history of head injury or high-speed impact, then a cervical spine injury should be assumed. In patients who have had their cervical spine immobilized prehospital, this should be maintained until ruling out the injury. In patients who are apneic or who have a Glasgow coma scale (GCS) less than 8, the airway should be secured, most commonly with orotracheal intubation. Induction of general anesthesia using a rapid sequence induction (RSI) may be useful in patients requiring airway support. Breathing and air entry are then assessed by auscultating breath sounds and observing chest movement. Several serious chest injuries, like hemothorax or pneumothorax, can be identified by this physical exam only. Other injuries may need further assessment or imaging.

All clothing should be removed, and the patient's respiratory rate, work of breathing, tracheal position, and chest symmetry should be noted. The chest and axillae should be examined for abrasions, bruising, open wounds, and evidence of trauma. Auscultation and percussion of the chest will indicate the symmetry of ventilation and resonance. If a tension pneumothorax is suspected, needle decompression should be employed by inserting a wide-bore needle into the second intercostal space in the midclavicular line on the affected side. If a tension pneumothorax is suspected, treatment should not be delayed while awaiting for radiological confirmation. Hemothorax and pneumothorax both warrant the insertion of a wide-bore thoracostomy tube. Pulse oximetry will indicate arterial oxygenation, but supplemental oxygen may mask hypoventilation. Ultrasound of the chest may suggest the location of air pockets in the chest indicative of pneumothoraxes.

Bleeding is a common and potentially severe consequence of polytrauma [4,5]. The most likely cause of shock in trauma patients is a hypovolemic shock due to bleeding. Clinical signs of hypovolemic shock include tachycardia, prolonged capillary refill, cool peripheries, a drop in pulse pressure, increased respiratory rate, and a drop in conscious level. In some patients, a decrease in systolic pressure may not become apparent until they have lost up to 30% of their blood volume. The clinical presentation of hypovolemic shock follows the four classes of shock (Class I, II, III, and IV).

In patients presenting with hemorrhagic shock, access to the vascular system (usually venous access) must be obtained, and appropriate volume replacement should be initiated. Following intravenous access, blood should be drawn for cross-matching, along with the necessary blood tests. Patients should be connected to continuous ECG monitoring, non-invasive blood pressure monitoring set to an appropriate interval, so that response to resuscitation is measurable. If the patient has had a significant blood loss, non-invasive blood pressure measurement may not be recordable, in which case palpation of central pulses can help identify if there is any cardiac output. Volume resuscitation usually starts with a bolus of crystalloid fluids. If the patient does not respond adequately to this initial fluid replacement, or if the patient has sustained major injuries, then blood transfusion should be urgently considered [5,6]. Evidence suggests that a ratio of 1:1:1 for packed red cells, fresh frozen plasma, and platelets may provide the greatest survival advantage. Pharmacological adjuncts such as tranexamic acid should merit consideration in trauma patients. Tranexamic acid inhibits both plasminogen and plasmin, thus acting as an antifibrinolytic and reducing clot breakdown. Administration of 1 g bolus within three hours of injury followed by a further 1 g over the following eight hours is a commonly observed regime.

Control the source of bleeding is essential. Efforts of identifying the bleeding source should be concomitant with the volume resuscitation. External bleeding sources are usually easier to identify and control. Scalp laceration and open long bone fractures are common causes of eternal fractures. Internal bleeding sources are more challenging to identify and control. The thorax, abdomen, or pelvis may all be sources of bleeding that the clinician cannot immediately see. Appropriate examination and imaging, such as chest x-ray and focused assessment with sonography (FAST), will assist in the diagnosis of hemorrhage [7]. The clinical pelvic stability assessment may be performed once; further attempts may dislodge clot from an active bleeding point. A pelvic splint can be used to tamponade bleeding due to pelvic fractures. Definite treatment by an orthopedic surgeon should follow. Severe uncontrolled hemorrhage can drive the lethal triad in trauma patients of hypothermia, coagulopathy, and acidosis.

External hemorrhage control requires pressure on the bleeding source. Alternatively, proximal limb tourniquets are an option in uncontrolled bleeding. Tourniquets are designed to occlude arterial flow to a limb through compression and should be employed to control blood loss when methods such as compression, elevation, and topical hemostats have failed. They should only be used by practitioners trained in their proper use. They are usually applied 10 cm proximal to the injury,

directly on top of the skin and not over a joint. They should be used for a maximum of two hours to reduce the risk of ischemic damage to the limb. A proper and complete neurological function assessment is an essential component in the evaluation of polytrauma patients [8-10]. A normal initial exam is critical to document, so any consequent deterioration can be identified and properly managed. The assessment of neurological function includes central and peripheral deficits or injuries. Glasgow coma scale, pupils status/reaction, and the peripheral neurologic exam are parts of the assessment. In patients suspected of neurological injury, it is crucial that patients are not subjected to uncontrolled movement, and should be immobilized on a spinal board with appropriate cervical spine precautions. Spinal surgeons should be involved early in the case of suspected spinal injuries.

An open fracture, also called a compound fracture, is a fracture in which there is an open wound or break in the skin near the site of the broken bone. Most often, this wound is caused by a fragment of bone breaking through the skin at the moment of the injury.

An open fracture requires different treatment than a closed fracture, in which there is no open wound. Once the skin is broken, bacteria from dirt and other contaminants can enter the wound and cause infection. For this reason, early treatment of an open fracture focuses on preventing infection at the site of the injury. The wound, tissues, and bone must be cleaned out in a surgical procedure as soon as possible. The fractured bone is also usually stabilized with a surgical procedure to allow the wound to heal. The severity of an open fracture depends on several factors, including:

- The size and number of the fracture fragments,
- The damage to surrounding soft tissues (muscles, tendons, etc.),
- The location of the wound and whether the soft tissues in the area have good blood supply.

Open fractures pose an immediate risk of infection. In general, the greater the damage to the bone and soft tissues, the higher the risk of infection.

A bone infection can be difficult to treat. The patient may require long-term antibiotics and multiple surgical procedures. In extreme cases, where the infection cannot be cured and the patient's life is threatened, amputation may even be necessary. For this reason, preventing infection is the focus of an early treatment [10]. Almost all open fractures are treated with surgery. It is important to undergo surgery as soon as possible to allow the open wound to be cleaned, helping to prevent infection.

These are the first steps in controlling the risk for infection. During debridement, your doctor will remove all foreign and contaminated material, as well as damaged tissue, from the wound. If the wound is small, your doctor may need to create a larger incision to access all affected areas of bone and soft tissue. The wound will then be irrigated, or washed with fluid.

Once the wound has been cleaned, your doctor will evaluate the fracture and stabilize the bones. Open fractures are treated with either internal or external fixation.

If your wound and broken bones are not yet ready for a permanent implant, your doctor may apply external fixation to your injured limb. Most severe open fractures are first stabilized with external fixation. In this operation, the doctor inserts metal screws or pins through the skin into the bone above and below the fracture site. The pins and screws project out (stick out) of the skin where they are attached to metal or carbon fiber bars.

The external fixator has the advantage of stabilizing the broken bone while your doctor cares for the wound. In some cases, the wound may need further debridement or skin and tissue grafting to cover the injured bone. In most cases, an external fixator is kept in place only until it is safe to perform internal fixation. Sometimes, however, an external fixator is used to stabilize the bones until healing is complete. It is then removed in a subsequent (later) procedure when the fracture is healed.

Conclusion

This case underscores the challenges faced by trauma teams in balancing aggressive intervention with the potential for adverse outcomes. It highlights the importance of a multidisciplinary approach, incorporating trauma surgeons, thoracic surgeons, plastic and reconstructive surgeons and intensive care specialists to navigate the intricacies of polytrauma care. Ultimately, the case illustrates the necessity of individualized treatment plans that consider both immediate survival and long-term functional recovery.

Conflict of interests: None declared.

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Case report

HYPERTENSION IN PRIMARY HYPERPARATHYROIDISM: A CASE REPORT OF ECTOPIC PARATHYROID ADENOMA

ХИПЕРТЕНЗИЈА ПРИ ПРИМАРЕН ХИПЕРПАРАТИРЕОИДИЗАМ: СЛУЧАЈ НА ЕКТОПИЧНА ПАРАТИРЕОИДНА АДЕНОМА

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Abstract

Primary hyperparathyroidism is a condition characterized by excessive production of parathyroid hormone, often caused by an adenoma or hyperplasia of the parathyroid gland. One of the rare presentations of primary hyperparathyroidism is the development of an ectopic parathyroid adenoma, which can be challenging to diagnose and localize. Hypertension is observed seen in many cases of primary hyperparathyroidism (PHPT), although the exact mechanism is yet unclear. This paper aims to provide a comprehensive review of the current understanding of primary hyperparathyroidism, with a focus on the presentation of ectopic adenomas and the relationship between primary hyperparathyroidism and resistant hypertension. While most parathyroid adenomas are located in the normal anatomical position of the parathyroid glands, a small percentage (approximately 2-5%) can occur in ectopic locations, such as the thyroid gland, carotid sheath, or mediastinum. The atypical location of ectopic parathyroid adenomas can make them more difficult to identify and remove surgically, which can be difficult diagnostic challenge. The prevalence of primary hyperparathyroidism has been estimated to be around 0.1-0.4% in the general population, with a higher incidence in postmenopausal women.

The imaging tests consist mainly of an initial 99mTc-sestamibi SPECT/CT. Sensitivity and specificity of sestamibi scans are between 73%-80%, respectively. Clinical sensitivity is increased to 96% when sestamibi scans are combined with ultrasound.

Keywords: hypercalcemia, hyperparathyroidism, hypertension, ectopic parathyroid adenoma

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Абстракт

Примарниот хиперпаратироидизам е состојба која се карактеризира со прекумерно производство на паратироиден хормон, често асоцирано со аденом или хиперплазија на паратироидната жлезда. Една од ретките манифестации на примарен хиперпаратироидизам е ектопичен аденом на паратироидната жлезда, кој може да биде предизвик да се дијагностицира и локализира. Хипертензија е забележана во многу случаи на примарен хиперпаратироидизам (PHPT), иако точниот механизам сè уште е нејасен. Овој труд има за цел да обезбеди сеопфатен преглед на примарниот хиперпаратироидизам, со фокус на презентацијата на ектопични аденоми и корелацијата помеѓу примарниот хиперпаратироидизам и резистентната хипертензија. Повеќето паратироидни аденоми анатомски локализирани на параторироидната жлезда,мал процент (2-5%) се локализирано на тироидната жлезда, каротидната обвивка или медијастинумот. Атипичната локација на ектопичните паратироидни аденоми може да го отежне нивното идентификување и отстранување хируршки, што може да биде тежок дијагностички предизвик. Преваленцата на примарен хиперпаратироидизам е проценета на околу 0,1-0,4% кај општата популација, со повисока инциденца кај жените во постменопауза. Визуелизирачки метод за дијагноза на паратироидните аденоми е 99mTc-sestamibi SPECT/CT. Сензитивноста и специфичноста на скенирањата со 99mTc-sestamibi SPECT/CT. се помеѓу 73%-80%, соодветно. Клиничката сензитивност се зголемува на 96% кога 99mTc-sestamibi SPECT/CT се комбинира со ултрасонографски преглед.

Клучни зборови: хиперкалцемија, хиперпаратироидизам, хипертензија, ектопичен паратироиден аденом

Introduction

Primary hyperparathyroidism leads to increased levels of parathyroid hormone (PTH), which can alter calcium metabolism. Elevated calcium levels are associated with an increased risk of cardiovascular diseases by promotion of calcification and impairment of endothelial cell function. Hypertension is a common comorbidity associated with primary hyperparathyroidism, with studies suggesting that 40% of patients with primary hyperparathyroidism have hypertension [1]. Effective management of PHPT requires a multidisciplinary approach that includes comprehensive treatment of both hypertension and hypercalcemia, and surgical removal of parathyroid adenoma. Recognizing this association is vital for timely diagnosis and management, which may help to mitigate cardiovascular complications in patients with PHPT [2]. While parathyroidectomy remains to be primary treatment, it is important not to overlook the role of medications, such as bisphosphonates, cinacalcet and vitamin D, during the preoperative period [3].

Case Report

A 55-year-old female presented to the University Clinic for Cardiology with a hypertensive crisis; the blood pressure was measured at 250/160 mmHg, and the patient had a poor response to antihypertensive medications. She underwent echocardiography and carotid artery ultrasonography, both of which were unremarkable. Then, the patient was referred to the University Clinic for Nephrology. Renal ultrasound depicted a calculus in the right kidney. Blood test results revealed increased total and ionized calcium levels, as well as a low phosphate level along with increased PTH levels, findings consistent with a diagnosis of PHPT. Therefore, the patient was referred to our clinic.

Table 1. Initial laboratory results of the patient

Laboratory findings	Patient's results	Reference range
Inorganic phosphate	0.62	0.8 – 1.4 mmol/L
Calcium	3.2	2.1 -2.6 mmol/L
Ionized calcium	1.85	1.12 -1.31 mmol/L
PTH	301	10-69 pg/ml

A thyroid ultrasound revealed no significant findings. An ECG indicated left ventricular hypertrophy. DEXA scan confirmed high-grade osteoporosis in the spine and in the femur neck on both sides. The patient's antihypertensive regimen included calcium channel blockers (nifedipine 40 mg), angiotensin II receptor antagonists (valsartan 320 mg), mineralocorticoid receptor antagonists (spironolactone 25mg), and a loop diuretic (furosemide 40 mg).

In order to alleviate hypercalcemia, we administered 4 mg of zoledronic acid intravenously. Consequently, the ionized calcium level returned to normal, and PTH levels increased further. Following that, blood pressure control occurred. However, the duration of the effect was short, and severe hypertension ensued several days later when hypercalcemia reappeared.

Table 2. Laboratory results after administration of zoledronic acid

Laboratory findings	Patient's results	Reference range
Inorganic phosphate	0.35	0.8 – 1.4 mmol/L
Calcium	2.56	2.1 -2.6 mmol/L
Ionized calcium	1.28	1.12 -1.31 mmol/L
PTH	1034	10-69 pg/ml

A parathyroid scan using 99mTc-MIBI with SPECT/CT revealed hyperfunctional parathyroid tissue ectopically located in the retrosternal region. While waiting for surgical treatment, cinacalcet was initiated, with favorable outcomes in reducing calcium levels and controlling blood pressure.

Table 3. Laboratory results under treatment with cinacalcet

Laboratory findings	Patient's results	Reference range
Ionized calcium	1.38	1.12 -1.31 mmol/L
PTH	454	10-69 pg/ml

Discussion

Due to high blood pressure, worsened by hypercalcemia, the patient was treated with four antihypertensive medications, such as calcium channel blockers (nifedipine 40 mg), angiotensin II receptor blockers (valsartan 320 mg), a mineralocorticoid receptor antagonist (spironolactone 25 mg), and a loop diuretic (furosemide 40 mg). Intravenous zoledronic acid is the choice of treatment for severe hypercalcemia. However, as seen in our case, under extreme circumstances, its effects may be temporary. Consequently, therapy with cinacalcet 60 mg twice daily was initiated, resulting in significant improvement in calcium levels and patient's blood pressure control as well.

We also provided vitamin D supplementation. Vitamin D supplementation is important in terms of preventing postoperative hypocalcemia in the setting of "hungry bone disease". The patient underwent a CT scan of the neck and chest to determine the precise location of the parathyroid adenoma. Subsequently, the adenoma was excised via transsternal thoracotomy. Pathohistological analysis indicated the presence of oxyphilic adenomas. Following surgery, the patient was treated with 1500 mg of calcium carbonate and 2000 IE of vitamin D, and the patient's calcium and PTH levels returned to the reference range. Primary hyperparathyroidism is a

challenging diagnosis given that one of the symptoms, hypertension, is often treated at primary care and many neglect ordering further tests to investigate the true cause. With the given symptoms and laboratory results, an ectopic adenoma takes time to diagnose given the unusual location and the limited resources available in small countries. Conservative treatment may ease symptoms and make little improvements in the patient's daily life, but ultimately surgery is the mainstay treatment along with lifelong supplementation.

Conclusion

Based on the patient's history and laboratory investigations, elevated total and ionized calcium, and abnormally high levels of PTH, accompanied by low inorganic phosphates, a diagnosis of PHPT was made. Further investigations with SPECT/CT revealed an ectopically located parathyroid adenoma in the mediastinum. Primary hyperparathyroidism is a condition characterized by the overproduction of PTH, mostly due to a parathyroid, adenoma although it may also arise from ectopic parathyroid adenoma [9]. The prevalence of asymptomatic hypercalcemic patients is significant [8]. Treatment of hypertension in the setting of PHPT can be quite challenging and complicated in cases of ectopic parathyroid adenoma. Bisphosphonates are an effective treatment for hypercalcemia; however, their efficacy may be temporary in cases of severe PHPT. The combination of bisphosphonates, cinacalcet, has proven to be an effective treatment for hypercalcemia [4-7]. We recommend a level of consideration for hypercalcemia to be maintained in patients presenting with refractory hypertension or hypertensive crises. Treatment should not be overlooked but surgery should not be delayed if the improvements we want are not achieved.

Conflict of interests: None declared.

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Case report

PRENATAL DIAGNOSIS OF NON-INHERITED OSTEOGENESIS IMPERFECTA: A CASE REPORT

ПРЕНАТАЛНА ДИЈАГНОЗА НА НЕ-НАСЛЕДНА ОСТЕОГЕНЕЗИС ИМПЕРФЕКТА: ПРИКАЗ НА СЛУЧАЈ

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Abstract

Osteogenesis imperfecta (OI) is a group of rare inherited disorders of connective tissue, characterized by excessive bone fragility. It results from mutations in the *COL1A1* and *COL1A2* genes, which encode the alpha 1 and alpha 2 chains of type I collagen, respectively. Severe OI is perinatally lethal, while mild OI can sometimes be unrecognized until adulthood.
Severe or lethal forms of OI can typically be identified through antenatal ultrasound and confirmed using advanced imaging techniques and genetic testing. A combination of imaging modalities, including ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI), allows for accurate detection of OI and can also aid in predicting lethality before birth. Furthermore, prenatal genetic testing-whether noninvasive or invasive-can definitively confirm the diagnosis, providing crucial information for early medical intervention and parental counseling. We present a case of non-inherited osteogenesis imperfecta caused by a *de novo* mutation (c. 1094G>T, p.(Gly365Val)) in the *COL1A1* gene, diagnosed prenatally in the early second trimester. Early detection of skeletal dysplasia using ultrasound, followed by confirmation with genetic testing, allowed for a timely diagnosis and appropriate counseling.

Keywords: Osteogenesis imperfecta, skeletal dysplasia, fetal ultrasound, de novo mutation, prenatal diagnosis

Апстракт

Остеогенезис имперфекта (OI) претставува група на ретки наследни нарушувања на сврзното ткиво, кои се карактеризираат со прекумерна кршливост на коските. Ова нарушување настанува како резултат на мутации во гените COL1A1 и COL1A2, кои ги кодираат алфа 1 и алфа 2 синџири на колаген тип I. Тешките форми на OI се перинатално смртоносни, додека поблагите форми може да останат непрепознаени сè до зрелата возраст. Тешките или смртоносни форми на OI најчесто можат да се детектираат преку антенатален ултразвук и да се потврдат со напредни техники на снимање и генетско тестирање. Комбинацијата на различни методи за снимање, како што се ултразвук, компјутерска томографија (CT) и магнетна резонанца (MRI), овозможува прецизна детекција на OI и може да помогне да се предвиди леталитетот пред породување. Дополнително, пренаталното генетско тестирање-било да е неинвазивно или инвазивно-може дефинитивно да ја потврди дијагнозата, овозможувајќи навремена медицинска интервенција и советување на родителите. Претставуваме случај на ненаследна остеогенезис имперфекта, предизвикана од de novo мутација (c.1094G>T, p.(Gly365Val)) во COL1A1 генот, која беше дијагностицирана пренатално во раниот втор триместар. Раната детекција со ултразвучен преглед, проследена со потврда преку генетско тестирање, овозможи навремена дијагноза и соодветно советување.

Клучни зборови: Остеогенезис имперфекта, скелетна дисплазија, фетален ултразвук, de novo мутација, пренатална дијагноза

Introduction

Osteogenesis imperfecta (OI) is a group of rare genetic disorders primarily affecting connective tissue and characterized by fragile bones prone to fractures [1,2]. OI is commonly caused by mutations in the *COL1A1* and *COL1A2* genes, which encode the alpha 1 and alpha 2 chains of type I collagen [3]. Over 1,500 mutations have been identified in these genes [4]. Rear autosomal recessive and additional X-linked mutations have been

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found in other genes, such as *LEPRE1*, *CRTAP*, and *PPIB* [5,6].

The incidence of OI is approximately 1 in 20,000 live births, with manifestations observed across all racial and ethnic groups [1]. While OI is typically inherited in an autosomal dominant manner, autosomal recessive forms have also been documented [6,7]. The clinical classification of OI, as proposed by Sillence *et al*. [8], divides the disorder into four types: **Type I**: Mild OI, **Type II**: Perinatal lethal OI, **Type III**: Progressive deforming OI, **Type IV**: Moderately severe OI. This classification is based on specific phenotype although within the same genetic mutations, different phenotype may be seen.

With an increase in the discovery of the number of gene mutations responsible for causing OI, the classification of OI subtypes has expanded up to OI type XX to date [9]. The International Nomenclature Group of Constitutional Disorders of the Skelton (INCDS) divides the disorder into five groups. This classification adds OI type V to the Sillence classification, which is characterized by calcification of the interosseus membrane and is phenotypically distinct from the other four types [10].

The timing of the diagnosis depends on the severity, and it can be done prenatally, after birth, in childhood or in adulthood [6].

Common features of OI include short stature, multiple fractures, often occurring *in utero* or during the perinatal period, demineralization of the bones, blue sclera, otosclerosis with hearing loss, a high arched palate, joint hyperlaxity, dentinogenesis imperfecta (defective dentition), scoliosis, and growth retardation [6]. Wormian bones may also be observed on skull X-ray. Importantly, cognitive development is typically unaffected [11].

Due to the complexity and large variety of these diseases, accurate prenatal diagnosis of skeletal dysplasias remains a challenge, with only approximately 65% of cases being accurately diagnosed by conventional 2D US [12]. Prenatal diagnosis of OI is usually achieved in second trimester during the second trimester anomaly scan [13]. Given the advances in ultrasound technology, if the ultrasound exam is performed by a skilled sonographer, bone abnormalities and skeletal dysplasias can be detected in late first and early second trimester [13].

Prenatal diagnosis is primarily achieved through ultrasound examination and invasive genetic testing [14,15]. The first ultrasound marker that raises suspicion of OI is a shortened femur [16]. Other ultrasound features are long bone shortening, no posterior acoustic shadowing from the long bones, fractures of the long bones and ribs and bowing of the long bones [17].

The aim of this case report is to present an early prenatal diagnosis of OI with *de novo* mutation in the *COL1A1* gene.

Methods

The fetal ultrasound imaging was performed using a 5 MHz transabdominal transducer from Voluson E10 ultrasonography device.

Case Report

We present the case of a 38-year-old patient, gravida two, parity one. She was referred to our Clinic at 14+6 weeks of gestation for a fetal morphological ultrasound exam. The first trimester anomaly scan was negative; however, the combined biochemical screening test showed a high risk for Down syndrome (1:234). Obstetric history revealed one healthy live-born baby via C-section 7 years earlier. There was negative family history. Past medical history included hypothyroidism, managed with Euthyrox.

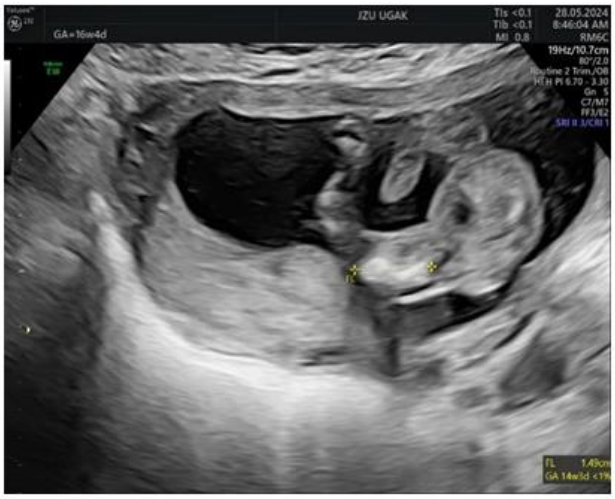

Fig. 1. Curved and short femur

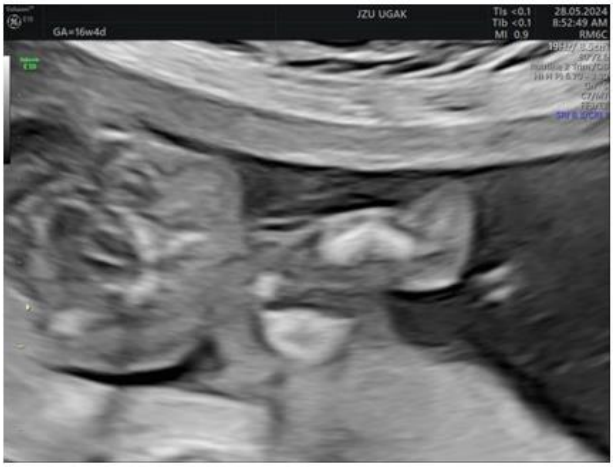

Fig. 2. Curved humerus

The first obstetric ultrasound exam at our Clinic revealed a single fetus with positive heart activity. The fetus had shortened bones in both lower and upper ex-

tremities. The lengths of the humerus and femur were <1st percentile for the referred gestational age. The femoral bones were extremely short and curved. The lower extremities were flexed, without extension movements. The spine looked normal., and the skull had a normal appearance. Both placenta and amniotic fluid were within normal limits. These ultrasound findings were consistent with the differential diagnosis of skeletal dysplasias and possible osteogenesis imperfecta.

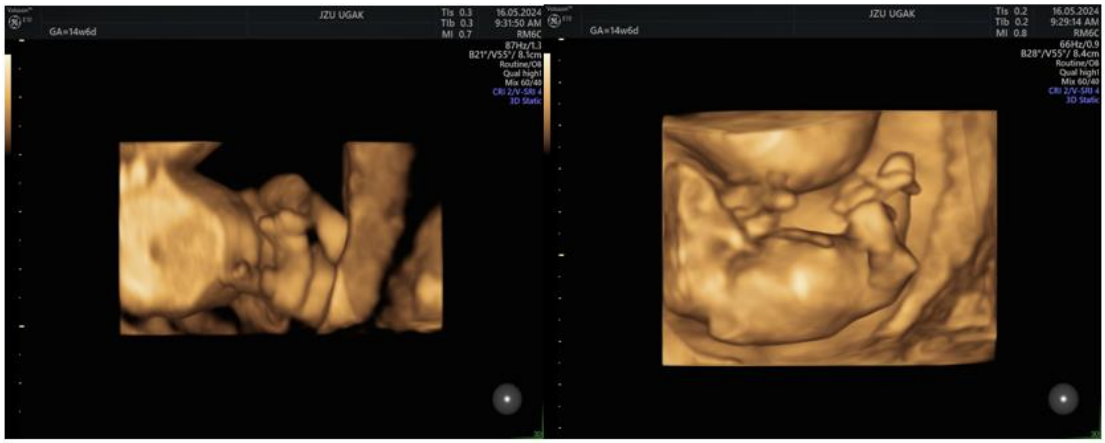

Fig. 3 and 4. 3D image of lower extremities

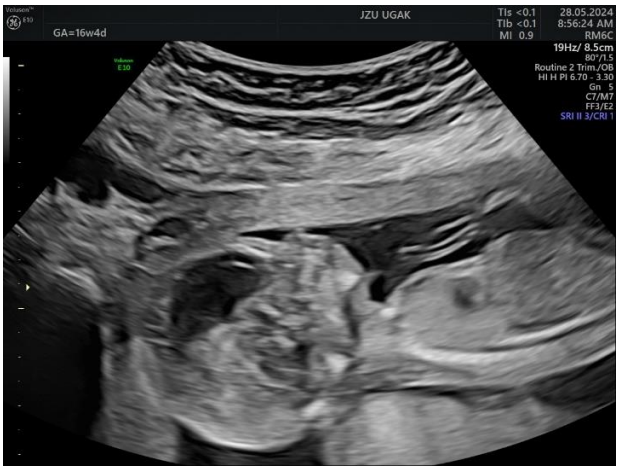

Fig. 5. Small fetal chest

Amniocentesis was performed at 16 weeks of gestation. Genetic testing of the amniotic fluid revealed the presence of the pathogenic *COL1A1* mutation (c. 1094G>T, p. (Gly365Val)) in a heterozygous state in the fetus. Further genetic analysis of the maternal and paternal genomes did not reveal the presence of this variant, confirming the mutation was *de novo* occurrence in the fetus. This genotype was consistent with a diagnosis of osteogenesis imperfecta.

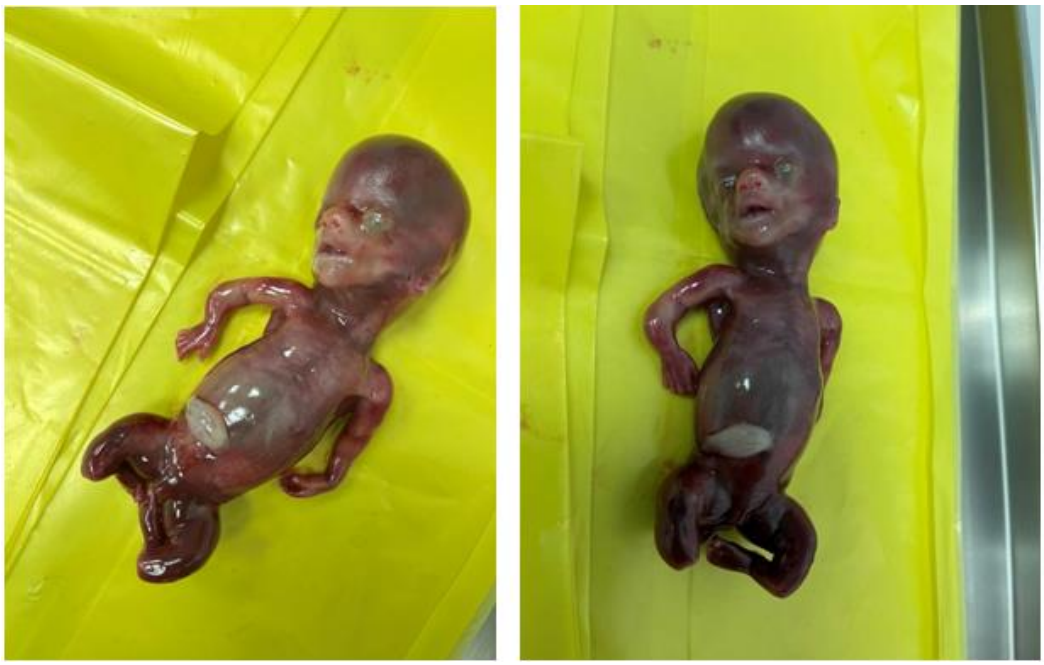

Fig. 6 and 7. Fetus with short and curved extremities

The second ultrasound exam at 17+4 weeks of gestation showed a short, curved femur, and a short humerus that was also curved with fractures by this time. A small chest and easily compressible calvaria were also observed. The femur-to-abdominal circumference ratio was 0.13. This ratio is below the threshold of 0.16, which is shown to be associated with lethal skeletal dysplasias [18].

Given the severity of the condition and the prognosis for the fetus, the pregnancy was terminated with medical abortion at 17+6 weeks of gestation. The phenoltype of the fetus correlated with the prenatal ultrasound findings.

Discussion

This case represents a rare occurrence of a non-inherited, *de novo* mutation in the *COL1A1* gene causing osteogenesis imperfecta. While OI is most commonly inherited in an autosomal dominant pattern, the presence of a *de novo* mutation in this case underscores the genetic complexity and variability of this disorder.

Prenatal diagnosis played a crucial role in this case, enabling early detection and providing the parents with critical information to make informed decisions regarding the pregnancy. Early detection of skeletal dysplasia using ultrasound, followed by confirmation with genetic testing, allowed for a timely diagnosis and appropriate counseling.

Diagnostic modalities, including ultrasound (US) and molecular testing, are crucial for the prenatal diagnosis of OI. The diagnostic approach depends on the presence or absence of a family history. In known familial cases, genetic counseling and inheritance pattern analysis (autosomal dominant or recessive) guide expectant couples. Without a family history, clinicians typically suspect fetal OI during second-trimester US based on decreased femoral length.

Prenatal diagnosis of osteogenesis imperfecta can be achieved by ultrasound examination, computed tomography (CT) and magnetic resonance imaging (MRI), genetic testing-noninvasive (NIPT test) or invasive (amniocentesis).

Ultrasound (US) is a standard antenatal procedure, typically performed at least twice-once in the first trimester of pregnancy and second dating around 20 weeks to detect congenital anomalies. Since fetal skeletal development begins at eight weeks, with secondary ossification centers visible by 20 weeks, second-trimester screening is ideal for diagnosing OI and other skeletal dysplasias. Femoral length (FL) is the most reliable indicator of skeletal dysplasia, and fetuses with FL below the fifth percentile or two standard deviations below the mean should undergo further evaluation by fetal medicine specialists.

Prenatal or neonatal lethality is one of the most significant concerns at the time of US diagnosis. Because OI is a rare condition, the criteria for lethality are usually examined in conjunction with other skeletal dysplasias. Several case series have demonstrated that an FL (femur length) to AC (abdominal circumference) ratio could predict the fetal outcome (18). The ratio of FL to AC <0.16 is a predictor of lethal skeletal dysplasia [18-20]. Ramus *et al*. demonstrated that 92% of fetuses with FL/AC ratio <0.16 had lethal skeletal dysplasia and none of the cases with FL/AC ratio >0.16 had lethal form of skeletal dysplasia [19]. In our case, the FL/AC ratio was 0.13, which strongly indicated that the fetus had a lethal form of skeletal dysplasia.

Definitive diagnosis is made by amniocentesis or non-invasive genetic testing (NIPT). Non-invasive prenatal testing (NIPT) analyzes circulating cell-free fetal DNA (cffDNA) in maternal blood for genetic testing [21]. Since 2011, it has become widely available for aneuploidy screening and is now a common prenatal diagnostic tool. Non-invasive prenatal diagnosis (NIPD) for single-gene disorders is also advancing, though at a slower pace due to technical challenges and a smaller market share [22,23].

Once imaging suggests a prenatal diagnosis of OI, laboratory investigations and genetic counseling are recommended [24]. In cases of lethal OI, termination of pregnancy may be considered, though legal and cultural restrictions affect access in many regions. If termination is not an option, discussions on postnatal resuscitation become crucial. These decisions are often ethically and emotionally challenging for families.

Counseling plays a key role in presenting options, discussing risks and benefits, and ensuring parents receive balanced information while respecting their reproductive autonomy. However, some studies suggest that counseling can feel directive due to staff attitudes. Research indicates that shared decision-making reduces decisional conflict and regret in parents facing similar diagnoses [25].

Although *de novo* mutations in OI are rare, this case emphasizes the importance of prenatal genetic screening, particularly in high-risk pregnancies or in those with a history of genetic disorders. Moreover, the ability to identify OI early *in utero* provides an opportunity for parents to consider all potential outcomes and make decisions that are in line with their preferences and understanding of the condition. Early detection of skeletal dysplasia using ultrasound, followed by confirmation with genetic testing, allows for a timely diagnosis and appropriate counseling.

Conclusion

This case represents a rare instance of a non-inherited, *de novo* mutation causing osteogenesis imperfecta, diagnosed prenatally. Prenatal ultrasound plays a crucial role in detecting fetal skeletal abnormalities and assessing the possible outcome given that even within

the same genetic mutations, different phenotype may be seen. In this case, early detection allowed for informed decision-making, illustrating the importance of prenatal diagnosis in managing complex genetic conditions such as osteogenesis imperfecta.

Conflict of interests: 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. Вариа е (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.

ТЕКСТ НА РАКОПИСОТ

Сите ракописи се испраќаат во електронска форма на електронската адреса (mld@unet.com.mk ; info@mld.mk) 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. email); 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.

1. **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.

2. **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 Index Medicus):

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.

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

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.