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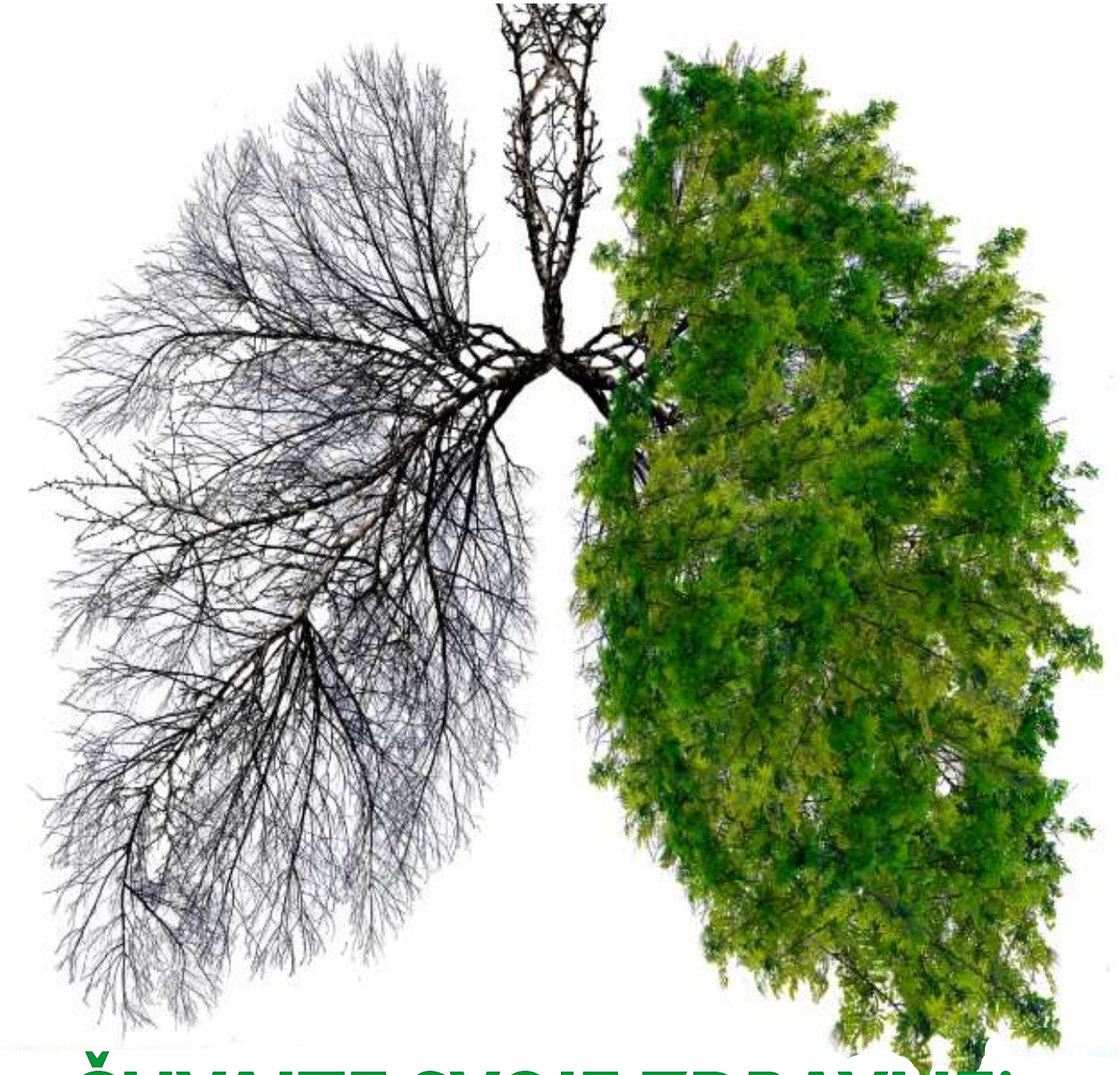

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Novi Evropski vodič za prevenciju tromboembolizma kod A Fib

CHA_2DS_2-VASc skor za procjenu rizika od tromboembolizma kod A Fib!

Risk factor-based point-based scoring system - CHA_2DS_2 -VASc

Risk factor	Score
Congestive heart failure/LV dysfunction	1
Hypertension	1
Age ≥75	2
Diabetes mellitus	1
Stroke/TIA/thrombo-embolism	2
Vascular disease*	1
Age 65–74	1
Sex category (i.e. female sex)	1
Maximum score	**9**

*Prior myocardial infarction, peripheral artery disease, aortic plaque. Actual rates of stroke in contemporary cohorts may vary from these estimates.

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Major i non-major riziko fakori za procjenu tromboembolizma kod A Fib!

Risk factors for stroke and thrombo-embolism in non-valvular AF

Major risk factors	Clinically relevant non-major risk factors
Previous stroke	CHF or moderate to severe LV systolic dysfunction [e.g. LV EF ⊠ 40%]
TIA or systemic embolism	Hypertension
Age ⊠75 years	Diabetes mellitus
	Age 65-74 years
	Female sex
	Vascular disease

AF = atrial fibrilation; EF = ejection fraction (as documented by echocardiography, radio nudide ventriculography, cardiac catheterization, cardiac magnetic resonance imaging, etc.); LV = left venticular; TIA = trasient ischaemic attack.

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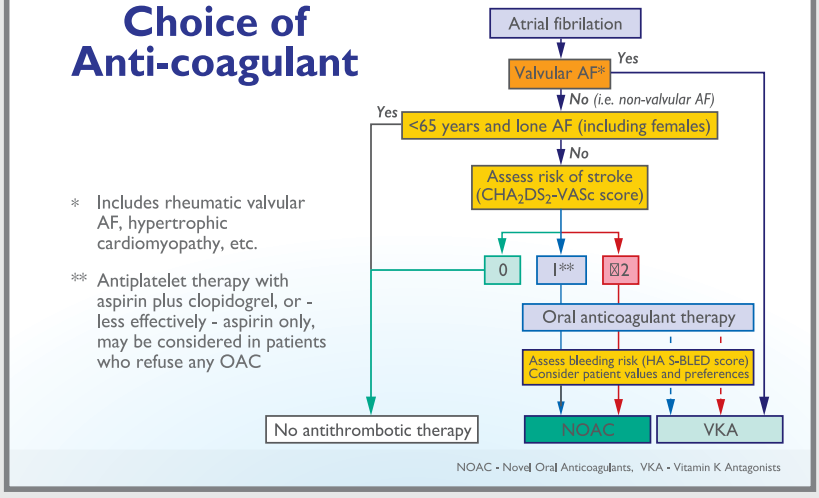
Algoritam antikoagulantne terapije nakon procjene CHA_2DS_2VASc i major risk faktora!

Choice of Anti-coagulant



* Includes rheumatic valvular AF, hypertrophic cardiomyopathy, etc.

** Antiplatelet therapy with aspirin plus clopidogrel, or - less effectively - aspirin only, may be considered in patients who refuse any OAC

NOAC - Novel Oral Anticoagulants, VKA - Vitamin K Antagonists

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AIMS AND SCOPE

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Address:
Medical Journal, Discipline for Research and Development
Clinical Center University of Sarajevo,
71000 Sarajevo,
Bolnička 25,
Bosnia and Herzegovina,
Phone: +387 33 298 514
Web. www.kcus.ba
Technical secretariat: svjetlana.barosevcic@kcus.ba

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Original articles

Case reports

Epilepsy and pregnancy: clinical characteristics and outcomes

Epilepsija i trudnoća: kliničke karakteristike i ishodi

Mohammad Abou El-Ardat[1]*, Sebija Izetbegović[2], Lana Lačević[1]

[1]Clinic of Gynecology and Obstetrics, Clinical Center University of Sarajevo, Jezero, 71000 Sarajevo, Bosnia and Herzegovina
[2]Clinical Center University of Sarajevo Management, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Introduction: epilepsy is a common chronic disorder that affects approximately more than one million women of reproductive age. Reproductive function can be adversely affected in women with epilepsy by reducing fertility, increasing the risk of polycystic ovary syndrome, abnormal menstrual cycles, and altering antiepileptic drug (AED) metabolism. Most epileptic seizures during pregnancy occur in women with pre-existing epilepsy. Aim: to investigate and present the clinical characteristics and outcomes of epilepsy during pregnancy. Materials and methods: the research included the clinical characteristics of 26 women with epilepsy who were pregnant at the Clinic of Gynecology and Obstetrics of the Clinical Center University of Sarajevo, in the period from 2020 to 2022. Results: the average age at which seizures occurred was 21.90±2.42 years. All of their first seizures occurred in pregnancy, specifically 16 (61.50%) in the first trimester, 7 (27.00%) in the second trimester, and 3 (11.50%) in the third trimester. The largest number of patients had a focal attack (11/26, 42.30%). Regarding the frequency of seizures in female patients, sporadic seizures were found in the majority of patients (17/26, 65.40%). CSE was found in 4 patients (15.40%), who did not take therapy regularly. The patients were advised to start antiepileptic drug (AED) therapy, while all 26 of them took AED (levetiracetam in 4, carbamazepine in 15 and intravenous diazepam in 7 CSE) during pregnancy. Conclusion: the conducted study showed that epileptic seizures in the largest number of pregnant women occurred in the first trimester, including focal seizures. Sporadic seizures were found in most pregnant women. The patients were advised to take antiepileptic drug therapy, and all examined pregnant women took it.

Keywords: epilepsy, pregnancy, seizures, antiepileptic therapy, outcomes

SAŽETAK

Uvod: epilepsija je čest hronični poremećaj koji pogađa otprilike više od milijun žena u reproduktivnoj dobi. Reproduktivna funkcija može negativno utjecati na žene s epilepsijom smanjenjem plodnosti, povećanjem rizika od sindroma policističnih jajnika, abnormalnim menstrualnim ciklusima i promjenom antiepileptika (AED) metabolizma. Većina epileptičkih napada tokom trudnoće javlja se kod žena s već postojećom epilepsijom. Cilj: istražiti i prikazati kliničke karakteristike i ishode epilepsije tokom trudnoće. Materijali i metode: istraživanjem su obuhvaćene kliničke karakteristike 26 žena sa epilepsijom koje su vodile trudnoću na GAK Sarajevo, u periodu od 2020. do 2022. godine. Rezultati: prosječna dob u kojoj su nastupili napadaji bila je 21,90 ± 2,42 godine. Svi njihovi prvi napadi dogodili su se u trudnoći, uključujući 16 (61,50%) u prvom tromjesečju, 7 (27,00%) u drugom tromjesečju i 3 (11,50%) u trećem tromjesečju. Najveći broj pacijentica ima fokalni napad (11/26, 42,30%). Što se tiče učestalosti napada pacijentica, sporadični napadi su pronađeni u većine bolesnika (17/26, 65,40%). CSE je pronađen kod 4 pacijentice (15,40%), koje nisu redovno uzimale terapiju. Pacijenticama je savjetovano započeti terapiju antiepilepticima (AED), dok je svih njih 26 uzimalo AED (levetiracetam u 4, karbamazepin u 15 i intravenski diazepam u 7 CSE) u trudnoći. Zaključak: Sprovedena studija je pokazala da su se epileptični napadi kod najvećeg broja trudnica dogodili u prvom tromjesečju, uključujući fokalni napad. Sporadični napadi su pronađeni u većine trudnica. Pacijenticama je savjetovano da uzimaju terapiju antiepilepticima, te su je sve ispitane trudnice uzimale.

Ključne riječi: epilepsija, trudnoća, napadi, antiepileptična terapija, ishodi

INTRODUCTION

Epilepsy is one of the common chronic disorders affecting women of reproductive age (1). The occurrence of seizures during pregnancy is really a challenging situation which risks the health of both mothers and fetuses (2). Epilepsy is a serious neurological problem that is most often encountered in obstetric practice (3). While many women with epilepsy experience no change in seizure frequency during pregnancy, up to one-third of women have an increase in seizure frequency (4).

The physiological changes that occur to maintain homeostasis continue throughout pregnancy and the new hormonal balance has the potential of altering neuronal excitability and the seizure threshold (5). In addition, it is well known that anticonvulsant/antiepileptic drugs (AEDs) interact with female sex hormones (endogenous as well as exogenous) by decreasing their levels (6). Further, these pharmacological agents may also induce

major malformations in the fetus (7). The teratogenic effects of these drugs indeed pose a serious concern for the patients and their healthcare providers. Moreover, the pharmacokinetics of these drugs keep on changing throughout the pregnancy because of the changes in the water balance of the body (8).

Previous studies showed that structural and metabolic changes may precipitate new onset seizures during pregnancy, including intracranial hemorrhage, cerebral venous sinus thrombosis, ischemic stroke, brain tumor, hydrocephalus, infection, hypoglycemia, acute intermittent porphyria, and so on (9,10). However, most of these seizures were only the acute symptoms of underlying diseases; rare would develop into epilepsy, especially without obvious structural abnormalities. There is a condition that some women may have their first seizures during pregnancy and continue to get spontaneous recurrent seizures after delivery, which is called new onset epilepsy during pregnancy (11).

It's difficult to predict how pregnancy will affect epilepsy. More than 95% of pregnant women with epilepsy give birth to a healthy child (12). It is important to convince women of the likelihood of a good pregnancy outcome with epilepsy, but it is also crucial to provide counseling before conception (13). A recent study showed that only 46% of women with epilepsy remembered information about interactions between AEL and contraceptives, 63% about the need to plan pregnancy, and only 56% about the need for folic acid supplementation (14).

In the US, more than one million women with epilepsy are of childbearing age and have over 20.000 babies each year. Patients with epilepsy who become pregnant are at risk of complications, including changes in seizure frequency, maternal morbidity and mortality, and congenital anomalies due to antiepileptic drug exposure. Appropriate management of epilepsy during pregnancy may involve frequent monitoring of antiepileptic drug serum concentrations, potential preconception switching of antiepileptic medications, making dose adjustments, minimizing peak drug concentration with more frequent dosing, and avoiding potentially teratogenic medications. Ideally, preconception planning will be done to minimize risks to both the mother and fetus during pregnancy. It is important to recognize benefits and risks of current and emerging therapies, especially with revised pregnancy labeling in prescription drug product information (15).

Planning pregnancy is the most optimal and safest method for both the woman with epilepsy and her future child. This allows for optimization of pharmacotherapy in order to reduce the risk of fetal anomalies on one hand and, on the other, to best control seizures during pregnancy, which reduces the risk of subsequent developmental disorders in the child. However, the US studies indicate that 50% of pregnancies are not planned, and 40% of patients diagnosed with epilepsy do not consult a physician before becoming pregnant (17,18). Although the risk of pregnancy complications is higher in women with epilepsy than in the non-epileptic ones, more than 90% of pregnancies have a normal course, and these women deliver healthy babies (16).

The key element in the care of women with epilepsy of childbearing age is pregnancy planning. While the number of planned pregnancies is rising as a result of increased awareness and knowledge of epilepsy, 40% are still unplanned. The risk of major fetal malformations is higher in women with epilepsy taking AEDs (19,20). The concerns of a woman with epilepsy about the adverse effects of AEDs on her child may prompt her to arbitrarily discontinue or reduce doses of AEDs, increasing the risk of seizures and even resulting in SUDEP syndrome (sudden unexpected death in epileptic patients) (21,22,23). It is therefore important to promote early care of women of childbearing age (15-44 years) who are treated for epilepsy by specialized neurologic and gynecological clinics (24).

AIM

The aim of this study was to investigate and present the clinical characteristics and outcomes of epilepsy during pregnancy.

MATERIALS AND METHODS

The retrospective study included 26 pregnant women with epilepsy hospitalized at the Clinic for Gynecology and Obstetrics of the Clinical Center University of Sarajevo (CCUS) over the period of two-year. The respondents were women with epilepsy and a reproductive history from 2020 to 2022, selected from the pregnancy registry of patients with epilepsy. Inclusion criteria were: pregnant women from Sarajevo Canton; age between 18-40 years, regardless of parity; women with the first attack during pregnancy. Exclusion criteria were: women with first seizure before pregnancy; women with first seizure after delivery; women with evidence of eclampsia; women with other severe neurological, psychiatric or systemic diseases.

Detailed demographic data, clinical characteristics, neurologic status, associated tests, management modalities, seizures, and pregnancy outcomes were recorded and monitored.

Statistical analysis was performed using SPSS (version 20.0, SPSS Inc., Chicago, IL). Descriptive analysis was applied to process demographic data, clinical characteristics and pregnancy outcomes, presented as percentages, means and standard deviations.

RESULTS

The study examined the pregnancy histories of 26 patients with epilepsy from 2020 to 2022. Clinical characteristics, treatment and outcome are presented in the following tables.

Clinical characteristics

The sociodemographic and clinical characteristics of the 26 women included in the study are presented in Table 1.

Table 1 **Clinical characteristics of patients with epilepsy.**

Variable	Number (f)	Proportion (%)	Mean	SD
Female	26	100.00 %		
Previous pregnancy				
0	0	0.00 %		
1	13	50.00%		
2	10	38.50%		
3	3	11.50%		
Age at seizure onset (yr) Min-max (18-27)	-	-	21.90	2.42
Seizure type				
FS (focal seizures)	11	42.30%		
GTCS (generalized tonic-clonic seizures)	9	34.60%		
sGTCS (secondary generalized tonic-clonic seizures)	2	7.70%		
CSE (convulsive status epilepticus)	4	15.40%		
Seizure frequency in pregnancy				
Sporadic	17	65.40%		
Frequent	3	11.50%		
Very freguent	2	7.70 %		
CSE (convulsive status epilepticus)	4	15.40%		
Pregnancy period of first seizure				
First trimester	16	61.50%		
Second trimester	7	27.00%		
Third trimester	3	11.50%		

The largest number of patients had one previous pregnancy, 13 of them (50.00%), 10 of them had 2 pregnancies (38.50%), and three patients had a third pregnancy (11.50%). The mean age at onset of seizures was 21.90±2.42 years (range: 18–27 years). All of their first seizures occurred during pregnancy, including 16 (61.50%) in the first trimester, 7 (27.00%) in the second trimester, and 3 (11.50%) in the third trimester. Therefore, the greatest number of attacks occurred in the first trimester of pregnancy.

The largest number of patients has a focal attack (11/26, 42.30%), while 9 of them have primary GTCS during pregnancy (34.60%), and 2 of them have secondary GTCS during pregnancy (7.70%). Furthermore, 4 patients (15.40%) had CSE (15.40%).

Regarding the frequency of seizures in female patients, sporadic seizures were found in most patients (17/26, 65.40%), while frequent and very frequent seizures were found in only 5 patients. CSE was found in 4 patients (15.40%), who did not take therapy regularly.

Routine antenatal examinations were performed in almost all patients, including physical examination, laboratory tests and prenatal ultrasound. There was no evidence of preeclampsia, including hypertension, proteinuria, thrombocytopenia, or hepatic and renal dysfunction. A chromosomal test was performed in 22 patients (84.60%), and no evident abnormality (trisomy 21 syndrome) was found in any fetus.

Treatment and outcome

Table 2 **Treatment and pregnancy outcome of patients with epilepsy.**

Variable	Number (f)	Proportion (%)
Female	26	100.00%
AED during pregnancy		
LEV (levetiracetam)	4	15.40%
CBZ (carbamazepine)	15	57.70%
DIA iv (diazepam intravenozno)	7	26.90%
Folate supplement (0,4 mg/d) and multivitamin	18	69.20%
Neuroimaging finding		
Negative	2	7.70%
Positive	16	61.60%
HS (hippocampus sclerosis)	3	11.50%
Possible FCD (focal cortical dysplasia)	5	19.20%
Abnormal situation in pregnancy		
Fetal distress	6	23.10%
Induced abortion	10	38.50%
Mild harelip	1	3.80%
Atresia of the anus	2	7.70%
Trisomy-21 syndrome	0	0.00 %

Folate (0.4mg/day) was supplemented during the first trimester in 18 patients (69.20%). The patients were advised to start antiepileptic drug (AED) therapy, while all 26 of them took AED (levetiracetam in 4, carbamazepine in 15 and intravenous diazepam in 7 CSE) during pregnancy. The neurologist's report was negative in the case of 2 patients, while it was positive in 16 patients (61.60%), HS was detected in 3 patients, and focal cortical dysplasia was possible in 5 of them (19.20%).

Out of the total number of patients, 7 (7/26, 26.90%) had easy deliveries and gave birth to healthy babies, while 19 (73.10%) faced abnormal situations. Six babies suffered fetal distress before birth and had to be treated in the neonatol unit, fortunately, without serious complications. Two of these occurred in patients with CSE in the third trimester. However, the other 3 were much worse. One patient had to choose an induced abortion in the first trimester due to the absence of a fetal heartbeat. One baby was diagnosed with mild cleft lip. Atresia of the anus was observed in two babies. Trisonomy syndrome was not observed.

DISCUSSION

One in six women with epilepsy live in India, accounting for 2.73 million patients in the country, 52% of whom are of reproductive age (15-49 years) (25). In the US, approximately 1.5 million women with epilepsy are of childbearing age and give birth to approximately 25.000 children per year, that is, 3-5 children per 1000 born. The results obtained by Pennel PB, et al., may indicate that primary causes of reduced fertility seem social rather than biological (26). They evaluated 197 women- 89 with epilepsy and 108 as a control group. Among women with epilepsy, 60.7% achieved pregnancy and among the control group, 60.2% within 21 months.

Most women with epilepsy do not have seizures during pregnancy, whereas a third of them may experience an increase in the number of epileptic seizures occurring during this period (27). The data from the Epilepsy Pregnancy Registry report that seizures are not observed in 50-67% of women (28,29,30). The EURAP study covering 42 countries and involving 3784 pregnant women with epilepsy found that 66.6% were seizure-free, with 58.2% treated with LTG, 75% with VPA, 67.35% with CBZ, and 73.4% with phenobarbital (PB). Generalized tonic-clonic seizures were more frequently observed with LTG treatment (21.1%) than with VPA (11.5%), CBZ (12.6%), or PB (14.0%).

In our 26 patients, the mean age at the onset of seizures was 22.90±2.42 years (range: 18-27 years), which was in a favorable reproductive stage and indicated that age may not be a risk factor for new onset epilepsy during pregnancy. However, the gestation period of their first seizures varied, including 16 (61.50%) in the first trimester, 7 (27.00%) in the second trimester, and 3 (11.50%) in the third trimester. It seems that new-onset epilepsy during pregnancy tends to occur in early and mid-pregnancy (23/26, 88.5%).

The largest number of patients had a focal attack (11/26, 42.30%), while 9 of them had primary GTCS during pregnancy (34.60%), and 2 had secondary GTCS during pregnancy (7.70%). Furthermore, 4 patients (15.40%) had CSE (15.40%).

Clinical studies have shown that folic acid supplementation can prevent malformations in children of women taking AEDs, hence the recommendation of a dose of 5 mg/day of folic acid for women considering pregnancy and during the first months of pregnancy (31,32). High doses of folic acid (above 5 mg/day) are not recommended as they may lower the seizure threshold.

Women with epilepsy who experience generalized tonic-clonic seizures may be at a relatively higher risk of harming the fetus during a seizure, even though the absolute risk remains very low and the level of risk may depend on seizure frequency (33). It should be noted that the risk of experiencing major congenital malformations (MCMs) in the general population varies between 1.6% and 3.2%, and WWE who do not receive AEDs show similar MCM rates. Hence, exposure to AED leads to teratogenic effects.

Regarding the frequency of seizures in female patients, sporadic seizures were found in most patients (17/26, 65.40%), while frequent and very frequent seizures were found in only 5 patients. CSE was found in 4 patients (15.40%), who did not take therapy regularly.

Regarding the type of seizure in pregnancy, other studies on preexisting epilepsy have shown that generalized epilepsy is more common than focal epilepsy (34,35). CSE was observed in 4 patients (4/26, 15.40%) in the third trimester, which was more common than in pre-existing epilepsy. It has been reported that only 1% to 2% of women with pre-existing epilepsy may experience CSE during pregnancy (34). Although CSE during pregnancy has been associated with high morbidity and mortality, timely and appropriate treatment could lead to a satisfactory outcome. In addition, most patients (17/26, 65,4%) had only sporadic seizures (less than 1 seizure per month) during pregnancy. New onset seizures in pregnancy appeared to be either mild or severe.

Providing care for women with epilepsy in the preconception period is intended to reduce the risk of fetal abnormalities and subsequent disorders of child development by optimizing pharmacological treatment and implementing folate supplementation. This may also provide desirable seizure control during pregnancy. It is important to explain the issue to the patient and her relatives and to emphasize that a potential teratogenic factor, such as the use of AEDs, is already present in the first days after conception. To reduce the risk of birth defects, it is recommended to administer the lowest effective dose of the most appropriate AED until at least the end of the first trimester of pregnancy (16,19). Discontinuation of medication may be considered in women after a three-year seizure-free period (16). Pregnant women who have had seizures a year before conception require increased monitoring of their epilepsy treatment. Most women (67%) do not experience seizures during pregnancy. Between 74% and 92% of women who have been seizure-free for at least 9 months to 1 year before pregnancy will remain free of seizures during pregnancy (36). The data from EURAP (European Registry of Antiepileptic Drugs and Pregnancy) have shown that pregnant women with idiopathic generalized epilepsy are more likely to be free of seizures (74%) than those with focal epilepsy (60%) (21).

Additionally, in polytherapy, teratogenicity risks have been associated with drug combinations that were not documented when each individual drug was used as monotherapy. When used in polytherapy, the malformation rate of topiramate dramatically increases to 14.1% versus 2.4% when used as monotherapy (37). This was recently reported in the Australian Pregnancy Registry but had not been reported prior. It is important to put the risk of teratogenicity into perspective; in general, the risk of significant fetal malformation is approximately 3% if one AED is prescribed and up to approximately 17% if polytherapy is recommended (38,39). Drug teratogenicity risk should be routinely considered throughout pregnancy when treating a WWE.

In addition to new-onset seizures, AED was another problem that patients with new-onset epilepsy had to face during pregnancy. To avoid maternal and fetal risk associated with seizures, AED therapy was often maintained during pregnancy, despite the increased risk of congenital malformations and adverse cognitive development in infants (40). In this study, all 26 patients started AED therapy during pregnancy. Levetiracetam (LEV) was prescribed in 4 patients, carbamazepine in 15 and intravenous diazepam in 7 patients.

Structural and metabolic changes were thought to trigger new seizures during pregnancy (41). Most of the provoked seizures, however, could not be diagnosed as epilepsy, except that spontaneous recurrent seizures occurred.

In this study, 16 positive neuroimaging findings from brain MRI images (16/26, 61,50%) were detected, including hippocampal sclerosis (HS) in 3 patients and possible focal cortical dysplasia (FCD) in 5 patients. This was different from common gestational cerebral complications, such as cerebral venous sinus thrombosis, intracranial hemorrhage, and ischemic stroke. Furthermore, eclampsia, the most common disease to be distinguished in pregnancy, is defined as the onset of generalized seizures that may not be attributable to other causes in pregnant women with preeclampsia (42).

CONCLUSION

The present study showed that epileptic seizures in the largest number of pregnant women occurred in the first trimester, including a focal seizure. Sporadic seizures were found in most pregnant women. The patients were advised to take antiepileptic drug therapy, and all examined pregnant women took it.

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Reprint requests and correspondence:
Mohammad Abou El-Ardat, MD, PhD
Clinic of Gynecology and Obstetrics
Clinical Center University of Sarajevo
Jezero, 71000 Sarajevo
Bosnia and Herzegovina
Email: ardatdrm@hotmail.com
ORCID ID: 0000-0003-3753-958X

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Primary distal hypospadias repair with Snodgrass technique: a prospective cohort study

Primarna rekonstrukcija distalnih hipospadija Snodgrass tehnikom: prospektivna kohortna studija

Asmir Jonuzi[1]*, Zlatan Zvizdić[1], Nermir Granov[2], Verica Mišanović[3], Benjamin Kulovac[4]

[1]Clinic of Pediatric Surgery, Clinical Center University of Sarajevo, Patriotske lige 81, 71 000 Sarajevo, Bosnia and Herzegovina
[2]Clinic of Cardiovascular Surgery, Clinical Center University of Sarajevo, Bolnička 25, 71 000 Sarajevo, Bosnia and Herzegovina
[3]Pediatric Clinic, Clinical Center University of Sarajevo, Patriotske lige 81, 71 000 Sarajevo, Bosnia and Herzegovina
[4]Clinic of Urology, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Introduction: hypospadias is one of the most common congenital anomalies of the penis. Different methods of hypospadias management are described in the literature. In this study, we assessed national trends and compared them with international practice. Aim: to report our experience in using the tubularized incised plate - TIP (Snodgrass) urethroplasty in distal hypospadias forms. Materials and methods: in the period from January 2016 to May 2019, 40 patients, aged 13 months to 13 years underwent distal hypospadias repair using TIP procedure. Results: the majority reported an overall complication rate of ≤10% for distal hypospadias form. Three children (7.5%) developed a meatal stenosis and 1 child (2.5%) had urethral fistula. Conclusion: TIP repair is the preferred technique for distal hypospadias form. It became the first choice in repairing this malformation.

Keywords: hypospadias, TIP urethroplasty, urethral fistula

SAŽETAK

Uvod: hipospadija je jedna od najčešćih kongenitalnih anomalija penisa. U literaturi su opisane različite metode liječenja hipospadije. U ovoj studiji procjenjivali smo nacionalne trendove i upoređivali ih sa međunarodnom praksom. Cilj: izvijestiti o našem iskustvu korištenja tubularizacije incidirane ploče - TIP (Snodgrass) uretroplastike kod distalnih formi hipospadija. Materijali i metode: u periodu od januara 2016. do maja 2019. godine, 40 pacijenata, starosti od 13 mjeseci do 13 godina, su podvrgnuti rekonstrukciji distalne hipospadije primjenom TIP procedure. Rezultati: ukupna stopa komplikacija je ≤10% za distalne forme hipospadija. Troje djece (7,5%) je razvilo stenozu meatusa, a 1 dijete (2,5%) imalo je uretralnu fistulu. Zaključak: TIP rekonstrukcija je poželjna tehnika za rekonstrukciju distalnih formi hipospadija. Postala je prvi izbor za popravku ove malformacije.

Ključne riječi: hipospadija, TIP uretroplastika, uretralna fistula

INTRODUCTION

Hypospadias is one of the most common congenital anomalies of the penis, a ventral opening of the external urethral meatus with or without chordee, and a deficiency of the ventral penile skin (1). There are many publications in the literature describing the methods of hypospadias management. Factors that can influence choices in clinical practice include hypospadias severity, surgeon's background, and preference (2). Since its introduction in 1994, Tubularized Incised Plate Urethroplasty (TIPU) has gained widespread popularity because of its versatility, low complication rate, and good cosmetic results (3).

The technique relies on an incision of the glandular urethral plate to permit a tension-free tubularisation of the neourethra. Snodgrass (3) postulated that healing occurs by re-epithelialisation, with no evidence of neourethral stenosis or stricture formation, whereas many others consider that it heals by epithelial creeping, which theoretically increases the incidence of complications because of healing by primary intention if allowed to contract (4,5). This can be prevented by separating the two sides of the gapped urethral wound; therefore, regular daily dilatation of the neourethra was advised to prevent meatal stenosis and fistula formation (6). In the standard technique, Snodgrass and others (7,8,9) affirmed that it was better not to incise the apical part of the glans for fear of meatal stenosis. Hypospadias repair focuses on achieving three main objectives: voiding in an upright position, an appropriate voiding stream, and normal penile appearance and function (10,11).

In this study, we evaluate the trends of national practice toward distal hypospadias repair at the Clinical Center University in Sarajevo.

AIM

The aim of this study was to report our experience in using the tubularized incised plate (TIP) urethroplasty in distal hypospadias forms.

MATERIALS AND METHODS

This prospective study was performed in the period from January 2016 to May 2019 on 40 patients aged 13 months to 13 years who underwent primary distal hypospadias repair using the Snodgrass technique (TIP). The following inclusion criteria: 1. apply 0.6 to 18 years of age, 2. Patients with primary distal hypospadias repair. The exclusion criteria were as follows:1. Patients with proximal hypospadias, 2. megameatus intact prepuce, and 3. those with a previously failed repair. All the patients received preoperative local therapy 2.5 % dihydrotestosterone. Informed consent was obtained from all patients' parents, and the procedure and possible risks were explained thoroughly, according to the Helsinki declaration. The study was approved by the Ethics committee of the Clinical Center University of Sarajevo.

Statistical analysis

Mean and median were used as a measure of central tendency and standard deviation and range as measures of dispersion for continuous variables. The values of categorical variables were presented as numbers or percentages. Significance was assumed at a p-value of less than 0.05.

RESULTS

Intraoperative preference

A tubularized incised plate (TIP) is the preferred procedure for distal penile hypospadias repair (Figure 1). The preferred suture material, size, and suturing technique are summarized in (Table 1). Tourniquet and bipolar diathermy is used by the majority to achieve hemostasis. All pediatric surgeons use a form of the second layer to cover their repairs (Table 2). Urethral Stents and cistofix are always used following hypospadias surgery. Table 3 summarizes the preferred stent type and duration kept following distal penile hypospadias repair. The native meatus was coronal in 10 (25%), subcoronal in 8 (20%) and distal penile in 22 (55%) of the patient. Mean follow-up was 35.90 months (SD ±29.58) postoperatively (range 12-162 months). Cephalosporins were used in 33 patients on antibiotic therapy (Table 4). There were 4 complications, three children developed meatal stenosis and 1 child had fistulae (Table 5).

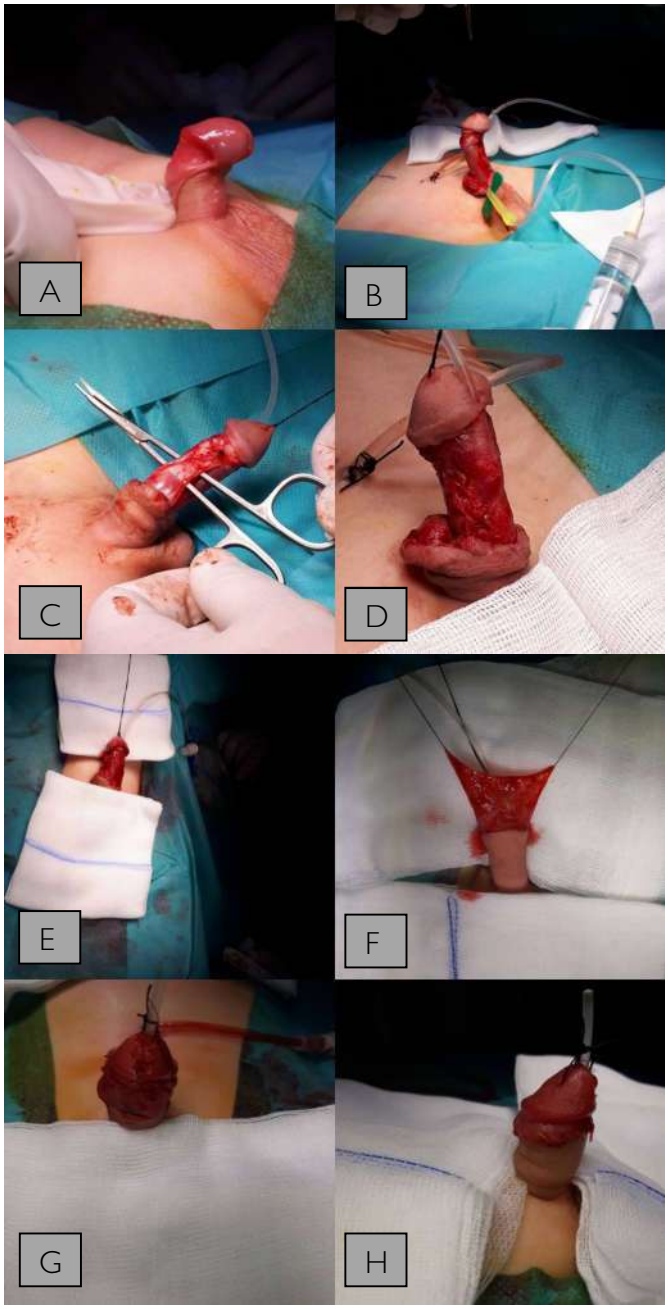

Figure 1 **TIP procedure: A - Distal hypospadias form; B -Ventral penile curvature; C - Preparation of the dorsal penile nervous plexus; D - Curvature correction with Yahia tunica albuginea plication; E - Urethroplasty; F - Dorsal dartos flap preparation; G -Glansplasty; H - Skin closure.**

Table 1 **Preferred suture material, size, and suturing technique.**

Suture	Percentage (%)
1. Suture material	
Monofilament	40 (100.0)
2. Suture size	
6/0	40 (100.0)
3. Suturing techinque	
Through and through	35 (87.5)
Subcuticular	5 (12.5)

Table 2 **Preferred second layer type and technique.**

Second layer	Percentage (%)
Buttonhole dartos flap	27 (67.5)
Ventral dartos flap	3 (7.5)
Spongioplasty	3 (7.5)
Spongioplasty + butonhole dartos flap	7 (17.5)

Table 3 **Preferred stent type and duration of stenting.**

Stent	Percentage (%)
1. Type	
Hypospadias stent	40 (100.0)
2. Duration of stenting	
7 days	40 (100.0)
3. Duration of cistofix	
8 days	40 (100.0)

Table 4 **Postoperative antibiotic choice and regimen**

Antibiotic	Percentage (%)
Cephalosporins	33 (82.5)
Amoxicillin + clavulanic acid	3 (7.5)
Trimethoprim/Sulfamethoxazole	4 (10.0)

Table 5 **Postoperative complications.**

Complications	Early (%)	Late (%)
Fistula	0	1 (2.5)
Meatal stenosis	0	3 (7.5)

DISCUSSION

Hypospadias is one of the most common anatomical congenital malformation of the penis (1). Different techniques of repair and protocols of postoperative care and follow up are described in the literature. We tried in this study to evaluate the national practice and compare it with the international practice.

As in every surgery, the experience of the surgeon and number of cases operated on per year is an important factor for surgical outcomes (2). Another important factor for success is the careful dissection and tissue handling. This can be facilitated by the use of magnifying surgical loupes (12).

Since Snodgrass published his initial description of TIP repair in 1994, this procedure became popular, especially for the repair of distal penile hypospadias (3). This procedure has an additional advantage of its simplicity and versatility to correct different variants of hypospadias. A Canadian multicenter evaluation of technical preferences for primary hypospadias repair showed that TIP is the preferred technique for distal penile hypospadias repair which is comparable to our results (13).

Sutures size, composition, and techniques of placement may contribute significantly to the outcomes of hypospadias surgery (12). Nearly 100% of our participants use monofilament sutures for their urethroplasties, which is comparable to international practice (14).

The use of an intervening layer between the neourethra and skin significantly reduces urethracutaneous fistula (UCF), the most common complication (15). All of our participants use one for their repairs. The most commonly used flap is the buttonhole dartos flap. To overcome rotation penis, some use a ventral dartos flap or a double dartos flap (16).

To reduce the rates of surgical site infections, surgeons commonly use perioperative antibiotics. The type, dose, and regimen have not been standardized for hypospadias repairs (17). TIP repair is recommended as the primary treatment for anterior hypospadias, mid-shaft hypospadias and possible selected proximal ones (18).

Holland AJ, et al. (19) suggested that the use of the TIP in boys with inherently narrow urethral plates (< 8 mm) leads to a greater risk of complications. They noted that the possibility of a UCF increases with a shallow glans of <8 mm wide and there was no objective evidence that the relaxing incision actually increased the true width of the final neourethra. Snodgrass WT (20) stated that a relaxing incision also varies from a deep incision for a shallow groove to no incision for a deep groove, and that the results of Holland et al (19) might be because the relaxing incisions were not made deeply enough.

Assessment of outcome includes: complication rate, cosmetic appearance of the penis, functional outcome (micturition, sexuality), and psychological factors such as quality of life and psychosexual life. Validated scales are at the advantage of allowing surgeons to more objectively assess postoperative outcomes, as well as providing a platform for discussion about the outcomes with colleagues. However, analysis of long-term outcomes may also be readily performed by the use of scales, which can also be used as a screening tool, emailed as an online survey, or posted to evaluate which patients require further clinical consultation (21,22).

The UCF has been shown the most frequent complication of hypospadias repair, in almost all studies. In our study, the overall UCF incidence was noticed in 1 case (2.5%). There is wide variation in the UCF frequency in different studies, mentioning its overall rate as 12.5% (23), 17.24% (24), 12.6% (25), 26% (26) and 4.14% (27).

Numerous studies have advocated vascularized dartos flap coverage to repair, to decrease the incidence of UCF (3,23,28,29).

This is an additional procedure that requires skill to dissect the fascia away from the skin, which may subject the skin to necrosis. The chances of vascularity compromise may become more when the dorsal skin is transported ventrally as Byar flaps to resurface the ventral skin deficient area in certain cases and later on accompanied by compressive dressing leading to skin loss and dehiscence, making subsequent surgery more difficult.

Meatal stenosis stood second in the list of complications, which occurred in 3 (7.5%) cases and this is comparable with other reported incidences of 9.7% (27), 2.1% (24), and 3% (30).

The established cases of meatal stenosis underwent routine postoperative dilatation. In our study, there was no case of flap necrosis, urethral diverticulum, or residual chordee which required correction.

The TIP repair has the advantage of technical simplicity. Every case is an interesting operation on border of pediatric surgery, urology and plastic surgery. It is not a simple urethroplasty, it is a reconstruction of the malformated penis. The TIP may be accepted as having an important role in hypospadias treatment (31).

We had to make a good degloving for a good orthoplasty and to prepare a vascularized tissue, as an intermediary layer between the neourethra and the skin or glans. We harvested tissue from the dorsal hooded prepuce or from ventral dartos as a coverage layer for the tubularized urethra. A catheter was let in every case, for 7 days, and cystifix for 8 days. Periodic neourethral calibrations were performed in 3 cases.

CONCLUSION

Snodgrass (tubularized incised plate) urethroplasty is a simple, quick, single stage procedure suitable for distal penile hypospadias repair. It provides excellent functional neourethra, cosmetically normal looking glans and meatus and is associated with very few complications.

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Reprint requests and correspondence:
Asmir Jonuzi, MD, PhD
Clinic of Pediatric Surgery
Clinical Center University of Sarajevo
Patriotske lige 81, 71000 Sarajevo
Bosnia and Herzegovina
Phone: + 387 33 250345
Email: jonuziasmir@hotmail.com
ORCID ID:0000-0002-5637-9510

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Comparison of real-time reverse transcription-polymearse chain reaction (rtRT-PCR) with isolation in cell culture for detection of influenza virus from severe acute respiratory illness and influenza-like illness cases

Komparacija reverzne transkripcije-polimeraza lančane reakcije u stvarnom vremenu (rtRT-PCR) s izolacijom u ćelijskoj kulturi za detekciju virusa influence iz teške akutne respiratorne bolesti i slučajeva bolesti sličnih influenci

Edina Zahirović*, Amela Dedeić-Ljubović, Azra Čamdžić, Irma Salimović-Bešić

Clinical Microbiology, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Introduction: Influenza (flu) is an infectious respiratory disease caused by Influenza A and B viruses in humans. In the Federation of Bosnia and Herzegovina (FB&H), influenza ranks first on the list of leading infectious diseases. Cell culture is the dominant and indispensable tool for virus isolation. Aim: comparison of diagnostic methods for isolation and detection of circulating subtypes of influenza A virus (H3N2 and H1N1pdm09) and influenza B virus (Victoria strain) during two influenza surveillance seasons (2018/19 and 2019/20). Materials and methods: this retrospective study included nasal and throat swabs from all patients who met the SARI (severe acute respiratory illness) and ILI (influenza-like illness) case definitions according to WHO influenza surveillance guidelines. Samples were screened for influenza A and B virus strains using real-time reverse transcription polymerase chain reaction (rtRT-PCR). rtRT-PCR positive samples with Ct (threshold cycle) value ≤30 were inoculated in two types of cells cultures (MDCK for influenza A/H1N1/pdm09 and Influenza B virus and MDCK SIAT 1 for influenza A/H3, respectively). Results: during the research period from 2018 to 2020, out of 948 samples, 333 (35.1%) were positive for the influenza virus. Among them, 94.9% were positive for influenza A and 5.1% for influenza B virus. Both influenza seasons in Bosnia and Herzegovina were characterized by co-circulation of (H1N1) pdm09 and (H3N2) Influenza A virus subtypes. A total of 249 samples (Ct≤30) positive for influenza A and B viruses were inoculated into cell cultures, of which 83 (33.3%) showed cytopathogenic effect (CPE). Conclusion: it is necessary to continuously monitor the etiology of influenza in order to assess the burden of the influenza virus, the eventual emergence of a new pathogenic variant, in order to opportunely define risk groups and formulate effective control measures. Due to the high rate of antigenic variation and the seasonality of influenza virus strains, there is a need for constant evaluation of test performance to ensure accurate diagnosis.

Keywords: influenza, rtRT-PCR, MDCK cell culture

SAŽETAK

Uvod: influenca (gripa) je zarazna respiratorna bolest uzrokovana virusima influence A i B kod ljudi. U Federaciji Bosne i Hercegovine (FBiH) influenca zauzima prvo mjesto na listi vodećih zaraznih bolesti. Ćelijska kultura je dominantan i nezamjenjiv alat za izolaciju virusa. Cilj: poređenje dijagnostičkih metoda za izolaciju i detekciju cirkulirajućih podtipova virusa influence A (H3N2 i H1N1pdm09) i virusa influence B (soj Victoria) tokom dvije sezone nadzora influence (2018/19 i 2019/20). Materijali i metode: ova retrospektivna studija uključila je briseve nosa i grla kod svih pacijenata koji su ispunjavali definicije slučaja SARI (teška akutna respiratorna bolest) i ILI (bolest slična gripu) prema smjernicama SZO za nadzor influence. Uzorci su testirani na podtipove/sojeve virusa influence A i B koristeći lančanu reakciju polimeraze reverzne transkripcije u realnom vremenu (rtRT-PCR). rtRT-PCR pozitivni uzorci sa Ct vrijednošću (prag ciklusa) ≤30 inokulirani su u dvije vrste ćelijskih kultura (MDCK za infuencu A/H1N1/pdm09 i virus influence B i MDCK SIAT 1 za influencu A/H3, respektivno). Rezultati: tokom perioda istraživanja od 2018. do 2020. godine, od 948 uzoraka, 333 (35,1%) su bila pozitivna na virus influence. Među njima je 94,9% bilo pozitivno na influencu A i 5,1% na virus influence B. Obje sezone influence u Bosni i Hercegovini karakterizirala je ko-cirkulacija (H1N1)pdm09 i (H3N2) podtipova

virusa influence A. Ukupno 249 uzoraka (Ct≤30) pozitivnih na viruse influence A i B inokulirano je u ćelijske kulture, od kojih je 83 (33,3%) pokazalo citopatogeni efekat (CPE). Zaključak: potrebno je kontinuirano pratiti etiologiju gripe kako bi se procijenila opterećenost virusom influence, pojava nove patogene varijante virusa, blagovremeno definisale rizične grupe i formulisale efikasne mjere kontrole. Zbog visoke stope antigenskih varijacija i sezonskog karaktera sojeva virusa influence, postoji potreba za stalnom evaluacijom performansi testa, kako bi se osigurala tačna dijagnoza.

Ključne riječi: influenca, rtRT-PCR, ćelijska kultura MDCK

INTRODUCTION

As novel influenza viruses regularly emerge in human population, influenza virus continues to pose serious medical and economic challenges to global public health (1). The influenza A and B viruses are responsible for seasonal influenza epidemics which typically occur between November and April each year (2). Influenza is an acute viral respiratory disease caused by infection of the respiratory tract with influenza viruses that circulate among people worldwide (3). The spectrum of disease severity ranges from mild forms of disease to those with severe complications, including death. The worldwide, the annual influenza epidemics affect 5%-10% of the world's population, resulting in about 3-5 million cases of severe illness, and about 250000-500000 deaths (4). The European Centre for Disease Prevention and Control (ECDC) estimates that nearly 40000 people in the European Union die prematurely each year due to influenza-related causes (4). In the Federation of Bosnia and Herzegovina (FB&H), influenza ranks first on the list of leading infectious diseases (5).

Influenza viruses constantly change their surface glycoproteins, responsible for binding to receptors and antigenic characteristics of the virus, which is why the characterization of non-typed, potentially new influenza viruses is of great importance. Reference standards for laboratory confirmation of influenza virus infection in respiratory samples are real-time reverse transcription polymerase chain reaction (rtRT-PCR) or virus culture (6).

Several different approaches are currently available for the diagnosis of influenza viruses. The rtRT-PCR is the standard approach for the identification of influenza viruses in most laboratories. It is a molecular assay of high sensitivity and specificity for detection of influenza virus RNA in respiratory specimens (6, 7). Previous studies have demonstrated that rtRT-PCR shows superior sensitivity compared to viral culture and is now accepted as the new gold standard test for influenza diagnostics (7). The MDCK (Madin-Darby canine kidney) cell culture has been the most used cell line since its establishment in 1958 for influenza research. MDCK cells are considered universal and the most sensitive cells from the kidney of a healthy cocker spaniel dog for the primary isolation of influenza virus from clinical samples (8).

MDCK SIAT-1 cell line is produced by genetic engineering by stable transfection of MDCK cells with human 2,6-sialtransferase (SIAT1) cDNA. MDCK SIAT1 cells are suitable for testing human influenza virus sensitivity to neuraminidase inhibitors (NAIs) due to their overexpression of sialyl-α2,6-galactose moieties. They are used for the isolation/amplification of influenza A/H3 viruses (9). It may take 3-10 days to receive cell culture results. However, cell culture allows extensive antigenic and genetic characterization of influenza viruses. Collection of representative respiratory samples for cell culture is necessary for surveillance and antigenic characterization of new strains of seasonal influenza A and B viruses that may be included in next year's influenza vaccine (10). The procedure for viral cultivation begins with the inoculation of a clinical specimen into a cell culture flask. Cell culture consists of cells of human or animal origin and medium (e.g. MEM -minimum essential medium: buffer system, vitamins, amino acids, sugars, animal serum, small amounts of antibiotics and indicators to show the pH of the medium). Proteolytic enzymes like trypsin (trypsinization) selectively break down the protein matrix that binds the cells in the culture, thus enabling the separation of the cells (11). The virus proliferates in the cell line, causing changes in the cells. The microscopic examination of the unstained cell culture monolayer has been the standard approach for detecting viral proliferation. The spectrum of change is broad, ranging from swelling, shrinking, and rounding of cells to clustering, syncytium formation, and, in some cases, complete destruction of the monolayer, collectively called the cytopathogenic effects or cytopathic effect (CPE). However, observing CPE is quite complex, it takes a certain period of time for CPE to develop, and experience is necessary to observe and monitor the process. Dramatic CPE may be easily detected, but the subtle CPE of many viruses, early CPE, or CPE that is not typical may go unrecognized unless the observer has considerable expertise, so confirmatory testing is required to make a definitive identification of the virus (9).

AIM

The aim of this study was to evaluate the performance and the effectiveness of influenza virus isolation using MDCK and MDCK SIAT 1 cell cultures with rtRT -PCR from human respiratory samples.

MATERIALS AND METHODS

The study was conducted in the period from October 2018 to March 2020 and it included the total of 948 specimens of severe acute respiratory illness (SARI) or influenza-like illness (ILI) patients. Oropharyngeal and/or nasopharyngeal swabs were collected using universal viral transport collection kits (BD, Fisher Scientific, USA) containing 3 ml of the virus transport medium (VTM). The specimens were stored in VTM at -70°C until testing by rtRT-PCR following the standard CDC (US Centers for Disease Prevention and Control) protocol. Total nucleic acids were extracted from nasopharyngeal/oropharyngeal swabs using QIAamp Viral RNA Mini Kits (Qiagen, Hamburg, Germany) according to the manufacturer's instructions (QIAamp Viral RNA Mini Handbook 03/2018). Following the instructions, 220 µl of VTM of each specimen was used for nucleic acid extraction. Approximately 60-70 µl of the total RNA eluate was recovered into a nuclease-free tube and tested immediately by one step rtRT-PCR. The purified RNAs were amplified by Applied Biosystem 7500 system using SuperScript III One-Step RT-PCR System with Platinum™ (Invitrogen, MA USA). rtRT-PCR was performed for screening of influenza virus A and B and subtyping. All samples tested positive for influenza A virus were further analyzed for influenza A/H3 and influenza A/pdmH1 subtypes. Samples positive for influenza B virus were tested for influenza B lineage determination (Yamagata and Victoria). Cycling conditions were as follows: cDNA synthesis at 50°C for 30 minutes, an initial denaturation at 95°C for 10 minutes, followed by 45 cycles

of 95°C for 15 seconds and 55°C for 30 seconds. The interpretation of the results was done according the threshold cycle (Ct) value measurement for each target in the sample. The result was considered positive when Ct value was < 37 for each target and negative if Ct value was above 37. The Ct value of the positive control should be detectable at ≤ 37, and not detected for the negative control. The specific primers and FAM-labeled TaqMan probes sequences were obtained from Influenza Reagent Resource (IRR)- CDC (US) developed at the CDC for the detection of influenza A and B viral RNA. The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel consists of nucleic acid amplification assays for detection of influenza A and B viruses and identification of influenza A subtypes A/H1, A/H1pdm09, A/H3, and influenza B lineages B/Victoria and B/Yamagata (12).

Table 1 **Components of the PCR reaction mixture.**

Reagents	Volume
Nuclease free water	5.5 µl
*Forward, Reverse- primers; Probe	1.5 µl
SuperScript™ III RT/Platinum® Taq Mix	0.5 µl
2X PCR Master Mix	12.5 µl
Virus RNA	5.0 µl
Total reaction volume	25.0 µl

*Specific for each target RNA (influenza A, A/H3, A/pdmH1; influenca B, B/Victoria, V/Yamagata).

Isolation of influenza virus in MDCK (MDCK-SIAT1) cell culture

Standard MDCK (FR-58 - MDCK London, IRR) cell lines were used for influenza A/H1 and Influenza B virus, while MDCK SIAT1 cells were permissive for isolation and propagation of influenza A/H3 subtype.

MDCK and MDCK SIAT1 cell cultures were grown in plastic flasks (75 cm2), on a temperature of +37°C, in a previously prepared nutrient medium for MDCK growth (DMEM - Dulbesco's Modified Eagle's Medium; Sigma-Aldrich, Burlington, MA, US). The inoculation of the influenza virus was performed in cultures that showed a confluent monolayer of cells of about 90-95% of the flasks surface.

All steps were performed in a Class II biosafety cabinet, in a clean room, to avoid cell contamination, following the instructions from Manual for the laboratory diagnosis and virological surveillance of influenza (13). MDCKs were grown in an incubator at a temperature of +37°C, with 95% humidity and 5% CO_2 in a nutrient medium D-MEM.

After the adequate cell confluency was established, the influenza virus inoculation procedure was started. Into a T-25 flask, 250 µl of each sample of the nasopharyngeal swabs-VTM suspension was inoculated. The flasks were placed in a flat lying position for 30-60 minutes at 35°C, to ensure adsorption of the monolayer covered with the inoculum. Then 6 ml of D-MEM virus growth medium (containing 2 µg/ml TPCK trypsin) was added to the flask. The inoculated cells were incubated at 35°C, and CPE was observed daily on an inverted microscope.

The cells were harvested up to 6 or 7 day, even if no CPE was observed. Upon finding a CPE intensity of 3+ or 4+ (75-100%), the supernatant was removed and 0.5% stabilizer such as glycerol was added. The cell culture medium from the flasks was poured into a 50 ml conical tube, which was then centrifuged at 1800-2000xg at 4°C/5 minutes. After harvesting, an aliquote of the virus isolate (250 µl) was stored at +4°C, and the rest was stored with cryomedia in cryotubes at -70°C for further analyzis (haemagglutination assay (HA), the hemagglutination inhibition assay (HAI), sequencing).

For the purpose of detailed antigenic and genetic characterization, representative positive samples for A(H1N1)pdm09 and A(H3) from the 2018/2019 and 2019/2020 seasons, as well as positive samples for the influenza B virus from the 2019/2020 season were sent to the WHO referral laboratory for FB&H - The Crick Worldwide Influenza Centre, The Francis Crick Institute, London, United Kingdom.

RESULTS

During the two seasons of 2018/2019 and 2019/2020, a total of 948 nasopharyngeal swab samples in VTM were tested. Influenza viruses were detected in 35.1% (333/948) of samples (Table 2). No statistically significant difference in the representation of positive samples by season was observed, in relation to the total number of tested samples (p=0.493). Across two research seasons, influenza A(H1N1)pdm09 and A/H3 subtypes were detected. Distribution of influenza virus subtypes showed a significant difference in the representation of the A(H1N1)pdm09 subtype (205/333) compared to other virus subtypes. In the case of influenza B virus, which was proven only in the second season of the research, the Victoria strain was identified in all samples. Of the total number of positive samples, influenza A virus was significantly more prevalent (94.9%; 316/333; p<0.001) compared to influenza B virus (5.1%; 17/333).

Table 2 **Seasonal distribution of influenza virus types and subtypes.**

Samples		2018/19 N (%)	2019/20 N (%)	Total N (%)	p value
Analyzed samples		410 (43.2%)	538 (56.8%)	948 (100%)	0.493
Positive samples		149/410 (36.4%)	184/538 (34.2%)	333/948 (35.1%)	
Influenza A positive		149/149 (100%)	167/184 (90.8%)	316/333 (94.9%)	p<0.001
Influenza B positive		0 (0%)	17/184 (9.2%)	17/333 (5.1%)	
Influenza A positive	(H1N1)pdm09	71/149 (47.6%)	134/167 (80.2%)	205/316 (64. 9%)	p<0.001
	(H3)	78/149 (52.4%)	33/167 (19.8%)	111/316 (35.1%)	
Influenza B positive	Victoria	0 (0%)	17/17 (100%)	17/17 (100%)	p<0.001

The efficiency of influenza virus isolation in MDCK and MDCK-SIAT1 cell cultures depended to a large extent on the initial virus concentration in the sample estimated by the Ct value of the previously performed rtRT-PCR assay (Table 3). The results showed that the success of virus isolation was higher in the samples with the Ct value lower than 30 (<30).

During the research period, the 249/333 (74.7%) of samples had a Ct value <30. MDCK cell cultures were inoculated with 158 influenza A (H1) pdm09 and 13 influenza B viruses, while MDCK-SIAT1 cell cultures were inoculated with 78 influenza A (H3) virus samples (Table 3).

Table 3 **Results of Ct values obtained by rtRT-PCR test.**

Season	2018-2019	2019-2020	Total period (2018-2020)
	Influenza A (H1N1)pdm09		
Ct value ≤30	56/71 (78.8%)	102/134 (76.1%)	158/205 (77.1%)
	Influenza A (H3)		
Ct value ≤30	49/78 (62.8%)	29/33 (87.9%)	78/111 (70.3%)
	Influenza B		
Ct value ≤30	0(0)	13/17(76.5%)	13/17 (76.5%)
TOTAL	**105/149 (70.5%)**	**144/184 (78.3%)**	**249/333 (74.7%)**

After inoculation, the intensity of CPE caused by the inoculated virus in MDCK and MDCK-SIAT1 cell lines was monitored (Figure 1). A total of 83 (33.3%) samples showed clear CPE, of which 37 were with a maximum intensity of 4+, i.e. 100% infected cells (Table 4).

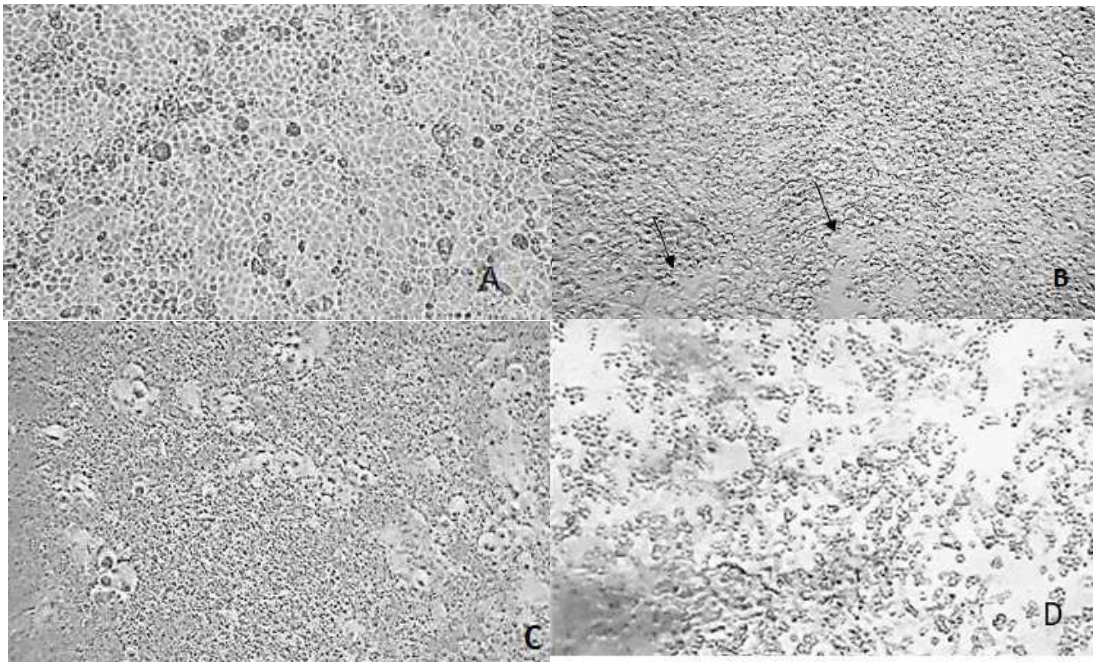

Figure 1 **(A) MDCK cell culture infected with influenza A(H1N1)pdm after 24h - cells are still confluent, no visual CPE; (B) Cell monolayer after 48h indicates slight rounding of cells. Cells change in shape. Holes appear in the monolayer (arrows); (C) Cell monolayer after 96h shows rounding and peeling of cells. Clumping, detachment of cells from the monolayer, holes in the monolayer are observed; (D) Appearance of MDCK infected with influenza on day six. CPE ++++ = ~ 100% infected; The cells are (almost) completely detached from the monolayer, floating on the surface of the medium. Magnification 10x, inverted microscope.**

Table 4 **Results of evaluation of the amount of CPE influenza virus on MDCK and MDCK-SIAT cultures.**

CPE intensity	Season 2018-2019		Season 2019-2020		
	Influenza A virus		Influenza A virus		Influenza B virus
	(H1N1)pdm09	(H3)	(H1N1)pdm09	(H3)	
+	0	0	0	0	0
++	5	3	3	0	1
+++	13	13	5	1	1
++++	20	6	9	2	1
	38	**22**	**17**	**3**	**3**
Total		**83**			

DISCUSSION

Several different approaches have been currently available for the diagnosis of influenza viruses. The reference standards for laboratory confirmation of influenza virus infection in respiratory samples have been the rtRT-PCR or virus culture, so far.

rtRT-PCR is the most traditional and powerful approach for the identification of influenza viruses in most laboratories. It is a molecular assay that can identify influenza virus RNA or nucleic acids in respiratory specimens, and it has very high sensitivity and specificity (13,14). Previous studies have demonstrated that rtRT-PCR shows superior sensitivity compared to viral culture and is now accepted as the new gold standard test for diagnostic of influenza (15,16).

In our study, rtRT-PCR has high specificity for diagnosing both influenza A and B, also subtyping was 100% successful during the research period.

Referral laboratories, including ours, have the obligation to send samples to the WHO reference center that performs HA, HAI, sequencing and data analysis for final confirmation of the sequence. By sending representative samples to the WHO referral center for influenza, our laboratory participated in the preparation of the vaccine for the next season for the Northern Hemisphere. Representative positive samples (samples of a specific subtype, selected to represent different phases of the epidemic, as well as different clinical manifestations of influenza) for A(H1N1)pdm09 and A(H3) from the 2018/2019 and 2019/2020 seasons, as well as positive samples for the influenza B virus from the 2019/2020 season

were sent to the WHO referral center for influenza on the regular bases two times during the season.

In the report from the WHO referral laboratory, for all samples included also in this study, the results of influenza typing and subtyping by the rtRT-PCR were confirmed. In this study, a total of 249/333 influenza A and B viruses were inoculated onto MDCK cell lines whose Ct value of the rtRT-PCR was less than 30. The success of virus isolation was higher for the samples whose Ct value was less than 30. After inoculation, we monitored the intensity of CPE caused by the inoculated virus on MDCK and MDCK-SIAT1 cell cultures. The success of virus propagation in cell culture depends on the number of viable virions, the number of adequate receptors present on the cells, as well as on the affinity of the hemagglutinin (HA) of the tested virus to the receptors present (17, 18). A total of 83 (33.3%) samples showed clear CPE, of which 37 were with a maximum intensity of 4+, i.e. 100% infected cells. Dramatic CPE may be easily detected, but the subtle CPE of many viruses, early CPE, or CPE that is not typical, may go unrecognized unless the observer has considerable expertise. For this reason, confirmatory testing is required to make a definitive identification of the virus (9). Data from the literature showed the different percentages of greater or lesser success of the isolation of the influenza virus in the cell culture.

In the research by Zaki AM, et al. a total of 20/71 positive samples previously proven by the rtRT-PCR method were negative in cell culture (19). Such discrepancies were explained by Zambon M et al. who state that culture can miss up to 46% of positive samples for influenza, especially in patients with an advanced clinical course of the disease (20).

Other explanations, such as the use of antiviral drugs or the presence of non-viable virus in the samples, the quality of the sample collected, the titer of the virus in the clinical sample, may affect the propagation and isolation of the virus in the cell culture where intact virus is required (21). Vontas et al. reported that 76% (387/510) of the influenza A viruses caused clear CPE in MDCK cell cultures (22). Viral disease diagnosis has traditionally relied on the isolation of viral pathogens in the cell cultures. Although this approach is often slow and requires considerable technical expertise, it has been regarded for decades as the "gold standard" for diagnosis of some viral infection (cytomegalovirus and other herpesviruses, enteroviruses, orthomyxoviruses etc.). With the development of nonculture methods for the rapid detection of viral antigens and/or nucleic acids, the usefulness of viral culture has been questioned. Today, virus isolation in cell culture remains a useful approach for viral disease diagnosis. Tomorrow, as more sophisticated, yet simpler-to-use broad-range molecular platforms become available for clinical diagnostics, virus isolation in cell culture may once again become mainly a research tool (9). In addition, regardless of all the above, the most important fact is that rtRT-PCR is favored because the results are obtained much faster.

CONCLUSION

Continuous and expanded monitoring of the etiology of influenza is necessary in order to assess the burden of influenza and define risk groups. Due to the high rate and seasonality of antigenic variation among influenza virus strains, there is a need for fast and continuous evaluation of the test efficiency to ensure accurate results and the possible emergence of the new pathogenic variants. For clinical diagnosis of influenza, molecular platforms, such as rtRT-PCR, are more suitable. Until cell culture becomes more sophisticated, yet easier to use, virus isolation in the cell culture remains largely a research tool within referral laboratories for influenza diagnostics.

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Reprint requests and correspondence:
Edina Zahirović, MSc, MLD
Clinical Center University of Sarajevo
Clinical Microbiology
Bolnička 25, 71000 Sarajevo
Bosnia and Herzegovina;
Email: edina_dervovic@yahoo.com
ORCID ID: 0000-0002-8838-1236

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Our contribution to the reduction of cardiovascular diseases in Bosnia and Herzegovina!
Naš prilog redukciji kardiovaskularnih bolesti u Bosni i Hercegovini!

Correlation of serum PTH, calcium, phosphorus and their product with demographic and inflammation parameters among hemodialysis patients

Korelacija serumskog PTH, kalcijuma, fosfora i njihovog produkta sa demografskim i upalnim parametrima kod hemodijaliznih pacijenata

Alma Mutevelić-Turković[1]*, Amela Bećiragić[1], Amela Dervišević[2], Nesina Avdagić[2], Aida Ćorić[1]

[1]Clinic of Hemodialysis, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina
[2]Department of Human Physiology, Faculty of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Introduction: alteration of mineral metabolism is a prevalent condition in chronic kidney disease (CKD). Large epidemiologic studies have shown a strong relationship between elevated levels of calcium (Ca), phosphorus (P), Ca-P product (CaxP), and parathyroid hormone (PTH) with cardiovascular morbidity and mortality, especially hyperphosphatemia as the most prominent one. Aim: to evaluate the metabolism of parathyroid hormone, serum levels of calcium, phosphorus and their product with their interrelationship with demographic, clinical and biochemical parameters in male and female hemodialysis (HD) patients. Materials and methods: this cross-sectional study involved 82 patients with kidney failure (47 male, 35 female) undergoing HD, over 18 years of age. Blood samples were drawn from each patient before breakfast in the morning after an overnight fast in the middle of the week before HD. Serum concentrations of Ca, P, intact PTH (iPTH), uric acid, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured. Body mass index (BMI) was calculated as weight divided by height squared. Results: serum Ca levels most strongly directly correlated with iPTH levels in male patients (rho=0.435,p=0.002). On the contrary, Ca levels inversely correlated with CRP serum levels in male and female patients (rho=-0.417; p=0.004 and rho=-0.458; p=0.006 respectively). In male patients, a statistically significant positive correlation was observed between uric acid and P level (p=0.003), and between iPTH and P level (p=0.007). CRP showed a significant negative correlation with dialysis duration in female patients (p=0.027) and with uric acid levels in male patients (p=0.001). Demographic characteristics showed that male patients had significantly higher CRP concentrations than female patients (p=0.014). Conclusion: the obtained results suggest that men undergoing HD are particularly vulnerable to mineral disorders and that their chronic inflammatory state leads to an increased cardiovascular risk.

Keywords: hemodialysis, parathyroid hormone, mineral metabolism, inflammation

SAŽETAK

Uvod: poremećaji mineralnog metabolizma je često stanje kod pacijenata sa hroničnom bubrežnom bolešću. Velike epidemiološke studije pokazale su snažne veze između povišenih nivoa kalcija (Ca), fosfora (P) njihovog umnožka (CaxP) i paratireoidnog hormona (PTH) sa kardiovaskularnim morbiditetom i mortalitetom Cilj: evaluacija metabolizma paratireoidnog hormona, serumskih nivoa kalcijuma, fosfora i njihovog umnožka te njihova korelacija sa demografskim, kliničkim i biohemijskim parametrima kod oba spola hemodijaliznih (HD) pacijenata. Materijali i metode: ova presječna studija je uključivala 82 pacijenta sa terminalnim stadijem hronične bubrežne bolesti (47 muškaraca, 35 žena) koji su na hroničnom programu hemodijalize stariji od 18. godina. Krvni uzorci za laboratoriju su uzeti na tašte u sredini sedmice prije drugog HD tretmana. Mjerene su serumske vrijednosti Ca, P, intaktnog PTH (iPTH), uričnе kiseline, C-reaktivnog proteina (CRP) i sedimentacija eritrocita. BMI se izračunavao kao težina podijeljenja sa kvadratnim metrom visine. Rezultati: serumski nivoi Ca je snažno korelirao sa nivoima iPTH kod muških pacijenata (rho=0.435,p=0.002). Nasuprot tome, nivoi Ca obrnuto su korelirali sa serumskim nivoima CRP-a kod muških i ženskih pacijenata (rho=-0.417; p=0.004 i rho=-0.458; p=0.006). Kod muških pacijenata, statistički signifikantna pozitivna korelacija je uočena između uričnе kiselina i nivoa fosfora (p=0.003), te između iPTH and P (p=0.007). Muški pacijenti su imali značajno veće vrijednosti CRP-a (p=0.014), te je ujedno i uočena signifikantna negativna korelacija sa dužinom HD tretmana kod ženskih pacijenata (p=0.027) i sa uričnom kiselinom kod muških pacijenata (p=0.001). Zaključak: prikazani rezultati navode da su muški hemodijalizni pacijenti posebno osjetljivi na poremećaj mineralnog metabolizma i da je njihovo hronično upalno stanje izraženije u odnosu na žene što ih čini podložnijim za veći kardiovaskularni rizik.

Ključne riječi: hemodijaliza, paratireoidni hormon, mineralni metabolizam, inflamacija

INTRODUCTION

Alteration of mineral metabolism is a prevalent condition in chronic kidney disease (CKD). Large epidemiologic studies have shown a strong relationship between elevated levels of calcium (Ca), phosphorus (P), Ca-P product (CaxP), and parathyroid hormone (PTH) with cardiovascular morbidity and mortality, especially hyperphosphatemia as the most prominent one (1). Vascular and coronary artery calcification has been suggested as the link between abnormal mineral metabolism in general, and hyperphosphatemia in particular, and cardiovascular events in this population in HD patients and predialysis population (2). Hyperphosphatemia has been pointed out as the primary culprit in the process of cardiovascular calcification, an event already presents in the early phases of renal failure. More interesting, a significant association between the progression of coronary artery calcification and serum P concentration was observed in patients with kidney failure, despite serum P being in the normal range (3). However, despite these previous findings, the mechanisms by which serum P contributes to cardiovascular disease are not completely known (4). According to the Renal Registry of Bosnia and Herzegovina, the mortality rate of dialysis patients in our country in the year 2018 was 12.6% (5). In comparison, more than 300.000 people in the United States are undergoing therapy for kidney failure with the majority on in-center hemodialysis.(6) Numerous studies have attempted to identify risk factors for mortality and morbidity in this population. Most have shown important relations among demographic factors (e.g., older age, male gender, white race) and mortality (7,8). Comorbid conditions (e.g., diabetes, cardiovascular disease) and laboratory proxies of nutritional status (e.g., serum albumin, prealbumin, creatinine) have also been consistently associated with mortality and morbidity. Compared with those factors mentioned above, relatively little attention has been paid to disorders of mineral metabolism (1).

AIM

The aim of this study was to evaluate the serum levels of PTH, Ca, Pa, and CaxP product and their interrelationship with demographic, clinical, and biochemical parameters in male and female patients with kidney failure treated with HD.

MATERIALS AND METHODS

This cross-sectional study involved 82 (47 male, 35 female) patients over 18 years of age with kidney failure undergoing HD at the Clinic of Hemodialysis of the Clinical Center University of Sarajevo. Enrollment criteria included: patients over 18 who were on HD treatment for at least three months, and available baseline serum Ca, P, and iPTH data obtained during the study period. Exclusion criteria were acute renal failure, pre-dialysis patients with other stages of chronic renal disease, and HD duration less than 3 months.

The method of collecting data was performed through medical records in the relevant period, where we monitored demographic data of patients: (age, gender, comorbidities, smoking habits, and duration of the HD treatment expressed in months).

Blood samples were drawn from each patient before the HD treatment after 8 to 12 hours overnight fast in the middle of the week or before the second HD treatment in that week. Those blood samples were sent to our referral laboratory for analysis. Serum concentrations of Ca, P (CaxP was calculated from this values), C-reactive protein (CRP), intact PTH (iPTH), uric acid and erythrocyte sedimentation rate (ESR) were measured. Minerals were measured by the ion-selective electrode diluted (indirect) method. PTH was measured by an immunometric immunoassay technique. CRP was measured by the turbidimetric/immunoturbidimetric method, while ESR was calculated and measured after the blood count analysis. Body mass index (BMI) was calculated by dividing body weight by body height in square meters.

Upon careful explanation of the study procedure, an informed consent in writing was obtained from all patients. The study was approved by the CCUS Ethics Committee. Investigations were carried out in accordance with the Helsinki Declaration as revised in 2000.

Statistical analysis

The normality and variance homogenecity of data for quantitative variables were tested using the Kolmogorov-Smirnov or Shapiro-Wilk test. Categorical variables were demonstrated as numbers and percentages and differences between them were assessed using the Chi-square test. Results of descriptive statistics for quantitative variables were presented as median with interquartile range (25-75 percentile).

The difference between the two groups was assessed by the Mann-Whitney U-test or Kruskal-Wallis test. Correlation coefficient between two quantitative variables was assessed using the Spearman's rank correlation test. Statistical significance of the obtained results was set at $p<0.05$. All statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) version 13.0 for Windows (Chicago, IL, USA).

RESULTS

Demographic characteristics of the study population are presented in Table 1.

Table 1 **Differences in demographic, clinical and biochemical characteristics between male and female dialysis patients.**

Variable	Male (n=47)	Female (n=35)	p
Demographic parameters			
Age > 65 years	21/44.7	13/37.1	0.47
Smokers (n/%)	14/29.8	4/11.4	0.086
Cardiovascular Disease (n/%)	21/44.7	11/31.4	0.224
BMI > 30 kg/m^2 (n/%)	3/6.4	6/17.1	0.132
Dialysis duration (months)	5.0 (3.0 - 7.0)	4.0 (3.0 - 7.0)	0.944
Inflammation parameters			
CRP (mg/L)	7.7 (3.7 - 10.8)	2.9 (1.1 - 10.2)	0.014
ESR (mm/h)	40.5 (17.8 - 56.3)	45.0 (21.0 - 67.0)	0.310
Biochemical parameters			
Uric acid (µmol/L)	343.5 (302.5 - 400.8)	323.0 (292.0 - 389.0)	0.288
Calcium (mmol/L)	2.2 (2.11 - 2.31)	2.24 (2.14 - 2.37)	0.167
Phosphorus (mmol/l)	1.87 (1.42 - 2.18)	1.8 (1.62 - 2.05)	0.892
Calcium/Phosphate ratio	1.18 (1.02 - 1.41)	1.22 (1.02 - 1.38)	0.963
iPTH (pg/mL)	314.0 (207.0 - 577.5)	380.7 (195.0 - 603.5)	0.553

Data are presented as median (25th and 75th percentiles); n (%).BMI - Body Mass Index; CRP - C-reactive protein; ESR - Eritrocite Sedimentation Rate; iPTH - Intact parathyroid hormone; p-probability.

Statistically significant negative association was found between serum Ca level and CRP concentration in both male (rho= - 0.417; p=0.004) and female patients (rho= - 0.458; p=0.006), while serum Ca level positively correlated with iPTH level in male patients (rho= 0.435; p=0.002) (Table 2).

Table 2 **Correlation of serum calcium levels with clinical and biochemical parameters.**

Variable	Serum calcium level (mmol/L)			
	Male		Female	
	rho	p	rho	p
Demographic parameter				
Dialysis duration (months)	0.088	0.556	- 0.278	0.105
Inflammation parameter				
CRP (mg/L)	- 0.417	0.004	- 0.458	0.006
ESR (mm/h)	- 0.261	0.080	- 0.261	0.130
Biochemical parameters				
Uric acid (µmol/L)	- 0.046	0.760	- 0.140	0.424
iPTH (pg/mL)	0.435	0.002	0.069	0.696

CRP - C-reactive protein; ESR - Eritrocite Sedimentation Rate; iPTH - Intact parathyroid hormone; rho = Spearman's correlation coefficient; p-probability.

In male patients a statistically significant positive correlation was observed between serum phosphorus levels and uric acid (rho= 0.433; p=0.003) and iPTH (rho= 0.386; p=0.007). In female patients, serum phosphorus levels statistically significant positively correlate with iPTH (rho= 0.315; p=0.007) (Table 3). Serum iPTH levels were correlated positively with dialysis duration in both male (rho=0.391; p=0.007) and female (rho=0.489; p=0.03) patients. Our results showed statistically significant positive association of calcium phosphate product with dialysis duration (rho= 0.281; p=0.022), uric acid (rho= 0.313; p=0.011) and iPTH serum levels (rho=0.453; p<0.001), and a negative correlation with CRP levels (rho= - 0.289; p=0.019) only in male patients.

Table 3 **Correlation of serum phosphate levels with clinical and biochemical parameters.**

Variable	Serum phosphorus level (mmol/L)			
	Male		Female	
	rho	p	rho	p
Demographic parameters				
Dialysis duration (months)	0.189	0.203	0.252	0.144
Inflammation parameters				
CRP (mg/L)	- 0.203	0.171	0.007	0.626
ESR (mm/h)	0.021	0.891	- 0.108	0.535
Biochemical paramteres				
Uric acid (µmol/L)	0.433	0.003	0.216	0.212
iPTH (pg/mL)	0.386	0.007	0.315	0.07

CRP - C-reactive protein; ESR - Eritrocite Sedimentation Rate; iPTH - Intact parathyroid hormone; rho = Spearman's correlation coefficient; p-probability

DISCUSSION

Bearing in mind that HD patients were in a chronic inflammatory state, our study had confirmed that levels of CRP and ESR were largely above the normal values.

In the current study, the serum CRP level was significantly higher in male than in female HD patients. Our results are in the accordance with the results of Stenvinkel P, et al., (9), who found a significantly higher median CRP level and a higher prevalence of patients with elevated CRP in males than in female ESRD patients. On the other side, Razeghiet E, et al. (10) concluded that HD patients had higher serum values of CRP but without a significant difference between gender.

Alsomaili MI, et al. also observed that ESR levels were increased in most of the stable patients on regular HD and that the mean post-dialysis ESR was significantly higher than the pre-dialysis ESR (11). A possible reason for this discrepancy in the results may be relat ed to the differences in the study design and sample size or might be due to anemia or some other hematologic abnormalities which can interfere with the results of measuring ESR.

Our findings showed a significant negative correlation between serum calcium levels and CRP both in both male and female HD patients and a positive correlation with iPTH level only in male HD patients. Those results are expected, because from the third stage of CKD, the phosphorus excretion by the kidneys is decreased, increasing the levels of PTH leading to a decreased regulation of vitamin D receptors and increased resistance to iPTH activity at the tissue level (12). Given that chronic kidney disease is a chronic inflammatory condition which inflammatory status is further exacerbated by hemodialysis itself, it is expected that CRP values are elevated. Accordingly, those patients had lower serum values of calcium, and knowing that they were in a chronic inflammatory state it was to be expected that their CRP was also elevated. Similar findings were noted in the study of Allaw AAD (13), who indirectly showed the correlation of consequences of these abnormal laboratory values of calcium and CRP. Based on the results of our study, serum phosphorus levels are not significantly correlated with the inflammation markers but a positive correlation with iPTH in both genders was recorded, which could be attributed to the fact that vitamin D deficiency leads to a mineral imbalance of calcium and phosphorus causing secondary hyperparathyroidism, whose consequence is renal osteodystrophy (14). Bone abnormalities can be found in almost all patients on hemodialysis, so these findings are expected in our study group and concur with the findings of the study of Arora K et al. (15), who also found that phosphorus levels positively correlated with PTH in that group of patients.

Our study also demonstrated a positive correlation between serum concentrations of phosphorus and uric acid in male HD patients. These results were in the accordance with the results of the study by Kim CS, et al. (16) who found that, serum uric acid levels were correlated with indicators of nutritional statuses, such as body mass index and phosphorus. Serum iPTH levels were significantly positively associated with dialysis duration in both genders. Data from a study by Ahmadi F, et al. (17) also suggested that serum iPTH levels and the duration of dialysis were strongly positively correlated.

The CaxP product showed statistically significant positive correlation with dialysis duration, uric acid and iPTH serum levels and a significantlly negative correlation with CRP levels, but only in male patients. As the time spent on hemodialysis of our patients was prolonged to several years due to a low transplantation rate, complications worsen which was manifested in a further deterioration of mineral metabolism, iPTH, and uric acid. These correlations were to be expected especially when patients spent a long time on this type of renal replacement therapy. Similar findings were noticed in the DOPPS study by Fuller DS, et al., where simultaneous consideration of Ca, P, and PTH may help in identifying patients on dialysis with a higher risk of major clinical outcomes related to CKD (18).

The strength of our study was reflected mostly in the fact that it included HD patients and it analysed one of their mostly frequent disorders that arose during their chronic hemodialysis program.

Limitations of our study was that we included a smaller number of study participants who were observed during a certain period of time, without correlating their mineral bone disorders with radiological findings since they were outpatients, which made it technically demanding.

CONCLUSION

Obtained results demonstrate that male patients presented an inflammatory state. Likewise, in those patients increased markers of mineral metabolism are noteworthy. The inter-relationship between both factors is very important because it is considered that abnormal mineral metabolism and inflammation are pivotal factors for the increased cardiovascular risk in CKD patients, where men are particularly vulnerable to this risk as shown in our study. In order to better understand all these mechanisms, further longitudinal studies with a greater number of patients is required.

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Reprint requests and correspondence:
Alma Mutevelić-Turković, MD
Clinic of Hemodialysis
Clinical Center University of Sarajevo
Bolnička 25, 71000 Sarajevo
Bosnia and Herzegovina
Email: amutevelic@yahoo.com
Phone: 00 387 33 297 038
ORCID ID: 0000-0002-3579-5689

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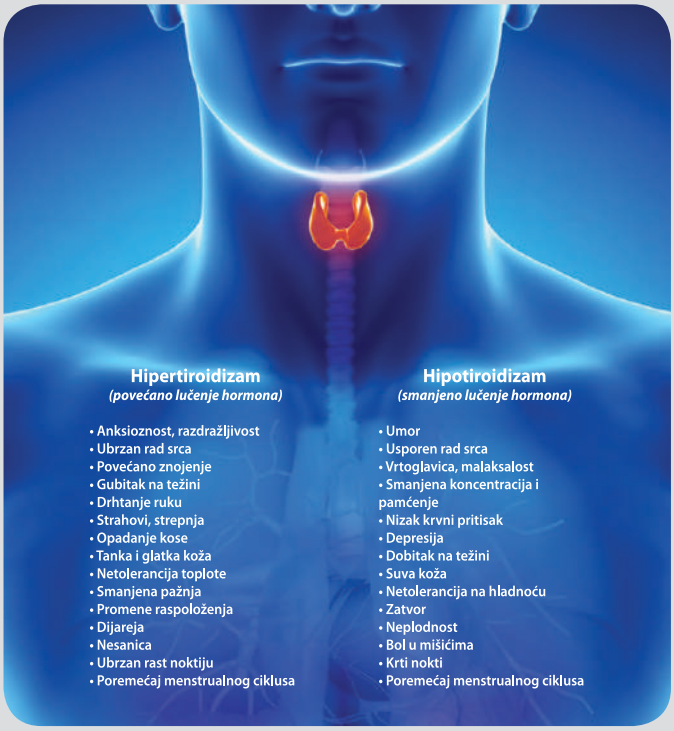

Comparison of aflibercept and bevacizumab analyzing central macular thickness on optical coherence tomography and best corrected visual acuity in patients with diabetic macular edema

Efekat djelovanja aflibercepta u odnosu na bevacizumab analizom parametara centralne debljine makule na optičkoj koherentnoj tomografiji i najbolje korigovane vidne oštrine kod pacijenata sa dijabetičkim makularnim edemom

Amila Alikadić-Husović[1], Emina Kujundžić-Begović[1]*, Alma Mutevelić-Turković [2]

[1]Eye Clinic, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina
[2]Clinic of Hemodialysis, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Introduction: intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF) agents blocks VEGF in forming of new vessels of retina and bleeding, slowing progression of a disease and improves visual acuity. Aim: we compared effect of two types of anti-VEGF injection: aflibercept and bevacizumab, and their impact on diabetic macular edema (DME). Materials and methods: the study included 100 patients, divided in two groups, one group of patients treated with aflibercept, and the other group of patients treated with bevacizumab. We compared central macular thickness (CMT) on optical coherence tomography (OCT) and best corrected visual acuity (BCVA) before and after three months of treatment. Results: the use of aflibercept after three months of treatment showed a statistically significant improvement of BCVA ($p<0.0001$, $p<0.009$) and CMT on OCT ($p<0.0001$,$p<0.001$) Conclusion: we consider that this research will help in choosing an effective anti-VEGF agent in the treatment of DME.

Keywords: aflibercept, bevacizumab, optical coherence tomography

SAŽETAK

Uvod: intravitrealne injekcije anti-VEGF učestvuju u blokiranju djelovanja endotelnog faktora rasta na stavranje novih krvnih žila, te krvarenja, i samim tim usporavaju progresiju oboljenja, te poboljšavaju vidnu oštrinu. Cilj: uporedili smo učinak dvije vrste anti-VEGF injekcija: aflibercepta i bevacizumaba te njihov utiecaj na dijabetički makularni edem (DME). Materijali i metode: u studiju je bilo uključeno 100 pacijenata, podijeljenih u dvije grupe, jedna grupa pacijenata koja je bila tretirana bevacizumabom, i druga grupa pacijenata tretrirana sa afliberceptom. Poredili smo parametre kao što su centralna debljina makule na optičkoj koherentnoj tomografiji, te najbolju korigovanu vidnu oštrinu, prije i nakon tromjesečnog tretmana. Rezultati: nakon tromjesečnog tretmana afliberceptom došlo je do poboljšanja najbolje korigovane vidne oštrine ($p<0.0001$, $p<0.009$), te poboljšanja nalaza centralne debljine makule na optičkoj koherentnoj tomografiji ($p<0.0001$, $p<0.001$). Zaključak: smatramo da će ovo istraživanje pomoći u odabiru najboljeg lijeka kod tretmana pacijenata sa dijabetičkim makularnim edemom.

Ključne riječi: aflibercept, bevacizumab, optička koherentna tomografija

INTRODUCTION

Diabetic macular edema (DME) is the main cause of vision impairment in diabetic patients (1). Vascular endothelial growth factor (VEGF) plays a main role in the pathogenesis of diabetic retinopathy (DR) and DME. Intravitreal injection of anti-VEGF agents remains as the first-line therapy in DME treatment due to the superior anatomic and functional outcomes (2). Anti-VEGF therapy can reduce edema, improve vision and prevent further visual loss (3). These drugs have replaced laser photocoagulation as the standard of care for people with DME (4,5). In clinical trials, this agent and the more recently developed drugs ranibizumab, aflibercept and

brolucizumab, have been shown to be able to reduce vascular leakage and proliferation and, in anywhere from 50–95% of eyes, improve vision (6). In our study we compared effect of two types of anti-VEGF injection: aflibercept and bevacizumab, and their impact on DME.

AIM

The aim of this study was to compare effect of two types of anti-VEGF injection: aflibercept and bevacizumab and their impact on diabetic macular edema (DME).

MATERIALS AND METHODS

This was a retrospective cohort clinical study conducted at the Eye Clinic of the Clinical Center University of Sarajevo, in the period from March to September 2022. The study included patients with diabetes mellitus who had DME, and who were treated with different kind of anti-VEGF therapy and triamcinolone.

Exclusion criteria were: patients with macular edema caused by thrombosis of central retinal vein, wet age macular degeneration (AMD), and other type of maculopathy. Based on the inclusion criteria the study included 100 patients. The first group consisted of patients treated with injection of bevacizumab intravitreal, in dose of 1.25 mg/0.05 ml, and the second group consisted of patients treated with aflibercept intravitreal, in dose of 2 mg/0.05 ml., during three months of follow up, one dose at month.

During three months of the treatment, 8 patients in aflibercept group and 4 patients in bevacizumab group were withdrawn from the study. At the end we analyzed data of 88 patients: 42 patients in aflibercept group and 46 in bevacizumab group. All patients signed the informed consent form. Patients from both groups had similar demographic characteristics at the beginning of the follow up period. We compared central macular thickness (CMT) on optical coherent tomography (OCT) and best corrected visual acuity (BCVA) measured on Snellen s chart, before and after three months of treatment. There were no statistical differences between the values of BCVA and CMT at the beginning of the study.

Statistical analysis

The normality and variance homogenecity of data for quantitative variables were tested using the Kolmogorov-Smirnov or Shapiro-Wilk test. The difference between the two groups was assessed by the Mann-Whitney U-test or Kruskal-Wallis test. Statistical significance of the obtained results was set at $p<0.05$. All statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) version 13.0 for Windows (Chicago, IL, USA).

RESULTS

Data were analyzed for 88 patients: 42 received aflibercept and 46 received bevacizumab. The mean age for all patients was 66.62+ 11.25 at the baseline, whereas a statistical age difference was noticed between the two study groups (Table 1).

Table 1 **Demographic and clinical characteristics of patients at the beginning of the study.**

Gender(m/f)		59/41	
Age (mean ± SD)		66.62+ 11.25	
	Aflibercept (mean + SD)	63.52 +9.88	p= 0.013
	Bevacizumab (mean + SD)	69.45 + 11.78	
BCVA right eye(mean ± SD)		0.39 ± 0.31	
	Aflibercept (mean + SD)	0.37± 0.27	
	Bevacizumab (mean + SD)	0.43 ± 0.33	p=0.598
*BCVA left eye (mean± SD)		0.41± 0.33	
	Aflibercept (mean + SD)	0.41 ± 0.34	
	Bevacizumab (mean + SD)	0.41± 0.33	p=0.956
*OCT right eye (mean ± SD)		340.83 ± 138.78	
	Aflibercept (mean + SD)	357.94 ± 154.38	p=0.354
	Bevacizumab(mean + SD)	319.46± 121.35	
OCT left eye(mean ± SD)		353.81 ± 137.35	
	Aflibercept (mean + SD)	394.94± 150.75	p=0.345
	Bevacizumab (mean + SD)	317.34± 118.70	

*BCVA-best corrected visual acuity, OCT-optical coherence thomography.

Differences before and after the three months treatment with aflibercept showed a statistical significant difference in the values of BCVA of both eyes ($p<0.0001$, $p<0.009$) and for CMT on OCT of both eyes ($p<0.0001$, $p< 0.001$) (Table 2).

Table 2 **Differences of the values of BCVA and OCT at the beginning and after the three months treatment with aflibercept.**

	Before therapy	After therapy	p value
BCVA right eye	0.37± 0.27	0.47 ± 0.30	**0.0001**
BCVA left eye	0.41 ± 0.34	0.47 ± 0.36	**0.009**
OCT right eye	357.94 ± 154.38	291.54± 103.49	**0.0001**
OCT left eye	394.94± 150.75	336.66± 131.12	**0.001**

*BCVA-best corrected visual acuity, OCT-optical coherence thomography.

There was no statistical significant difference before and after the three months treatment with bevacizumab in the values of BCVA of both eyes and of CMT on OCT of both eyes (Table 3).

Table 3 **Differences of the values of BCVA and OCT at the beginning and after the three months treatment with bevacizumab.**

	Before therapy	After therapy	p value
BCVA* right eye	0.43 ± 0.33	0.40 ± 0.33	0.179
BCVA left eye	0.41± 0.33	0.54 ± 0.90	0.859
OCT* right eye	319.46± 121.35	354.10± 153.90	0.122
OCT left eye	317.34± 118.70	345.24 ± 163.36	0.523

*BCVA-best corrected visual acuity, OCT-optical coherence thomography.

After the treatment period, a statistical difference was noticed between the two study groups in the values of CMT on OCT of the right eye (p <0.045) (Table 4).

Table 4 **Differences of the values of BCVA and OCT between two study groups after the three months treatment.**

	Aflibercept	Bevacizumab	p value
BCVA* right eye	0.47 ± 0.30	0.40 ± 0.33	0.253
BCVA left eye	0.47 ± 0.36	0.54 ± 0.90	0.627
OCT* right eye	291.54±103.497	354.10± 153.90	0.045
OCT left eye	336.66 ± 131.12	345.24 ± 163.36	0.800

*BCVA-best corrected visual acuity, OCT-optical coherence thomography.

DISCUSSION

Anti-VEGF therapy is first line therapy in the management of DME, along with a growing body of evidence to support the use of anti-VEGF drugs for proliferative DR (7). Our study showed that aflibercept had better effect on functional-visual acuity and anatomical-central macular thickness on OCT. We did not found a statistical significant improvement on bevacizumab treatment. Following the aflibercept treatment, the study showed a statistical significant improvement of the OCT values only on the right eye (p<0.045) compared to bevacizumab. This result was probably due to the fact that the right eye was more treated than the left eye. However, due to the results mentioned above, aflibercept showed an improvement of visual acuity and CMT in the treatment compared to bevacizumab. Our results were similar to the study of Bahrami B et al. which found that mean CMT reduced from 417 ± 91 µm (microns) at baseline to 380 ± 102 µm at 24 weeks (p <0.01). Mean BCVA improved from 67.8 ± 10.3 letters at baseline to 71.0 ± 10.1 letters at 24 weeks (p < 0.01). Eyes improving by ≥5 letters at 4 weeks following the first injection had improved vision outcomes at 24 weeks (p<0.01). Intravitreal aflibercept was effective in improving anatomical and visual outcomes among patients with incomplete response to intravitreal bevacizumab with 24 weeks of follow up (8).

In the study of Cai S, et al. in the first year all three anti-VEGF drugs improved visual acuity and there was no difference among drugs in mean change in visual acuity from baseline among eyes, but over 2 years, in post-hoc area-under-the-curve analysis, although with aflibercept vision outcomes were superior to bevacizumab or ranibizumab among these eyes. They concluded that when initial visual acuity loss was mild, there were no apparent differences in those three drugs for treating DME, but when visual acuity loss was moderate or worse, aflibercept was more likely to improve visual acuity (9).

In their meta-analysis Virgili G, et al. showed that anti-VEGF drugs were effective at improving vision in people with DME in one year. There was moderate-certainty evidence that aflibercept confered some advantage over ranibizumab and bevacizumab in people with DME at one year in visual and anatomic terms (10). Heier JS et al. in their study concluded that on average, all 3 anti-VEGF agents led to improved visual acuity in eyes with DME involving the center of the retina and visual acuity impairment. Worse visual acuity when initiating therapy was associated with greater visual acuity benefit of aflibercept over bevacizumab or ranibizumab 1 year later (11).

Limitations of our study were a smaller group of patients and a short period of follow up. Accordingly, in order to better understand these mechanisms a larger sample size with a longer period of follow up is needed.

CONCLUSION

Blockade of VEGF activity is currently the most effective strategy for reducing vascular permeability, which is frequently the main cause of visual acuity deterioration. In recent years, a number of other molecules have been developed to increase the efficacy and to prolong the durability of the anti-VEGF effect. Our research showed the benefit of aflibercept in the treatment of diabetic macular edema and therefore can help ophthalmologist in the decision which anti-VEGF agent should be given to those patients. Aflibercept had shown better effect on BCVA and CMT compared with bevacizumab in patients with diabetic macular edema. Based on the scientific results of our study, we suggest that aflibercept should be more available to patients in Bosnia and Herzegovina.

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Reprint requests and correspondence:
Emina Kujundžić-Begović, MD
Eye Clinic
Clinical Center University of Sarajevo
Bolnička 25, 71000 Sarajevo
Bosnia and Herzegovina
Email: emina0602@gmail.com
ORCID ID: 0000-0002-0702-801X

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Correlations of kidney function parameters in different phases of multiple myeloma

Korelacija parametara bubrežne funkcije u različitim stadijima multiplog mijeloma

Izeta Aganović-Mušinović*[1], Lejla Burnazović-Ristić[2], Enisa Ademović[3], Mirela Mačkić-Đurović[1], Maida Rakanović-Todić[2]

[1]Immunology Department, Faculty of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina
[2]Pharmacology and Toxicology Department, Faculty of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina
[3]Epidemiology and Statistics Department, Faculty of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Introduction: renal injury occurs as one of the most common causes of morbidity and mortality in patients with multiple myeloma (MM), as patients with MM are characterized by increased production of light chains of immunoglobulins. Aim: evaluation of correlation between potential parameters for early detection of renal injury in MM. Materials and methods: this cross-sectional study involved 62 patients with MM. They were characterized in three different stage groups of the disease using Salmon-Durie classification and by International Staging System. Blood samples were drawn to measure concentration of Cystatin C, Immunoglobulins, Free light chains (FLC), C-reactive protein (CRP), polyclonal chains, while urine is collected to measure creatinine level. All measurements were evaluated using the ELISA and nephelometry methods. Spearman correlation test was used to examine the relationship between the variables. Results: correlations between tested parameters in all patients regardless of MM classification were: Cystatin C significantly correlate with creatinine ($p<0.001$) and CRP ($p=0.004$), Cystatin C significantly correlated with serum kappa chains ($p<0.05$), and kappa chains significantly correlated with CRP ($p<0.05$) and serum λ chains ($p=0.002$). In the initial phase of MM, Cystatin C was observed to correlate with CRP ($p<0.001$). In MM at steady phase, statistically significant correlation was found between Cystatin C and creatinine ($p<0.001$) and creatinine and serum kappa and λ chain ratio ($p<0.05$). In MM at relapse phase, statistically significant correlation was found between Cystatin C and creatinine ($p<0.001$), Cystatin C and C reactive protein ($p<0.05$), Cystatin C and serum kappa chains ($p<0.05$) and creatinine clearance and serum kappa and λ chain ratio ($p<0.05$). In ISS stage 1, statistically significant correlation was found between Cystatin C and creatinine ($p<0.05$). In ISS stage 2, statistically significant correlation was found between Cystatin C and creatinine ($p<0.05$), Cystatin C and serum kappa chains ($p<0.05$) and Cystatin C and serum kappa and lambda chain ratio ($p<0.05$). In ISS stage 3, statistically significant correlation was found between Cystatin C and creatinine ($p<0.05$), Cystatin C and CRP ($p<0.05$) and CRP and creatinine ($p<0.001$). Conclusion: this study confirms that serum Cystatin C significantly correlates with creatinine level as well as with elevated serum κ chains prior to increase of serum creatinine levels, as a potential parameter of early detection of renal dysfunction and injury.

Keywords: Multiple myeloma, kidney injury, Cystatin C, κ chains, λ chains, CRP, creatinine

SAŽETAK

Uvod: oštećenje bubrežne funkcije se navodi kao jedan od najčešćih uzroka morbiditeta i mortaliteta kod pacijenata sa multiplim mijelomom (MM) kojeg karakteriše povećana proizvodnja lakih lanaca imunoglobulina. Cilj: evaluacija korelacija između potencijalnih parametara za ranu detekciju oštećenja bubrega kod pacijenata sa MM. Materijali i metode: studija je dizajnirana kao presječna sa uključenih ukupno 62 pacijenta sa dijagnozom MM. Koristeći Salmon-Durie klasifikaciju i Međunarodni staging system pacijenti su klasificirani u tri različite skupine prema težini bolesti. Uzorci krvi su korištni za mjerenje koncentracije cistatina C, imunoglobulina, slobodnih lahkih lanaca (FLC), C-reaktivnog proteina (CRP), poliklonskih lanaca, dok se urin prikuplja za mjerenje razine kreatinina. Mjerenja su rađena upotrebom ELISA i nefelometrijske metode. Spearmanov test korelacije korišten je za ispitivanje korelacija između varijabli. Rezultati: korelacije između testiranih parametara kod svih pacijenata bez obzira na MM klasifikaciju bile su: cistatin C značajno korelira sa kreatininom ($p<0,001$) i CRP ($p=0,004$), cistatin C značajno korelira sa serumskim kappa lancima ($p<0,05$), a kappa lanci značajno koreliraju sa CRP ($p<0,05$) i serumskim λ lancima (0,0). U početnoj fazi MM, uočeno je da je cistatin C u signifikantnoj korelaciji sa CRP ($p<0.001$). U MM u stabilnoj fazi, pronađena je statistički značajna korelacija između cistatina C i kreatinina ($p<0,001$) i odnosa kreatinina i serumskog kappa i λ lanca ($p<0,05$). U MM u fazi recidiva, statistički značajna korelacija je pronađena između cistatina C i kreatinina ($p<0,001$), cistatina C i C reaktivnog proteina ($p<0,05$), cistatina C i serumskih kappa lanaca ($p<0,05$) i klirensa kreatinina i serumskog kappa i λ lanca ($p<0,05$). U ISS stadiju I pronađena je statistički značajna korelacija između cistatina C i kreatinina ($p<0,05$). U ISS stadiju 2, uočena je statistički značajna korelacija između cistatina C i kreatinina ($p<0.05$), cistatina C i serumskih kappa lanaca ($p<0.05$) i citatina C i serumskog kappa i lambda odnosa ($p<0.05$). U ISS stadiju 3. signifikantna korelacija je uočena između cistatina C i kreatinina ($p<0.05$), i CRPa i kreatinina ($p<0.001$). Zaključak: ovo ispitivanje potvrđuje da serumski cistatin C signifikantno korelira sa nivoom kretinina i serumskim k lancima prije početka povećanja nivoa kreatinina, upućujući na ranu detekciju oštećenja bubrežne funkcije.

Ključne riječi: multipli mijelom, oštećenje bubrega, cystatin C, κ lanci, λ lanci, CRP, kreatinin.

INTRODUCTION

Several kidney features may predispose this particular organ to be affected by circulating paraproteins, an abundance of renal capillaries with large blood flow, the concentration of filtered solutes and the presence of a variety of exogenous and endogenous substances (1). Tumor burden is another important factor. Exes free light chains (FLC) filtration and overburdening of proximal resorptive capacity, with subsequent uromodulin interactions in distal tubules, may precipitate cast formation, inducing tubulointestinal damage and fibrinogenesis (2).

Immunoglobulin light chains have a very short half-life: kappa chain 2-4 hours at a rate of 40% glomerular filtration, and λ chain 3-6 hours with 20% glomerular filtration. Therefore, the concentration of free light chains in serum and urine will be increased if there is hyper-production, which is the case with monoclonal gammopathy, as well as with reduced renal clearance.

In healthy individuals, free light chains are rapidly filtered and metabolized, depending on their size, while in individuals with multiple myeloma, these processes can be extended to 2-3 days leading to complete renal failure (3). People with multiple myeloma (MM) are characterized by increased production of light chains of immunoglobulins, which is directly proportional to the increase in their serum concentration, filtration through the glomerular capillary wall, and thus increase in the concentration of proximal nephron tubule lumen. Excessive amounts of light chains exert a direct cytotoxic effect on the epithelial cells of the proximal tubules, which leads to vacuolation of the cytoplasm and apoptosis of cells (4).

Therefore, we assessed the correlation between several kidney damage paramethars: Cystatin C, CRP, serum creatinine concentration, creatinine clearance, serum κ light chains, serum λ light chains, the ratio between serum κ and λ light chains as well as serum polyclonal light chains in different phase of the MM and different classification, either Solomon-Durie or International Staging System.

AIM

The aim of this study was to evaluate association between renal injury markers and immunoglobulin fractions in patients with multiple myeloma at different stages of the disease.

MATERIALS AND METHODS

The study included 62 patients with multiple myeloma (MM). Patients with MM were divided into 3 groups based on the stage of the disease; 21 patients with MM at presentation, 21 patients with MM in "steady" phase of disease and 20 patients with MM in relapse phase.

Peripheral blood, i.e. serum collected from patients by venipuncture process, was used as a starting sample. Creatinine and CRP concentrations were determined using ARCHITEKTc Systems, Abbott Diagnostics. The method is based on the fact that a strong base like NaOH reacts with creatinine to form a red chromophore. The degree of increase in absorption at 510 nm as a consequence of the formation of this chromophore is directly proportional to the concentration of creatinine in the sample and was measured at a wavelength of 510 nm.

Based on the collected 24 h of urine and a certain concentration of creatinine within the urine, glomerular filtration of the kidneys and creatinine clearance were calculated with specific formulas.

ELISA test is an enzyme-linked immunosorbent assay that determines the presence and amount of antigen or antibody in the presence of enzymes as an indicator, and because it can detect very low concentrations of the target, substance is considered one of the most commonly used and powerful laboratory techniques. Immunological tests use a specific antibody or immunoglobulin to detect antigen. Monoclonal antibodies react with one specific, and polyclonal with several different epitopes on the antigen molecule. This test served to determine the concentration of Cystatin C as one of the important parameters used in the assessment of renal function.

Nephelometry is a modification of photo-optical end-point detection in which 90-degree or forward-angle light scatter, rather than optical density, is measured. A light-emitting diode produces incident light at approximately 600 nm, and a photodetector detects variations in light scatter at 90 degrees (side scatter) and 180 degrees (forward-angle scatter) used for measuring intact immunoglobulins, immunoglobulin light chains, immunoglobulin heavy–light chain pairs, and many other proteins in body fluids. Nephelometry is a modification of the basic precipitin reaction that relies upon light scattering by soluble immune complexes in solution.

Statistical analysis

Statistical analysis of data was done using computer SPSS - Statistical package for social sciences - programs, version 16.0. To estimate the correlation, the Spearman correlation coefficient (r) was used. The level of $p<0.05$ was considered significant.

RESULTS

Creatinine was observed to significantly correlate with Cystatin C ($r=0.628$, $p<0.001$) and CRP ($r=0.361$, $p=0.004$), Cystatin C was significantly correlated with CRP ($r=0.471$, $p<0.001$) and serum κ chains ($r=0.276$, $p<0.05$), and κ chains significantly correlated with CRP ($r=0.258$, $p<0.05$) and serum λ chains ($r=-0.384$, $p=0.002$) (Table1.)

Table 1 **Correlations between tested parameters in all patients regardless of MM classification.**

Spearman Correlations (n=62)		
	r	p-value
Cystatin C and CRP	0.471	<0.001
Cystatin C and Creatinine	0.628	<0.001
Cystatin C and Serum κ chains	0.276	0.03
Cystatin C and Serum κ/λ chain ratio	0.299	0.018
CRP and Creatinine	0.361	0.004
CRP and Serum κ chains	0.258	0.043
Creatinine clearance and Creatinine	-0.327	0.009
Serum κ chains and Serum λ chains	-0.384	0.002
Serum κ chains and Polyclonal chains	0.647	<0.001
Serum κ chains and Serum κ/λ chain ratio	0.852	<0.001
Serum λ chains and Polyclonal chains	0.250	0.05
Serum λ chains and Serum κ/λ chain ratio	-0.738	<0.001
Polyclonal chains and Serum κ/λ chain ratio	0.295	0.02

Legend: Cystatin C (mg/l); CRP (mg/l); creatinine (mmol/l); creatinine clearance (ml/s); serum κ chains (mg/l); serum λ chains (mg/l); serum κ/λ chains ratio (mg/l); polyclonal chains (mg/l).

When we analyzed the correlations between parameters according to the phases of Salmon-Durie classification, we got the following results.

MM at presentation

Table 2 **Spearman correlations of tested parameters in patients with MM at presentation.**

Spearman correlation (n=21)		
	r	p-value
Cystatin C and CRP	0.697	<0.001
Serum κ chains and Polyclonal chains	0.474	0.03
Serum λ chains and Polyclonal chains	0.492	0.023
Serum κ/λ chain ratio and Serum κ chains	0.797	<0.001
Serum λ chains and Serum κ/λ chain ratio	-0.760	<0.001

Legend: Cystatin C (mg/l); CRP (mg/l); creatinine (mmol/l); creatinine clearance (ml/s); serum κ chains (mg/l); serum λ chains (mg/l); serum κ/λ chains ratio (mg/l); polyclonal chains (mg/l).

Cystatin C was observed to correlate with CRP (0.697, p<0.001) (Table 2).

MM at steady phase

Table 3 **Spearman correlations of tested parameters in patients with MM at steady phase.**

Spearman correlation (n=21)		
	r	p-value
Cystatin C and Creatinine	0.740	<0.001
Creatinine clearance and Creatinine	-0.437	0.048
Serum κ chains and Polyclonal chains	0.716	<0.001
Serum λ chains and Serum κ/λ chain ratio	-0.752	<0.001
Serum κ/λ chain and Creatinine	0.442	0.045
Serum κ chains and Serum κ/λ chain ratio	0.864	<0.001

Legend: Cystatin C (mg/l); CRP (mg/l); creatinine (mmol/l); creatinine clearance (ml/s); serum κ chains (mg/l); serum λ chains (mg/l); serum κ/λ chains ratio (mg/l); polyclonal chains (mg/l).

In MM at steady phase, a statistically significant correlation was found between Cystatin C and creatinine (r=0.740, p<0.001) and serum κ/λ chain and Creatinine (r=0.442, p=0.045) (Table 3).

MM at relapse phase

Table 4 **Spearman correlations of tested parameters in patients with MM at relapse phase.**

Spearman correlation (n=20)		
	r	p-value
Cystatin C and CRP	0.512	0.021
Cystatin C and Creatinine	0.765	<0.001
Cystatin C and Serum κ chains	0.449	0.047
Cystatin C and Polyclonal chains	0.581	0.007
Creatinine clearance and Serum λ chains	-0.535	0.015
Serum κ chains and Polyclonal chains	0.623	0.003
Serum κ chains and Serum κ/λ chain ratio	0.923	<0.001
Serum λ chains and Serum κ/λ chain ratio	-0.603	0.005

Legend: Cystatin C (mg/l); CRP (mg/l); creatinine (mmol/l); creatinine clearance (ml/s); serum κ chains (mg/l); serum λ chains (mg/l); serum κ/λ chains ratio (mg/l); polyclonal chains (mg/l).

In MM at relapse phase, a statistically significant correlation was found between Cystatin C and creatinine (r=0.765, p<0.001), Cystatin C and CRP (r=0.512, p=0.021), Cystatin C and serum κ chains (0.449, p=0.047), and creatinine clearance and serum λ chains (r=-0.535, p=0.015) (Table 4).

When patients were divided according to International Staging System into three groups, the results were as follows:

Patients with MM at stage I using International Staging System for Multiple Myeloma.

Table 5 **Spearman correlations of tested parameters in patients with MM at stage I using International Staging System for Multiple Myeloma.**

Spearman correlation (n=21)		
	r	p-value
Cystatin C and Creatinine	0.446	0.043
Serum κ chains and Polyclonal chains	0.886	<0.001
Serum κ chains and Serum κ/λ chain ratio	0.635	0.002
Serum λ chains and Serum κ/λ chain ratio	-0.709	<0.001

Legend: Cystatin C (mg/l); CRP (mg/l); creatinine (mmol/l); creatinine clearance (ml/s); serum κ chains (mg/l); serum λ chains (mg/l); serum κ/λ chains ratio (mg/l); polyclonal chains (mg/l).

In ISS stage I, a statistically significant correlation was found between Cystatin C and creatinine (r=0.446, p=0.043) (Table 5).

MM at stage 2 by International Staging System classification

Table 6 **Spearman correlations of tested parameters in patients with MM at stage 2 using International Staging System for Multiple Myeloma.**

Spearman correlation (n=21)		
	r	p-value
Cystatin C and Creatinine	0.464	0.034
Cystatin C and Serum κ chains	0.454	0.039
Cystatin C and Serum κ/λ chain ratio	0.459	0.036
Serum κ chains and Serum L chains	-0.512	0.018
Serum κ chains and Serum κ/λ chain	0.84	<0.001
Serum L chains and Polyclonal chains ratio	0.54	0.012
Serum L chains and Serum κ/λ chain ratio	-0.836	<0.001

Legend: Cystatin C (mg/l); CRP (mg/l); creatinine (mmol/l); creatinine clearance (ml/s); serum κ chains (mg/l); serum λ chains (mg/l); serum κ/λ chains ratio (mg/l); polyclonal chains (mg/l).

In ISS stage 2, a statistically significant correlation was found between Cystatin C and serum κ chains (r=0.454, p=0.039), Cystatin C and serum κ and λ chain ratio (r=0.459, p=0.036) and Cystatin C and creatinine (r=464, p<0.034) (Table 6).

Patients at stage 3 of MM using International Staging System

Table 7 **Spearman correlations of tested parameters in patients with MM at stage 3 using International Staging System for Multiple Myeloma.**

Spearman correlation (n=20)		
	r	p-value
Cystatin C and CRP	0.469	0.037
Cystatin C and Creatinine	0.751	<0.001
CRP and Creatinine	0.477	0.033
Polyclonal chains and Serum κ/λ chain ratio	0.625	0.003
Serum κ chains and Polyclonal chains	0.789	<0.001
Serum κ chains and Serum κ/λ chain ratio	0.893	<0.001
Serum L chains and Serum κ/λ chain ratio	-0.670	0.001

Legend: Cystatin C (mg/l); CRP (mg/l); creatinine (mmol/l); creatinine clearance (ml/s); serum κ chains (mg/l); serum λ chains (mg/l); serum κ/λ chains ratio (mg/l); polyclonal chains (mg/l).

In ISS stage 3, statistically significant correlation was found between Cystatin C and CRP (r=0.469, p=0.037), Cystatin C and creatinine (r=0.751, p<0.001), and CRP and creatinine (r=0.477, p=0.033) (Table 7).

DISCUSSION

Recent meta-analyses have shown that Cystatin C can be favorable alone or combined with creatinine to predict mortality risk and end-stage renal disease in various populations (5).

Close associations between Cystatin C, serum creatinine and β2 M have been reported (6-8). The same correlation between Cystatin C and serum creatinine have been proven in our study, regardless which MM classification was used.

Maurer MJ, et al. (9), observed that elevated FLC was significantly associated with elevated creatinine and older age. Our study found significant correlation between age and serum λ chains (p<0.05) in ISS stage 3 and no correlation between FLC and creatinine. However, this study fou nd significant correlation between creatinine and kappa and λ chain ratio in SD steady phase. Other statistically significant correlations were found between serum kappa chains and cystatin C in ISS stage 2 as well as between Cystatin C and serum κ chains and serum λ chains and creatinine clearance in SD relapse phase. Creatinine is highly significant in prediction of renal injury regardless of classification model. Elevated FLC may also be a marker of disease burden (found an association of elevated FLC with stage of the disease).

This was not what we have found, beside elevation of Cystatin C and serum creatinine concentrations we noticed correlation between creatinine and κ/λ ratio in "steady" phase of MM and correlation between Cystatin C and κ/λ chains and in stage II MM by ISS. Neither of FLC concentrations alone regarding the renal injury was statistically significant (exceptions: SD classification serum κ chains at presentation, ISS classification κ chains at stage II.

Measured polyclonal FLC did not correlate with any of other measured parameters in either type of classification.

We found the statistically significant correlation between concentrations of Cystatin C and serum creatinine as well as correlation between the level of Cystatin C concentration and age of the patients in III phase of the disease. On the other hand, in patients at relapse phase Cystatin C correlated with serum creatinine and

CRP, while creatinine clearance correlated with concentration of serum λ chains.

In a study by Lamb EJ, et al. (8), it was shown that there were no significant differences between the Cystatin C / creatinine ratio in stage I, II, or III patients, but there was a statistically significant relationship between serum Cystatin C and serum creatinine. We found the correlation between Cystatin C and creatinine in stage II of MM using ISS classification.

The level of CRP in patients with multiple myeloma was positively correlated with the ratio of myeloma cells and the level of β2-microglobulin and from this it can be concluded that the level of CRP is closely related to the development of multiple myeloma (10). Concentration of CRP correlated with concentration of Cystatin C and creatinine in ISS stage III in our study, while statistically significant difference was found in CRP concentrations between ISS stage I and II, and between stage I and III.

Consistent with our results are the results obtained by Schanza M, et al. (11) in which there is a positive correlation between serum Cystatin C and CRP. Higher levels of serum Cystatin C were found in cases of elevated CRP levels, which is also consistent with the research conducted by Knight et al. (12). Schanza M, et al. (11), demonstrated a statistically significant correlation between Cystatin C log and acute renal injury ($p<0.0001$), while CRP log showed a significant correlation between II ($p=0.00073$) and III ($p=0.011$) degree of acute renal injury.

Contrary to ours and previous studies, a study conducted by Ferraninni M, et al. (13) showed that there was no correlation between serum Cystatin C and CRP, but a statistically significant correlation between Cystatin C and serum creatinine ($p<0.01$) was confirmed in acute renal injury.

We have found a significant correlation between CRP and Cystatin C, at presentation ($p<0.001$) and in relapse phase ($p<0.05$). In steady phase there was significant correlation between CRP and age ($p=0.009$). (Table 2-4).

In a study conducted by Bataille R, et al. (14), by assessing the correlation between serum CRP and other prognostic factors, it was concluded that no direct relationship was found with serum creatinine, β2-microglobulin, or tumor stage, although CRP alone ($p=0.002$) and β2-microglobulin ($p=0.01$) were shown to be statistically significant prognostic factors. In addition, that study demonstrated that: 1) induction of steady phase was associated with return of serum CRP levels to normal values and 2) disease progression was associated with increased serum CRP levels. That paper proposes a combination of CRP and β2-microglobulin as two strong prognostic factors for multiple myeloma (14).

In contrast to that study, a study conducted by Najjar SA, et al. in 2017 (15) showed that high specificity CRP strongly correlates with β2-microglobulin and using a Pearson correlation coefficient proved to be statistically significant ($p=0.0001$), just like the linear regression model ($p=0.0001$).

In our research, in the SD steady phase of the disease there is a significant correlation between several parameters. Cystatin C concentration correlated with serum creatinine concentration ($p<0.001$), and CRP concentration correlated with age and was statistically significant ($p=0.009$) (Table 3).

A study conducted by Terpos E, et al. in 2018 (16), came to similar results, and concluded that Cystatin C shows strong correlations with creatinine ($p<0.0001$), as well as with creatinine clearance ($p<0.0001$), and that Cystatin C concentration correlated with survival and showed statistical significance ($p<0.001$). Recent studies have shown that serum Cystatin C is superior to creatinine in the assessment of early renal impairment, including patients with multiple myeloma. Elevated Cystatin C levels were observed in 57.3% of newly diagnosed patients with multiple myeloma, while high serum creatinine levels were detected in only 23.5% of patients (17).

In the relapse phase of MM, we observed correlation between Cystatin C and CRP ($p<0.05$) as well as between Cystatin C and serum creatinine ($p<0.001$) (Table 4).

In a study conducted by Murty MSN, et al. (18), serum creatinine and Cystatin C were analyzed for the occurrence of early renal impairment. The study included two groups of patients, patients with acute renal impairment and a control group. Serum Cystatin C had a lower standard deviation (SD=1.1), while serum creatinine had a higher (SD=1.8) in acute renal impairment, indicating less variability in serum Cystatin C. The variation in serum creatinine concentration was significantly higher in relative to serum Cystatin C concentration in both groups. The standard deviation of serum creatinine (SD=0.23) was twice that of Cystatin C in the control group, indicating a wide fluctuation of serum creatinine compared to serum Cystatin C in a healthy population. Although the correlation between these two parameters was significant in both groups, they emphasize the correlation observed in the group of patients with renal impairment. That implied that small changes in serum creatinine were best reflected by a proportional increase in serum Cystatin C in acute injury of renal function, especially at lower values. Murty MSN, et al. (18) also found that 56.2% of patients with impaired renal function had normal serum creatinine levels in the early stages of the disease, while all patients had elevated Cystatin C levels at the same time. Serum Cystatin C is significantly elevated before serum creatinine levels begin to increase, thus aiding in the early detection of renal dysfunction (18).

Cystatin C correlates with glomerular filtration, and thus possesses greater diagnostic accuracy (19).

CONCLUSION

In addition to renal impairment and the correlation of parameters for the assessment of renal function in individual stages of the disease, our study examined the relationship between age and inflammatory markers. No relevant references were found in the literature for the relationship between creatinine clearance, age and multiple myeloma, since increased creatinine clearance occurs as an independent predictor of impaired renal function in various diseases regardless of age. This study concludes that Cystatin C correlated with creatinine in ISS stage I and in ISS stage II correlated with creatinine, κ chains and κ/λ chains ratio prior to increase of serum creatinine levels, as a potential parameter of early detection of renal dysfunction and injury.

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Reprint requests and correspondence:
Izeta Aganović-Mušinović, MD, PhD
Immunology Department, Faculty of Medicine
University of Sarajevo,
Čekaluša 90, 71000 Sarajevo
Bosnia and Herzegovina
Email: izeta.aganovic@mf.unsa.ba
ORCID ID: 0000-0001-5199-3709

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Hypothyroidism in patients with acute heart failure with reduced and preserved left ventricular ejection fraction

Hipotireoza kod pacijenata sa akutnim zatajenjem srca sa sniženom i očuvanom ejekcionom frakcijom lijeve komore

Azra Durak-Nalbantić[1*], Zarina Babić[2], Lejla Burnazović-Ristić[3], Samir Mehmedagić[1], Mirela Halilčević[1], Mirza Babić[2]

[1]Clinic of Heart, Blood Vessel and Rheumatic Diseases, Clinical Center University of Sarajevo, 71000 Sarajevo, Bosnia and Herzegovina
[2]Cantonal Hospital dr Irfan Ljubijankić, Darivalaca krvi, 67, 77000 Bihać, Bosnia and Herzegovina
[3]Department of Pharmacology and Toxicology, Faculty of Medicine, University of Sarajevo, Sarajevo, Čekaluša 90, 71000 Sarajevo , Bosnia and Herzegovina.

*Corresponding author

ABSTRACT

Introduction: hypothyroidism is a common comorbidity in patients with heart failure, but its prognostic role is not completely clear. Aim: to investigate the occurrence of hypothyroidism in patients with acute heart failure (AHF) with reduced ejection fraction (AHF rEF) and with preserved ejection fraction (AHF pEF), and to examine the difference in the occurrence of repeated decompensation and mortality depending on hormonal status and left ventricular EF. Materials and methods: this was a retrospective, clinical study which included 106 patients hospitalized due to acute heart failure (AHF) at the Clinic of Heart Diseases, Blood Vessels and Rheumatism of the Clinical Center University of Sarajevo (CCUS). Thyroid hormones and left ventricular EF were determined for each patient, and the patients were divided into 4 groups. Three clinical outcomes were monitored during the period of 12 months: 1. number of rehospitalizations due to decompensation, 2. time to first rehospitalization, and 3. mortality. Results: We found that 32.1% of patients had euthyroidism, AHF and reduced EF (Eu-rEF), 27.4% of patients had euthyroidism, AHF and preserved EF (Eu-pEF), 25.5% of patients had hypothyroidism, AHF and preserved EF (Hypo-pEF) and 15.1% of patients had hypothyroidism, AHF and reduced EF (Hypo-rEF). Patients in HFpEF group were predominantly females and they more often had hypertension ($p<0.05$) and hypothyroidism compared to patients with HFrEF. Decompensation was more frequent in the group with hypothyroidism and rEF compared to the hypo-pEF group ($p=0.05$). The shortest time to re-hospitalization was observed in the hypo-pEF group which was significantly shorter compared to the hypo-rEF group (105.27 vs 147.29 days, $p=0.045$). Mortality was higher in patients with hypothyroidism and pEF compared to the hypothyroidism and rEF group (29.6% vs 18.8%, $p=0.055$). Conclusion: patients with hypothyroidism and preserved EF (pEF) have a shorter rehospitalization time and higher mortality rate compared to patients with hypothyroidism and reduced EF. Determining the hormonal status of the thyroid gland in AHF, especially in patients with preserved EF, results in early recognition of a high-risk population.

Keywords: hypothyroidism, acute heart failure, hospitalization, mortality

SAŽETAK

Uvod: hipotireoza je često oboljenje kod pacijenata sa zatajenjem srca, ali njena prognostička uloga nije u potpunosti jasna. Cilj: ispitati zastupljenost hipotireoze kod pacijenata sa akutnim zatajenjem srca (AZS) sa sniženom ejekcionom frakcijom (AZS rEF) i očuvanom ejekcionom frakcijom (AZS pEF), te ispitati razliku u pojavi ponovnih dekompenzacija i mortaliteta ovisno od hormonalnog statusa, te EF lijeve komore. Materijali i metode: retrospektivna, klinička studija je provedena na 106 pacijenta hospliziranih na Klinici za bolesti srca, krvnih žila i reumatizam, KCU Sarajevo zbog AZS. Svakom pacijentu su određeni hormoni štitne žlijezde i EF lijeve komore, te su pacijenti svrstani u 4 grupe. Tokom 12 mjeseci su praćena tri klinička ishoda: 1. broj rehospitalizacija zbog dekompenzacije, 2. vrijeme do prve ponovne hospitalizacije i 3. mortalitet. Rezultati: 32,1% pacijenta su imala eutireozu, AZS i sniženu EF (Eu-rEF), 27,4% eutireozu, AZS i očuvanu EF (Eu-pEF), 25,5% hipotireozu, AZS i očuvanu EF (Hypo-pEF) i 15,1% hipotireozu, AZS i sniženu EF (Hypo-rEF). Pacijenti sa AZS sa pEF bili češće ženskog spola i češće imali hipertenziju ($p<0.05$), te hipotireozu u odnosu na pacijente sa AZS i rEF. Dekompenzacije su bile češće u skupini sa hipotiroezom i rEF u odnosu na hypo-pEF grupu ($p=0,05$). Najkraće vrijeme do ponovne hospitalizacije je bilo u AZS pEF grupi i vrijeme je bilo značajno kraće u odnosu na hypo-rEF grupu (105.27 vs 147,29 dana, $p=0.045$). Mortalitet je bio viši kod pacijenata sa hipotireozom i pEF u odnosu na hipotireozu i rEF grupu (29.6% vs 18.8% , $p=0.055$). Zaključak: pacijenti sa hipotireozom i očuvanom EF (pEF) imaju kraće vrijeme do rehospitalizacije i veći mortalitet u odnosu na pacijente sa hipotireozom i reduciranom EF. Određivanje hormonalnog statusa štitne žlijezde u ASZ, posebno kod pacijenata sa očuvanom EF vodi ranom prepoznavanju visoko rizične populacije.

Ključne riječi: hipotireoza, akutna srčana insuficijencija, hospitalizacija, mortalitet

INTRODUCTION

Heart failure (HF) represents a major public health care and economic problem around the world with very high incidence of MACE (major adverse cardiac events such as repeat hospitalisation due to decompesation, cardiovascular events) and mortality (1). Hypothyroidism is a risk factor for heart failure (HF) in the general population (2). However, the relationship between hypothyroidism and clinical outcomes in patients with established HF is still unclear (3).

AIM

The aim of this research was to examine the prevalence of hypothyroidism in patients with acute heart failure, reduced (HFrEF) and preserved ejection fraction (HFpEF). We also wanted to examine a possible difference in the occurrence of decompensation and mortality in groups with different thyroid status and LVEF values.

MATERIALS AND METHODS

This was a retrospective, clinical study which included 106 patients (67 female and 39 male), aged between 38 and 93, hospitalized due to acute heart failure (AHF) at the Clinic for Heart Diseases, Blood Vessels and Rheumatism of the CCUS in the period from 1 January to 30 April 2018. The diagnosis of AHF was made based on anamnestic data on dyspnea and symptomatic exertion intolerance, signs of pulmonary congestion and peripheral edema. All patients were tested for TSH, T3, T4 and hypothyroidism was diagnosed when TSH values were above 4.20 mU/L, T3 <1.3 nmol/L or T4 <66 nmol/L. LVEF was calculated for each patient with echocardiographic examination (end-diastolic volume minus end-systolic volume divided by end-diastolic volume (4). The examination was performed on a Philips IE30 device, manufactured by Philips, in the Echocardiography Department of the Clinic for Heart Diseases, Blood Vessels and Rheumatism of the CCUS.

HFrEF (heart failure with reduced ejection fraction) was diagnosed when LVEF was < 45% and HFpEF (heart failure with preserved ejection fraction) when EF was ≥45%.

Patients without thyroid hormones tested and without calculated LVEF were not included in study. Patients who were lost for follow up were excluded from study.

During the period of 12 months after hospitalization due to AHF patients were monitored for 3 endpoints: 1. hospitalisation due to decompensation, 2. the time to the first rehospitalization, and 3. mortality. Patients who were lost during the 12-month follow-up period and patients for whom we could not establish outcomes were excluded from the study.

The study was conducted in accordance with the Helsinki Declaration.

Statistical analysis

The results are presented in Tables by number of cases, percentage, arithmetic mean with standard deviation, standard error of the arithmetic mean and range of values. The difference between groups was tested by the Chi-square test, Ficher's exact test, one-way analysis of variance, and the Student's t-test for independent samples. The results of all tests were considered statistically significant at the 95% confidence level or with $p<0.05$. The analysis was performed using the statistical package IBM Statistics SPSS v 23.0 (Chicago, Illinois, USA) and MedCalc v12.3 (Antwerp, Belgium).

RESULTS

HFpEF patients were predominantly female with hypertensia and higher creatinine levels compared to HFrEF patients (Table 1).

Table 1 **Demographic data and comorbidities in HF patients with different left ventricle ejection fraction.**

Demografic data and comorbidities	HFrEF	HFpEF	Significance
Male	53.6%	16.1%	p=0.001
Female	35.75	83.9%	p=0.001
Age	69.8±10.85	73.44±94.8	p=0.09
Atrial fibrillation	54%	83.9%	p=0.8
Hypertension	62%	41%	p=0.01
Diabetes mellitus	30%	17.9%	p=0.3
COPD	16%	51.8%	p=0.8
Serum creatinine	153.3	161.3	p=0.000
Hemoglobin level	117.7	134.4	p=0.000

Out of the total number of patients 43 (40.6%) were diagnosed with hypothyroidism and hypothyroidism was more common in the HFpEF group compared to HFrEF (25.5% vs 15.1%, p=0.045). The oldest were patients with hypothyroidism and HFpEF, but the difference in age between the group was not significant (Table 2).

Table 2 **Age distribution of patients according to TSH and LVEF value.**

Group	Number (percentage)	Significance	Age	Significance
Eu-rEF	34 (32.1%)	p=0.065	69,24±11,84	p=0.70
Eu-pEF	29 (27.4%)		72,90±11,14	
Hypo-rEF	16 (15.1 %)	p=0.045	71,13±8,62	
Hypo-pEF	27 (25.5%)		74,04±7,48	p=0.65

There were more female patients in the total sample; male patients in hypo-rEF (62.5%) and in Eu-rEF group (58.8%), and more female patients in hypo-pEF (96.3%) and Eu-pEF group (72.4%) (Table 3).

Table 3 **Sex distribution in the group with different TSH and LVEF value.**

Patients	Male	Female	Significance
Total	39 (36.8%)	67(63.2%)	p<0.001
Eu-rEF	58.8%	41.2%	
Eu-pEF	27.6 %	72.4%	p=0.045
Hypo-rEF	62.5%	37.5%	
Hypo-pEF	3.7%	96.3%	p=0.001

The mean number of rehospitalization in 12 months follow-up period was 0.54. There was more rehospitalization in HFrEF group compared to HFpEF (0.74 vs 0.38, p=0,04). Hypo-rEF patients had more frequent rehospitalization compared to hypo-pEF group (p=0.05).

Table 4 **Number of rehospitalization according to TSH and LVEF.**

Number of rehospitalization in 12 months		Significance
HFrEF	0.74	
HFpEF	0.38	p=0.04
Hypo-rEF	0.88	
Hypo-pEF	0.52	p=0.05
Eu-rEF	0.67	
Eu-pEF	0.24	p=0.038

The longest period until the first rehospitalization due to decompensation was in hypo-rEF group (147.29 days) and the shortest period was in hypo-pEF group (105.27 days) (p=0.045, Table 5).

Table 5 **Time until hospitalization due to decompensation in a group with different TSH and LVEF values.**

Group	Days until rehospitalization	Significance
Total	11-383 (mean 124,15)	
Hypo-rEF	147,29	
Hypo-pEF	105,27	p= 0.045
Eu-rEF	125,80	
Eu-pEF	127,67	p=0.07

The number of patients who died within a year in the total sample was 31 (29.2%). Mortality in hypo-pEF group was higher compared to hypo-rEF group (Table 6). More patients died in Eu-rEF compared to Eu-pEF, but the difference was not significant (p=0.07).

Table 6 **Mortality rate in the different group according to TSH and LVEF.**

Group	Patients who died	Significance
All patients	31 (29.2%)	
Hypo-rEF	18.8%	
Hypo-pEF	29.6%	P=0.055
Eu-rEF	38.2%	
Eu-pEF	24.1%	P=0.07

DISCUSSION

Hypothyroidism is a common comorbidity in HF. However, the relationship between hypothyroidism and clinical outcomes in patients with HF with reduced and preserved LVEF is still inconclusive.

In our samples HFpEF there were significantly more female patients with arterial hypertension and higher creatinine. HFpEF patients were older and more often with atrial fibrillation, and COPD (chronic obstructive pulmonary disease), but not significantly. This was in accordance with ESC HF guidelines 2021 where HFpEF patients were older, more often female, with very high incidence of AF, arterial hypertension and non-CV comorbidities compared to HFrEF (1).

In our sample hypothyroidism was present in 40.6% of patients and the highest percentage of patients with hypothyroidism was in the group with HFpEF - 25.5%. In literature, a prevalence of 4-20% has been reported for hypothyroidism in the general population, while in HFpEF, the prevalence of hypothyroidism may be even higher as it is more common in women and the elderly - a group of individuals frequently diagnosed with HFpEF (5).

Hassan A, et al. (6) reported that 22% of patients with HFpEF had thyroid dysfunction. This implies that hypothyroidism could be a risk factor for diastolic dysfunction and HFpEF.

In our sample, there were more female patients in Hypo-rEF and Hypo-pEF groups. These results can be explained by the fact that hypothyroidism is a disease mostly found in women (7).

The highest percentage of females was in hypo-pEF group (96.3%) which indicates female sex and hypothyroidism as a strong risk factor for HFpEF.

When analysing repeated decompensation, there were more rehospitalizations in HFrEF group compared to HFpEF (0.74 vs 0.38, p=0,04, p<0,05). This trend was the same in hypothyroidism – hypo-rEF group patients had more frequent rehospitalization compared to hypo-pEF (p=0.05). It seems that low LVEF and high TSH are risk factors for decompensation.

In our sample the shortest period until decompensation was in hypo-PEF group - 105.27 days. This identifies hypo-pEF group as a target group for more frequent outpatient control, intensification of diuretic therapy and up-titration of beta-blockers and ACE inhibitors. This could be a strategy for early recognition of high-risk patients and reduction of decompensation and rehospitalization. In literature, we did not find studies that investigated time until decompensation so this could be a good idea for future studies.

Saad M, et al. (8) found a higher percentage of rehospitalization in 30 days follow-up after the first hospitalization in patients with sub hypothyroidism compared to patients with euthyroidism in patients with reduced ejection fraction (HFrEF) (57% vs. 39%, p=0.001). Rehospitalization within 30 days for patients with sub hypothyroidism versus euthyroidism did not differ in the group of patients with preserved ejection fraction (HFpEF) (49% vs. 43%, p=0.468). The authors concluded that subclinical hypothyroidism detected during the first hospitalization for acute heart failure was an independent predictor of re-hospitalization within 30 days for patients with HFrEF, but not for patients with HFpEF, and emphasized the importance of further research into this issue.

Sato Y, et al. (9) reported that rate of cardiac events was significantly higher in the group of patients with sub hypothyroidism compared to the group of patients with euthyroidism, regardless of the ejection fraction.

12 months mortality in our sample was very high - 31 (29.2%) patients died. Ezekowitz JA, et al. (10) reported that at one-year 2/3 of patients were hospitalized and 20% died within the first year.

Although in euthyreotic patient reduced LVEF means higher mortality, in our sample in hypo HF patients we had the oposite trend: mortality was higher in hypo-pEF compared to hypo-rEF, although not significantly (29.6% vs 18.8%, p=0.055). Therefore we should determine TSH in every HFpEF patient to identify one with bad prognosis. Early introduction of levothyroxine could lead to a better prognosis.

Saad M, et al. (8) reported higher mortality in patients with sub hypothyroidism compared to patients with euthyroidism in HFrEF (18.7% vs. 7%, p<0.001). However, mortality was similar for patients with sub hypothyroidism and for patients with euthyroidism in the group of patients with preserved ejection fraction (9.8% vs. 7.7%, p=0.727).

Based on the presented results, it is clear that the prognostic role of hypothyroidism in patients with AHF is still unclear. The differences in the results of the conducted studies can be explained by different sample sizes, patient follow-up periods, as well as differences in the design of each study.

Many studies tried to explain possible connection of hypothyroidism, LV diastolic dysfunction, heart failure, and worse survival (11,12,13). Overt hypothyroidism is associated with decreased cardiac output and contractility, lower heart rate, and higher systemic vascular resistance (14). However, both basic and clinical studies highlight that in hypothyroidism diastolic abnormalities predominate (15).

Several studies have shown a link between thyroid disorders (hyper - or hypothyroidism) and pulmonary hypertension without clear mechanisms and that treatment of these disorders leads to modification of pulmonary hypertension (16,17,18,19,20). Hypothyroidism reduces the volume and increases the pressure load of the heart, which consequently reduces the stroke and cardiac output. Anemia associated with hypothyroidism could be one of the causes of reduced exercise capacity.

Clinical studies have confirmed that patients with sub-clinical hypothyroidism had a higher risk of cardiovascular diseases perhaps due to increased low-density lipoprotein, elevated homocysteine, and hyper-coagulative blood (21). Interestingly, a previous animal study showed that sub-clinical hypothyroidism may lead to the accumulation of mucopolysaccharides in the myocardium, potentially leading to heart failure (22), suggesting that sub-clinical hypothyroidism may adversely affect cardiac function.

Hypothyroidism also causes changes in lipid metabolism, elevated C-reactive protein and a higher prevalence of aortic atherosclerosis and these mechanisms lead to increased prevalence of myocardial infarction and mortality (5). Therefore, it is necessary to emphasize the need to conduct additional research with a larger number of subjects and a longer follow-up period to better understand the role of hypothyroidism in the progression and outcome of acute heart failure. Neves JS et al. (5) speculate that thyroid hormones could lead to the modulation of diastolic function and this could be a promising target for heart failure with a preserved ejection fraction.

Correction of local tissue hypothyroidism with thyroid hormone supplementation improved diastolic function in animal models of HF (23,24). It is important to highlight that treatment with thyroid hormones may improve symptoms and morbidity in HFpEF, not only due to cardiac actions but also to extra-cardiac effects, including decreased adiposity and improved endothelial function, arterial compliance, and skeletal muscle function (25).

Epicardial fat tissue has also been proposed as a cardiovascular risk factor, and it has been shown to be increased in hypothyroidism and patients with HFpEF (26). Yazici D, et al. reported that epicardial fat tissue thickness in subclinical hypothyreosis decreased significantly following L-thyroxine treatment. Thus, the decrease of the epicardial fat tissue, and, possibly, the modulation of the profile of adipocytokines secreted by adipose tissue may contribute to the benefits of thyroid hormone supplementation in the reduction of cardiovascular risk in hypothyreosis (26). The limitation of the study of Yazici D, et al. is a small number of patients and a need for future studies with a higher number of participants and longer follow-up.

CONCLUSION

Hypothyroidism is more frequent in HF with preserved EF compared to HF with reduced EF. Patients with hypothyroidism and preserved ejection fraction (pEF) have a shorter time to rehospitalization and higher mortality compared to patients with hypothyroidisam with rEF. The highest mortality was found in the hypo-HFrEF group. Therefore we should test TSH in every HF patient to identify high-risk patients and to improve their prognosis through early levothyroxine administration.

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Reprint requests and correspondence:
Azra Durak-Nalbantić, MD, PhD
Clinic of Heart, Blood Vessel and Rheumatic Diseases
Clinical Center University of Sarajevo, 71000 Sarajevo
Bosnia and Herzegovina
Email: azradurak@yahoo.com
ORCID ID: 0000-0002-5175-8941

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Leptomeningeal carcinomatosis in gastric cancer: case report

Leptomeningealna karcinomatoza kod karcinoma želuca: prikaz slučaja

Nejra Mašić*, Nevena Mahmutbegović, Admir Mehičević, Enra Mehmedika - Suljić

Neurology Clinic, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Introduction: leptomeningeal carcinomatosis is a rare but frequently devastating complication of advanced cancer,most commonly lung cancer, breast cancer, and melanoma. Patients can present with a broadrange of signs and symptoms due to simultaneous involvement of multiple areas of thecranio-spinal axis. Aim: to present a case of a rare presentation of leptomeningeal carcinomatosis in a patient with gastric cancer. Case report: a 54-year-old patient felt gradually increasing weakness and tingling in the upper and lower extremities three weeks before admission. A month and a half before admission, she underwent a palliative gastrectomy, received four cycles of chemotherapy. She was admitted to Neurology Clinic with flaccid weakness of all extremities. Neuroimaging and laboratory analyzes suggested that it was most likely an infiltration of the meninges in the field of the underlying pathological process – gastric cancer. Conclusion: leptomeningeal carcinomatosis is traditionally a late-stage complication of advanced cancer. The main challenge in the diagnosis and management of leptomeningeal carcinomatosis is the wide range of symptoms, rapid deterioration and complex needs of patients. Additional clinical trials as well as an improved understanding of the pathophysiological processes are needed to achieve optimal treatment of this devastating disease.

Keywords: leptomeningeal carcinomatosis, leptomeningeal metastasis, gastric cancer

SAŽETAK

Uvod: leptomeningealna karcinomatoza je rijetka, ali često devastirajuća komplikacija uznapredovalog karcinoma, najčešće pluća, dojke i melanoma. Pacijenti mogu imati širok raspon znakova i simptoma zbog istovremenog zahvaćanja više niova kraniospinalne osovine. Cilj: prikazati slučaj rijetke prezentacije leptomeningealne karcinomatoze kod pacijentice sa karcinomom želuca.Prikaz slučaja: pacijentica u dobi od 54 godine osjećala je postupno pojačanu slabost i trnce u gornjim i donjim ekstremitetima tri sedmice prije prijema. Mjesec i po prije prijema podvrgnuta je palijativnoj gastrektomiji, te primila četiri ciklusa kemoterapije. Primljena je na Neurološku kliniku Kliničkog centra Univerziteta u Sarajevu sa flakcidnom slabosti sva četiri ekstremiteta. Neuroslikovne i laboratorijske analize ukazale su da se najvjerovatnije radi o infiltraciji moždanih ovojnica na terenu osnovnog patološkog procesa -karcinoma želuca. Zaključak: LMC je tradicionalna komplikacija u kasnom stadiju uznapredovalog karcinoma. Glavni izazov u dijagnozi i liječenju LMC-a je širok raspon simptoma, brzo pogoršanje i složene potrebe bolesnika.Potrebna su fokusirana klinička ispitivanja, uključujući kontrastni neuroimaging, te razumijevanje patofizioloških procesa kako bi se postiglo optimalno liječenje ove razorne bolesti.

Ključne riječi: leptomeningealna karcinomatoza, leptomeningealne metastaze, karcinom želuca

INTRODUCTION

Leptomeningeal carcinomatosis (LMC) is a rare but devastating complication of solid tumors (1). The disease occurs as a result of metastatic infiltration of the leptomeninges by malignant cells originating from an extra meningeal primary tumor site (2). It is estimated that LMC occurs in approximately 3-8% of solid carcinomas. Breast cancer, lung cancer and malignant melanoma remain the most common causes of LMC, while it is exceedingly rarely associated with gastric cancer, especially in its early stages(3). While significant advances in diagnostic approach have been made over the past years, this has unfortunately not been followed with corresponding advances in therapeutic options. Polymorphic and often subtle neurological signs represent a major diagnostic challenge as they are sometimes difficult to distinguish from brain metastases or adverse effects of cancer treatment (2). Low sensitivity of different diagnostic approaches additionally contributes to the challenging diagnosis. Brain and spine magnetic resonance imaging (MRI) as well as the cerebrospinal fluid (CSF) studies represent the first step in diagnostic approach. Most commonly described MRI signs

of LMC include leptomeningeal enhancement, hydrocephalus, subependymal nodules/deposits, patchy enhancement of nerve roots and extramedullary nodules. CSF studies usually show mild pleocytosis, an abnormally low glucose concentration within the cerebrospinal fluid and elevated protein levels (4). Taking into account the general condition of the patient, therapeutic options are both systemic and intrathecal chemotherapy, radiotherapy and palliative care (5). The poor prognosis in LMC caused by gastric cancer is the result of both late diagnosis and the absence of effective therapeutic options. The mean survival, even in the patients who underwent chemotherapy and radiotherapy, has been reported to be only approximately 4–6 weeks (6).

CASE REPORT

A 54 years old female patient was admitted to Clinic of Neurology due to gradually intensifying weakness and tingling in upper and lower extremities, started three weeks before admission. Weakness of the right arm was more pronounced compared to her left arm, while the weakness of lower extremities progressed to complete weakness and inability to move. A month and a half prior to admission the patient underwent a palliative gastrectomy, previously she was able to move on her on and well functioned. The surgical findings verified inoperable gastric cancer with associated regional lymphadenopathy, incipient peritoneal carcinomatosis and left hydronephrosis. In addition, histopathological findings confirmed the presence of gastric adenocarcinoma particles (Lauren: diffuse type; WHO: poorly cohesive carcinoma; PCC signet ring cell i non signet ring type).Postoperatively, the patient recovered, performed an oncologist checkup and a follow up abdominal CT which did not reveal any signs of residual underlying disease. After the patient underwent four cycles of chemotherapy, she developed progressive weakness of extremities. Neurological examination showed flaccid paralysis of all four extremities, and urine retention. Sensation was grossly intact. Laboratory findings showed negative immunological tests, as well as paraneoplastic antibodies. CSF evaluation revealed elevated protein concentration with lymphocytes domination. Bacteriological analysis of the CSF was negative, while the cytological analysis showed the presence of malignant cells. Furthermore we performed Computed Tomography (CT)scans of cervical and thoracic spine, which only showed the existence of a multi-level disc-osteophyte complexes, reduced bone structure and mineralization of the skeleton in terms of osteoporosis/osteopenia (Figure 1).

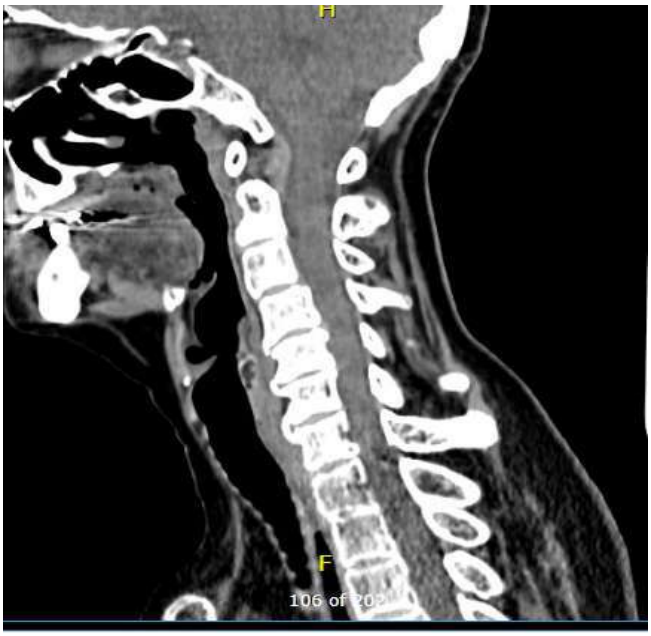

Figure 1 **Cervical and upper thoracic spine CT showed the existence of a multi-level disc-osteophyte complexes, reduced bone structure and mineralization of the skeleton in terms of osteoporosis/osteopenia.**

EMNG was performed: on upper extremities the amplitudes of the waves M were pathologically low, the extended latencies of the F waves with normal conduction velocities, while no sensory and motor responses on lower extremities were found.

Brain magnetic resonance imaging (MRI) revealed pathological increase in signal intensity in the area of the soft membranes of the cerebellum (Figure 2), while MRI of cervical and thoracic spine showed an increase in the signal intensity in the post-contrast series in the area of meninges (Figure 3).

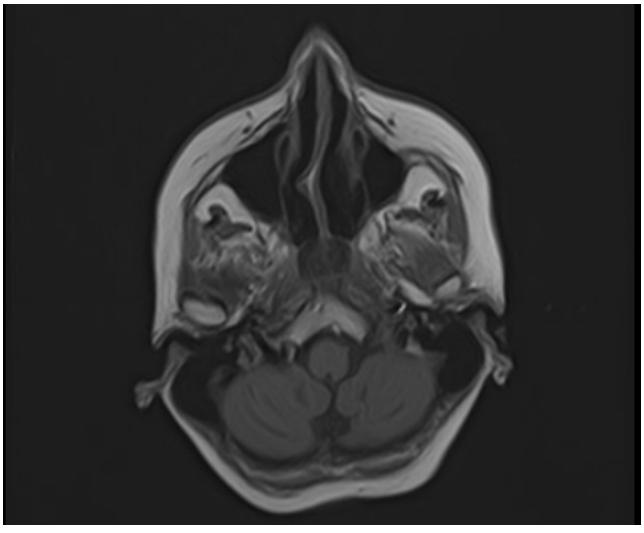

Figure 2 **Brain MRI (T1 weighted image) revealed pathological increase in signal intensity in the area of the soft membranes of the cerebellum**

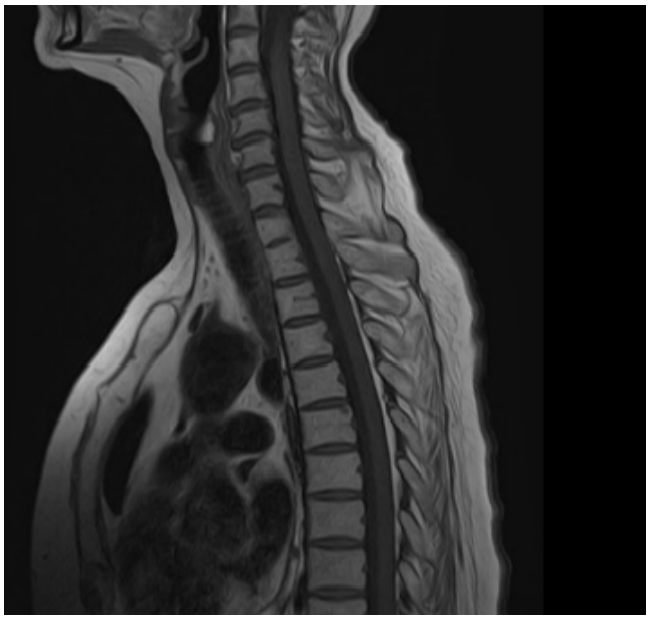

Figure 3 **Cervical and thoracic spine MRI (T1 weighted image) showed an increase in the signal intensity in the post-contrast series in the area of meninges**

During hospitalization, the patient was treated with symptomatic therapy and despite all the intensive care and supportive measures, she continued to deteriorate and died two weeks after admission to our intensive care unit (ICU).

DISCUSSION

Although first described in gastric cancers, LMC occurs incomparably more often in other solid tumors such as lung or breast carcinoma (7). Clinical manifestations depend on the localization of infiltrations (8). Lee JL, et al. analyzed 19 cases of cytological confirmed LMC due to gastric cancer, confirming once more that extra abdominal metastasis are relatively rare and the most common localizations were lungs and bones (9). Despite that, all patients with gastric cancer who suddenly develop neurological symptoms such as headache or cranial nerve dysfunction should be evaluated for possible metastatic changes. Regarding that, it is necessary to rule out other diagnoses, primarily iatrogenic causes due to chemotherapy, intracranial hypertension caused by venous sinus thrombosis, and deficiencies of vitamins B12 (10). Although there is still no sufficiently specific and sensitive test for definitive diagnosis, MRI and CSF pathology are extremely useful supports. MRI is relatively highly sensitive (65-75%), but meningeal staining with contrast is not a specific finding, while CSF cytology has a low sensitivity (about 54%), but is highly specific. The presence of malignant cells in the cerebrospinal fluid is necessary for the final confirmation of the diagnosis. The sensitivity increases by combining these two tests up to 91% (3). MRI of our patient showed gadolinium enhancement of the soft membranes of cerebellum as well as cervical and thoracic spine. CSF cytology was positive with the first performed lumbar puncture, which was enough to establish a firm diagnosis. Current research provides recommendations for the application of radiotherapy, as well as systemic and intrathecal chemotherapy (11). According to the recently established protocol (RANO - Response Assessment in Neuro-Oncology), the therapeutic response can be assessed through a negative cerebrospinal fluid cytological finding, improvement in neuroimaging as well as improvement in neurological findings/symptoms (12). Due to the very serious general condition of our patient and continuous deterioration, in consultation with the oncologist, we have decided not to apply chemotherapy or radiotherapy. The patient received palliative care and died 21 days after the admission. Nevertheless, the toxicity of the available therapies, as well as the limited effectiveness leads to the clear conclusion that not all patients will benefit from the currently available therapies. Even in the case of early diagnosis, the therapeutic approach is still based mainly on expert opinions due to the very small number of clinical studies (12). Waki F, et al. suggested that in patients with negative prognostic factors, including MRI-verified LMC, palliative care is the most acceptable therapeutic option, which was also applicable in our case. (13).

CONCLUSION

Leptomeningeal carcinomatosis is traditionally a late-stage complication of advanced cancer. The main challenge in the diagnosis and management is the wide range of symptoms, rapid deterioration and complex needs of patients. Additional clinical trials as well as an improved understanding of the pathophysiological processes are needed to achieve optimal treatment of this devastating disease.

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Reprint requests and correspondence:
Nejra Mašić, MD
Neurology Clinic
Clinical Center University of Sarajevo
Bolnička 25, 71000 Sarajevo
Bosnia and Herzegovina
Email: nejra.masic@gmail.com
ORCID ID: 0000-0001-9013-4165

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Cordectomy type Vd in a 68-year-old patient with primary modified false vocal fold reconstruction: case report

Hordektomija tip Vd kod 68-godišnjeg pacijenta sa primarnom modificiranom rekonstrukcijom lažne glasnice: prikaz slučaja

Meris Eminović[1,2]*, Mersudin Hadžić[3,2], Zehra Sarajlić[2], Velda Smajlbegović[4]

[1]Primary Health Center, Otorhinolaryngology Department, Vrazova 11, 71000 Sarajevo, Bosnia and Herzegovina
[2]Clinic of Otorhinolaryngology and Head and Neck Surgery, Clinical Center University of Sarajevo, Bolnička 25, 71 000 Sarajevo, Bosnia and Herzegovina
[3]Primary Health Center, Otorhinolaryngology Department, Zmaja od Bosne bb, 70101 Jajce, Bosnia and Herzegovina
[4]Clinic of Oncology, Clinical Centar University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

* Corresponding author

ABSTRACT

Introduction: malignant tumors of the larynx account for 1-3% of all malignant tumors in the body, with a strong increasing tendency in the number of newly found ed cases over recent years. Aim: to evaluate effective surgical techniques and their modalities, and highlight the importance of early detection and treatment of laryngeal cancer in order to improve the disease's prognosis and survival rate. Case report: a 68-year-old patient came to the ENT specialist for an examination due to hoarseness for the last 2 months. A biopsy of the tumor-altered right vocal cords was performed, and verrucous carcinoma of the right vocal cords was identified by histopatology. Following the clinical evaluation and radiological processing (CT), additional surgical treatment in the form of cordectomy of the right vocal cord type Vd with the primary modified recon struction of the false vocal cord was suggested with the agreement of the patient and the clinical oncologist. Postoperatively, the patient was able to continue oral nutrition without signs of aspiration. The patient was able to phonate [VHI score 10, GRBAS scale 6 (1.2 average value - mild), MFT 6.4 sec.], which led to a successful outcome of primary modified reconstruction of the false vocal cord. Postoperative endoscopic flexible laryngoscopy showed satisfying endolaryngeal mobility, slightly weaker on t he right with satisfactory approximation in the medial line during phonation, proper epithelization of the defect, and absence of clear signs of recurrence of the primary disease. Conclusions: the case report confirmes that primary or secondary modified false vocal fold reconstruction after extended cordectomy, due to laryngeal malignancy, enables the patient to have a better quality of life through satisfactory postoperative phonation and proper swallowing without aspiration. Additionally, early detection of larynx cancer, and timely surgical and/or radiation treatment improves the probability of survival and give a better prognostic outcome.

Keywords: laryngeal cancer, cordectomy, thyroplasty, vocal cord reconstruction

SAŽETAK

Uvod: maligni tumori larinksa čine 1-3% ukupnih malignih tumora organizma sa jasnom tendencom rasta broja novootkrivenih slučajeva posljednjih godina. Cilj: evaluacija dobre hirurške prakse kao i modaliteta iste, te značaj ranog otkrivanja i menadžmenta karcinoma larinksa u svrhu bolje prognoze bolesti i veće stope preživljavanja. Prikaz slučaja: 68-godišnji pacijent se javio ORL specijalisti na pregled radi promuklosti unazad 2 mjeseca. Načini se biopsija tumorski izmijenjene desne glasnice te se patohistološki verificira verukozni karcinom desne glasnice. Potom se uradi klinička evaluacija i radiološka obrada (CT), te se uz dogovor sa pacijentom i kliničkim onkologom indicira dalje hirurško liječenje u vidu hordektomije desne glasnice tip Vd sa primarnom modificiranom rekonstrukcijom lažne glasnice. Postoperativno pacijent je bio u mogućnosti nastaviti oralnu ishranu bez znakova aspiracije. Primarna modificirana rekonstrukcija lažne glasnice je dala zadovoljavajući rezu ltat te je pacijent bio u mogućnosti fonirati [VHI score 10, GRBAS 6 (1.2 prosječna vrijednost- blaga), MFT 6.4sec.]. Postoperativna endoskopska fleksibilna laringoskopija je pokazala zadovoljavajuću endolaringealnu pokretljivost, nešto slabije desno uz zadovoljavajuću aproksimaciju u medijalnoj liniji pri fonaciji, urednu epitelizaciju defekta te odsustvo jasnih znakova recidiva osnovnog oboljenja. Zaključak: prikaz slučaja potvrđuje da primarna ili sekundarna modificirana rekonstrukcija lažne glasnice nakon proširene hordektomije, a radi maligniteta larinksa omogućava pacijentu bolji kvalitet života kroz zadovoljavajuću postoperativnu fonaciju te uredno gutanje bez aspiracije. Također rano otkrivanje karcinoma larinksa kao i pravovrem eni hirurški i/ili radijacijski tretman povećava šansu za preživljavanjem te daje bolji prognostički rezultat.

Ključne riječi: karcinom larinksa, hordektomija, tireoplastika, rekonstrukcija glasnica

INTRODUCTION

One to three percent of all malignant tumors in the body are larynx cancers, which are among the most frequent cancers of the head and neck, and have a general tendency to become more common with time. The most frequent risk factors for their development include smoking, alcohol consumption, unfavorable living conditions, exposure to carcinogens, genetic predisposition, and HPV infection. They can develop up to five times more frequently in men, mainly in adulthood and after the age of 60. More than 90% of neoplasms of the larynx are squamous cell carcinoma of different differentiation, and slightly less frequent malignant forms are adenocarcinoma, fibrosarcoma, and chondrosarcoma (1). Two initial signs of laryngeal cancer are hoarse speaking and persistently dry throat. Based on morphology terms, they are categorized as vegetative, infiltrative, and ulcerative, and based on the localization as supraglottic, glottic and infraglottic (2).

Progress in the field of endoscopy led to earlier detection and differentiation of T1 and T2 malignant tumors of the larynx and thus enabled better management of the disease in the form of preservation laryngeal surgery compared to previous radical operations (3). Sparing partial laryngeal surgical procedures can be performed through a transoral approach (cold steel or CO2 laser), laryngofissure or thyrotomy approach. Both procedures considerably decrease postoperative complications and the need for a temporary tracheotomy. The success of sparing partial laryngectomies significantly depends on the experience and training of the surgeon and preoperative evaluation in the form of radiological examinations (CT) with the exclusion of invasion of the thyroid cartilage by a tumor process (4). The European Laryngological Association first proposed a classification of laryngeal endoscopic cordectomy in 2000, which included 8 types of cordectomy (5). Type I represents subepithelial cordectomy, type II subligamentary, type III transmuscular, type IV total, and type V extended cordectomy with four subtypes, depending on whether the tumor process affects the contralateral vocal cord, arytenoid, ventricular fold, or subglottis. The shortcoming of this classification is reflected in the fact that it does not include tumor lesions originating from the anterior commissure, which was subsequently designated as cordectomy type VI (6). The mentioned methods allow exceptional local control and laryngeal preservation. However, they have their own drawback, particularly cordectomy type IV and V, in the form of postoperative dysphonia and aspiration correlated with the amount of resected tissue (3). Ways of avoiding the mentioned complications, associated with surgical treatment of the disease, are reflected in primary or secondary thyroplasty. Modalities of thyroplasty include reconstruction with displacement of the false vocal cord, submucosal injection of autologous adipose tissue, reconstruction with cartilaginous graft originating from thyroid cartilage, and submucosal placement of muscle flap (4).

AIM

The aim of this case report was to evaluate effective surgical techniques and their modalities, and highlight the importance of early detection and treatment of laryngeal cancer in order to improve the disease's prognosis and survival rate.

CASE REPORT

A 68-year-old patient approached an ENT specialist for examination due to hoarseness in the previous 2 months, denying breathing and swallowing difficulties. The patient was a long-term smoker, consuming up to a pack and a half daily, nonalcoholic, with negative history of family malignancies. He suffered from DM, HTA, and dyslipidemia, for which he was taking regular therapy and Insulin. In 2015, a high amputation of the right leg was performed due to arterial occlusion.

Indirect and endoscopic laryngoscopy showed a completely altered, leukoplakia-coated, and tumor-infiltrated right vocal cord with subglottic propagation (Figure 1). A biopsy of the tumor-altered right vocal cord was performed and histopatlogy confirmed verrucous carcinoma. A detailed clinical and radiological evaluation (CT with contrast) was performed and a discrepancy was recorded, given that the CT did not describe a clear endoscopically visible subglottic propagation of the tumor on the right, but emphasized the radiologically highly suspicious infiltration of the anterior commissure (Figure 2a and 2b). The patient was presented to the oncology council, and with the agreement of the oncologist, and consent of the patient and family, further surgical treatment was indicated in the form of cordectomy of the right vocal cord type Vd with primary modified reconstruction of the false vocal cord.

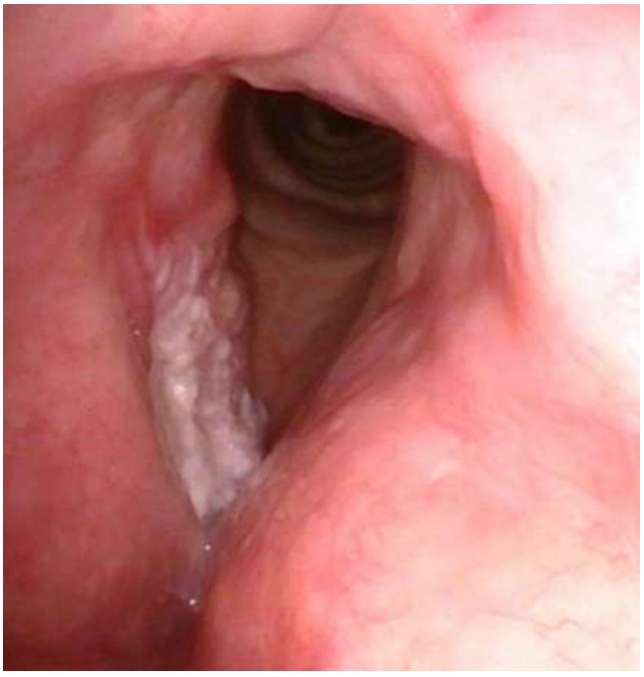

Figure 1 **Endolaryngeal view.**

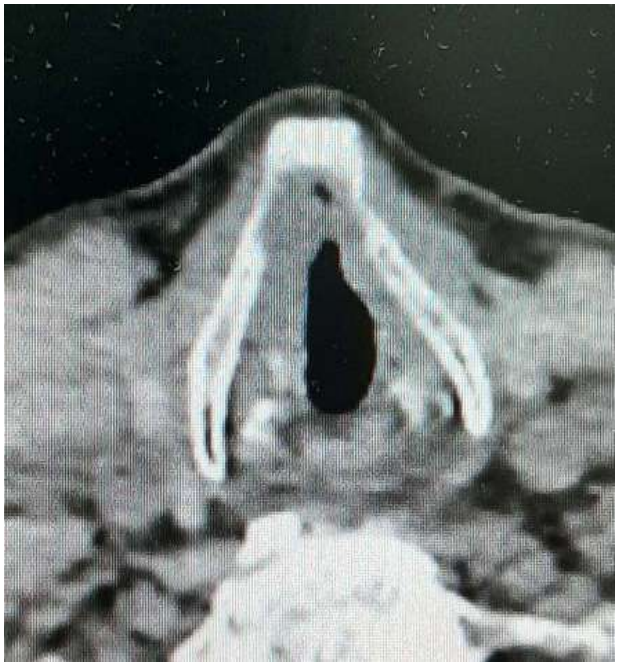

Figure 2a **CT of the larynx without contrast.**

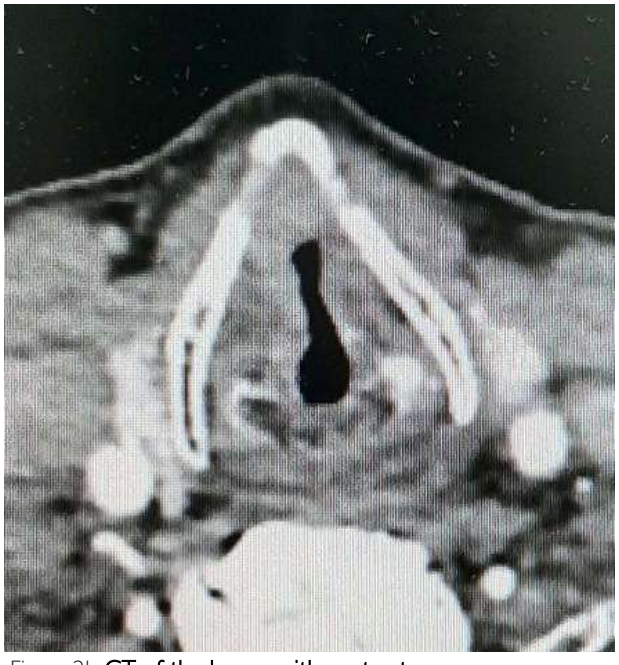

Figure 2b **CT of the larynx with contrast.**

The surgical technique included a vertical neck skin incision with a sharp/blunt preparation in layers in the medial line, deperichondrion of the thyroid cartilage, followed by laryngofissure/thyreotomy. Intraoperatively, clear subglottic cancer propagation was visualized (Figure 3).

Figure 3 **Intraoperative subglottic tumor propagation.**

The cancerous process of the right vocal cord with infraglottic propagation was resected to the macroscopically healthy tissue and sent for PH analysis, along with border preparations and resection of the anterior commissure. The rest of the false vocal cord on the right was freed and mobilized, and a primary modified reconstruction was performed in the form of thyroplasty, for medialization and caudal retraction of the false vocal cord. It was sutured caudally with the mucosa of the resection margin and with the anterior aspect of the thyroid cartilage with resorbable sutures. The laryngofissure was closed with 3-0 Vicryl suture, followed by suturing in layers. Correct hemostasis was achieved and there was no need for active or passive drainage. Intraoperative and postoperative procedures were with no indications for tracheotomy.

The postoperative procedure went smoothly, symptomatic, supportive with antibiotic therapy. Postoperatively, the patient was able to continue oral nutrition without signs of aspiration. The patient was able to phonate [VHI score 10, GRBAS scale 6 (1.2 average value - mild), MFT 6.4 sec.], which led to a successful outcome of primary modified reconstruction of the false vocal cord. Postoperative endoscopic flexible laryngoscopy showed satisfactory endolaryngeal mobility, slightly weaker on the right, with satisfactory approximation in the medial line during phonation, proper epithelization of the defect and the absence of clear signs of recurrence of the underlying disease (Figure 4). As a minor postoperative complication after the removal of sutures, a local wound infection developed, which was treated by regular dressing of the wound with previously prescribed antibiotic treatment (Figure 5). During the microbiological examination of the wound, presence of pathogens was not identified. The patient had been regularly monitored.

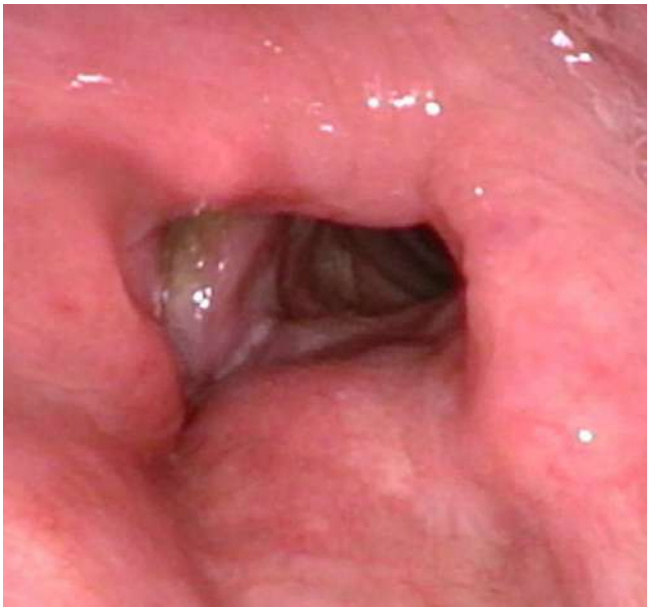

Figure 4 **Postoperative endolaryngeal view.**

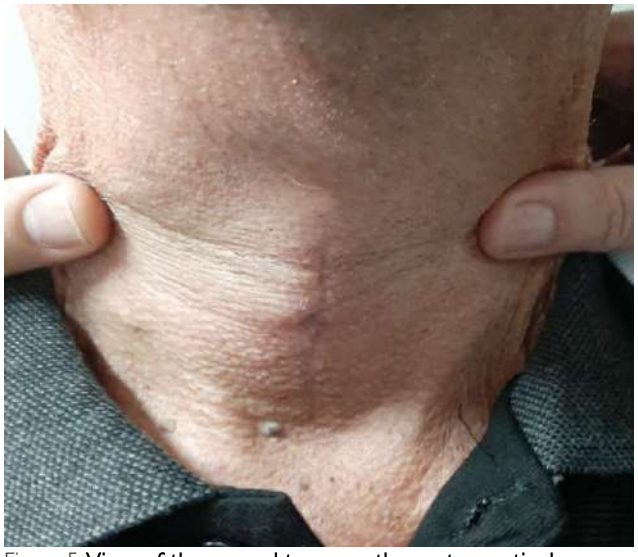

Figure 5 **View of the wound two months postoperatively.**

The previously verified verrucous carcinoma of the right hemilarynx stage I/II, pT2NxMxR1, was confirmed pathohistologically. Classification R1 was assigned due to the presence of LIN of mild degree in the infraglottic resection margin, although the tumor was located at a distance of 2 to 3 mm from the infraglottic space, which according to the available medical documentation presented a satisfactory resection margin. The resection margin towards the cranial or towards the ventricular fold was at a distance of 5 mm. The border preparation of the anterior commissure as well as the suspicious pretracheal lymph node did not show the presence of malignant cells. Followed by the results of PH analysis, the patient was again presented to the oncology council where, with the oncologist's consultation, further radiation treatment was suggested with the surgeon check-up every two months.

DISCUSSION

Advances in the field of endoscopic laryngoscopy have led to earlier detection and differentiation of T1 and T2 laryngeal carcinomas and enabled the advancement of minimally invasive laryngeal surgery as a treatment modality in comparison to earlier radical treatment. This is also shown by the increase in the number of studies on early larynx cancer after the classification of endoscopic cordectomy by the working committee of the European Laryngological Association in 2000. Results of a hypothetical and theoretical article by Mendelsohn AH, et al., statistically confirm this hypothesis and show the importance of the mentioned classification in relation to the pioneering study of Professor Steiner W, on transoral laser microsurgery in laryngeal cancer (7). According to the guidelines of the NIH (National Cancer Institute), the recommended treatment of newly diagnosed laryngeal cancer of the second stage includes radiotherapy with or without surgery or operative treatment itself (8). Different studies favor one modality of treatment over another. The choice of these depends to a large extent on the patient's wishes, the possibility and reliability of monitoring, the expected functional results, the experience and abilities of the surgeon, and the patient's general health condition.

Study by Elio K, et al. describes a case report with intermediate mucoepidermoid carcinoma of the right vocal cord treated with radiotherapy (35 fractions of 70Gy) with a five-year follow-up of the patient without signs of recurrence (9).

Study of Chung SY et al. compares radiotherapy and operative management of early laryngeal cancer. The results of the same study suggest a higher percentage of local control and recurrence-free survival in patients treated with radiotherapy compared to surgical treatment. The three- and five-year percentage of local control is 91.9% and 89.9% for the irradiation group, while the same is 82.8% and 73.2% for the cordectomy group. Three- and five-year survival rates without disease recurrence are 87.5% and 83.7% for the irradiation group, while the same is 79.2% and 68% for the cordectomy group. The study did not show a statistically significant difference in overall survival of both groups of subjects (10).

Earlier studies such as Yoo J, et al. and O'Hara J et al. showed that the treatment of early cancer of the larynx is equally effective with both surgical and radiation approaches and that the choice of treatment modality is based on patient and clinician preference as well as general health status (11,12).

Most of the available studies show a slightly higher percentage of laryngeal preservation during operative treatment as initial treatment.

Study by Bocciolini C, et al. shows an exceptional three-year percentage of overall survival (97.5%), survival without disease recurrence (89.9%), local control (92.4%) and laryngeal preservation (97.4%) in the surgical modality of early laryngeal cancer treatment (13).

In our case, after the presentation of the medical condition and modality of treatment, expected results as well as possible intraoperative and postoperative complications for the patient, and in agreement with the patient's family and the clinical oncologist, cordectomy type Vd through the laryngofissure with the primary modified reconstruction of the false vocal cord was performed, and susbsequently pathohistological diagnosis and radiation treatment by the oncologist was also performed.

Complications associated with surgical treatment of early laryngeal cancer are local injuries, bleeding from the larynx, postoperative edema, granulomas and adhesions, worse phonatory result, and aspiration of food or saliva (6). Various studies have

described the incidence of postoperative aspiration, usually ranging between 2% and 11% (3).

Study by Krengli M, et al. points to milder degrees of dysphonia based on electroacoustic voice analysis in patients treated with radiation treatment compared to a group of patients who underwent laser cordectomy without vocal fold reconstruction and concludes that the functional voice outcome should be significantly analyzed when choosing a treatment modality for laryngeal cancer (14).

More recent studies such as Lorenz KJ, et al. show that patients undergoing modified false vocal cord reconstruction after cordectomy have better vocal rehabilitation and fewer aspiration symptoms compared to transoral laser resection or conventional open resection without glottis reconstruction. The expected phonatory result was assessed through the GRBAS scale and the maximum phonation time as a mild or moderate voice disorder in comparison with the conventional method and laser excision without reconstruction, where the same was assessed as a moderate to severe voice disorder (4). Apart from the described reconstruction of the vocal cords, a satisfactory postoperative phonatory result after cordectomy type I-III can be achieved by medialization of the vocal fold with the help of autologous fat tissue.

Study by Zapata S, et al. describes medialization of the vocal cord with autologous fat tissue with dysphonia assessed as mild (GRBAS, VHI, and MFT) after cordectomy type Va (15). The most common complications of radiation management of early laryngeal cancer are reflected in local skin reactions as well as post-radiation pharyngitis and tissue edema. According to the study of Chung SY et al. the postradiation phonatory outcome assessed by subjective voice rating scales showed a score higher than 80 for most patients (scale from 0 to 100), while six patients (5.4%) complained of prolonged hoarseness with a mean value of 10 months (range, 3 to 44 months)(10).

In our case report, the primary modified false vocal cord reconstruction, after extended cordectomy (type Vd) due to laryngeal malignancy, enabled the patient to have a better quality of life through satisfactory postoperative phonation and proper swallowing without aspiration. In comparison to radiation treatment, the advantages include the possibility of analysis of resection margins pathohistologically, shorter hospital treatment, lower cost of treatment, the possibility of adequate rehabilitation and the maintenance of the quality of life shortly after surgery, and the possibility of radiotherapy as a treatment for disease recurrence.

Methods of reconstructive thyroplasty are widely described in medical databases, but only a few cases of extended cordectomy through the laryngofissure with primary modified false vocal cord reconstruction have been described. By searching some of the most popular medical databases (PubMed Central, Cochrane Library, Ovid, openmd, ResearchGate, Google Scholar, Wiley Online Library) it was established that no similar cases have been published in our region so far.

CONCLUSION

The case report confirms that primary or secondary modified false vocal fold reconstruction after extended cordectomy, due to laryngeal malignancy, enables the patient to have a better quality of life through satisfactory postoperative phonation and proper swallowing without aspiration. Additionally, early detection of larynx cancer, and timely surgical and/or radiation treatment improves the probability of survival and give a better prognostic outcome. In comparison to laser surgery, the advantage of this type of surgery is reflected in the fact that primary reconstruction of the glottis is not possible during laryngeal laser surgery. Further research is needed to confirm our experience, with the hope that new and improved modalities of surgical treatment of early laryngeal cancer will be developed.

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Reprint requests and correspondence:
Meris Eminović, MD
Family Medicine, ENT Department
Primary Health Center
Vrazova 11, 71 000 Sarajevo
Bosnia and Herzegovina
Email: meriseminovic@gmail.com
ORCID ID: 0000-0003-0438-311X

Declaration of patient consent: the authors certify that they have obtained appropriate patient's consent form.
Authors' Contributions: ME, MH, ZS and VS made major contributions to the writing of this paper; ZS, EŠ and RB are ENT specialists and project supervisors; AK is the chief of the Ear, Nose and Throat Clinic with Head and Neck Surgery of the CCUS; VS is the chief of the Department of Radiation Oncology and Clinical Oncologist who treated the patient; SIS participated in the clinical treatment and follow-up of the patient. Each author had a role in article drafting and in process of revision. Each author gave final approval of the version to be published and they agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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Treatment of patient with in-stent restenosis by ultra-high-pressure balloon and drug coating balloon as alternative to stent implantation

Tretman pacijenta sa instent restenozom sa balonom ultra-visokog pritiska i balonom obloženim lijekom kao alternativa implantaciji stenta

Mesud Jamaković*

Clinic of Heart Diseases, Blood Vessels and Rheumatism, Clinical Centre University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Introduction: although less frequent in era of drug eluting stents (DES) unlike previously used bare metal stents (BMS), in-stent restenosis (ISR) still remains most important long-term issue and cause of failure after percutaneous coronary interventions (PCI). Optimal treatment of ISR, suitable for all patients affected is not clearly established and a few concepts are evaluated. Treatment options include PCI, usually with implantation of a new DES inside the previously restenosed stent, coronary artery bypass grafting (CABG), or medical treatment optimization. Aggressive dilatation of ISR by dedicated balloons that tolerate ultra-high pressures followed by dilation with "drug coated balloons" (DCB) can be an attractive and eligible treatment option in considerable number of ISR cases. Aim: to consider optimal treatment of ISR lesions and show potential advantages of newer therapeutic strategies. Case report: a 61 old patient admitted to hospital due to non-ST-elevation myocardial infarction (NSTEMI). Nine years ago, patient suffered inferoposterior ST-elevation myocardial infarction (STEMI) and underwent to PCI with BMS implantation. Five years later, due to reinfarction also underwent to invasive coronary angiography that revealed patent previous stent and critical right coronary artery (RCA) stenosis proximally from recent stent. Treated again with another DES implanted in proximal RCA. During present hospitalization, coronary angiography findings revealed double vessel disease - again with culprit lesions in RCA including a critical ISR of earlier BMS and a new subocclusion in distal part of the vessel. After predilatation - DES implanted in distal lesion with good angiographic result. The ISR in mid RCA was dilated by OPN balloon with excellent angiographic result and optimal expansion. Procedure finished by DCB, thereby avoiding implantation of one more stent. Patient remains stable and discharged 3 days later. Conclusion: in this case we demonstrated successful treatment of ISR by super high-pressure balloon followed by DCB treatment, as alternative to new stent deployment. Such treatment can serve as default strategy in many cases of ISR instead of new stent implantation.

Keywords: PCI, coronary stent restenosis, myocardial infarction

SAŽETAK

Uvod: iako manje česta u eri "stentova obloženih lijekom" (DES) za razliku od ranije korištenih "čistih metalnih stentova" (BMS), instent restenoza (ISR) dugoročno i dalje ostaje najvažniji problem i uzrok neuspjeha tretmana nakon perkutane koronarne intervencije (PCI). Optimalan tretman ISR koji bi se mogao primijeniti na sve pacijente nije jasno definiran, a u tom smislu evaluirano je nekoliko strategija. Tretman uključuje PCI, obično sa ugradnjom novog DES-a u prethodno stenozirani stent, aorto-koronarno premoštenje ("bypass") ili optimizaciju medikamentoznog tretmana. Agresivna dilatacija specijalnim balonom koji toleriše vrlo visoke pritiske, praćena sa dilatacijom sa DCB može biti atraktivna i prikladna opcija tretmana kod značajnog broja pacijenata sa ISR. Cilj: razmotriti opcije tretmana lezija sa ISR i pokazati potencijalne prednosti novijih terapijskih strategija. Prikaz slučaja: 61-godišnji pacijent je primljen u bonicu radi infarkta miokarda bez ST-elevacije (NSTEMI). Devet godina ranije je imao inferoposteriorni infarkt miokarda sa ST elevacijom (STEMI) i podvrgnut je PCI sa ugradnjom stenta (BMS). Pet godina kasnije, radi reinfarkta miokarda je ponovo podvrgnut koronarnoj angiografiji koja je pokazala prohodan ranije implantiran stent i kritično suženje RCA proksimalno od ranijeg stenta. Tretiran je ponovo sa drugim DES koji je ugrađen u proksimalnu RCA. Tokom sadašnje hospitalizacije nalaz koronarne angiografije je ukazao na dvosudovnu koronarnu bolest, ponovo sa "ciljnim" lezijama na RCA, uključujući kritičnu ISR ranijeg BMS-a i novo subtotalno suženje u distalnom dijelu krvnog suda. Nakon predilatacije, na distalnu leziju je implantiran DES sa dobrim rezultatom. ISR u srednjoj RCA je dilatirana sa OPN balonom sa odličnim angiografskim rezultatom i optimalnom ekspanzijom. Procedura je završena sa DCB, na taj način izbjegavši implantaciju još jednog stenta. Pacijent je ostao stabilan i otpušten je nakon 3 dana. Zaključak: u navedenom prikazu slučaja prikazali smo uspješan tretman ISR balonom ultra-visokog pritiska praćen tretmanom sa DCB - kao alternativa ugradnji novog stenta. Ovakav tretman može poslužiti kao "default" strategija u mnogim slučajevima ISR umjesto implantacije novog stenta.

Ključne riječi: PCI, restenoza koronarnog stenta, infarkt miokarda

INTRODUCTION

Although in stent restenosis (ISR) is much less frequent in era of drug eluting stents (DES) unlike previously used bare metal stents (BMS), an ISR remains most important long-term issue and cause of failure after percutaneous coronary interventions (PCI) (1). Optimal treatment of ISR, suitable for all patients affected is not clearly established and a few concepts are evaluated in recent metaanalyses (2). Further treatment usually includes implantation of a new DES inside the previously restenosed stent, coronary artery bypass grafting (CABG), or even in some cases optimization of medical treatment is the last resort. Regarding to PCI treatment with a new DES inside the restenosed one, an appropriate preparation of affected site of the vessel has to be made before stent implantation, to achieve optimal stent expansion. Lesion preparation can be tried with use of NC balloons, cutting/scoring balloons, OPN balloons, rotablation, laser or intravascular lithotripsy balloon in selected cases (3), although some of these options are not routinely available in all cath-labs. Recent launching of balloons coated by drugs that inhibit neointimal hyperplasia is also utilized in ISR (4).

AIM

The aim of this study was to consider optimal treatment of ISR lesions and show potential advantages of newer therapeutic strategies.

CASE REPORT

A 61 old male admitted to hospital due to symptoms consistent to non-ST-elevation myocardial infarction (NSTEMI). Symptoms began 3 days before admission with chest pain after swimming in pool. Nine years ago patient suffered inferoposterior myocardial infarction (STEMI) and underwent to pPCI with implantation of BMS 2.5x15 mm. Five years later - due to reinfarction also underwent to invasive coronary angiography that revealed patent previously implanted stent and critical RCA stenosis proximally from recent stent. Treated again with PCI and DES 3.5x20 mm implanted in proximal RCA. Patient had grade II of arterial hypertension, type 2 diabetes mellitus with long term smoking and positive familiar history on cardiovascular diseases.

During present hospitalization, markers of cardiac necrosis were positive (CK: 1249 U/L; Hs troponin: 228 ng/L). Echocardiographic examination revealed left ventricle ejection fraction of 43% with inferoposterior and lateral hypokinesia. This time coronary angiography was performed by right radial approach with standard procedure. Findings revealed double vessel disease, borderline stenosis of OM2 and significant lesions in RCA; previously implanted DES was patent, but critical 90-95% ISR of BMS in middle part of RCA as well as new subocclusion in distal part of the vessel noticed (Figure 1,2). A distal lesion has been considered as culprit one. PCI performed by using Judkins right 4 (JR4) guiding catheter. After predilatation of a both lesions - DES 3.0x13 mm (Evermine, Meril) implanted in distal lesion (Figure 3) with good result (Figure 4). Thereafter, the lesion in mid RCA was dilated by OPN balloon (SIS medical) 2.5x15 mm (Figure 5) inflated to 40 atmospheres by obtaining excellent angiographic result with optimal ISR expansion. Procedure finished by treating the ISR with DCB 2.5x20 mm (Pantera Lux, Biotronic, Figure 6) with optimal angiographic appearance (Figure 7,8), thereby avoiding implantation of one more DES. Patient remains stable and discharged 3 days later with recommendation for DAPT consisting of acetylsalicylic acid and prasugrel, along with statins, beta blocker, antidiabetics and other appropriate therapy.

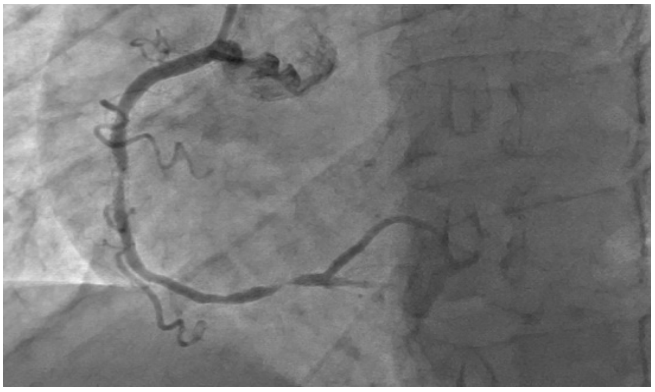

Figure 1 - **Critical stenosis of mid and distal RCA.**

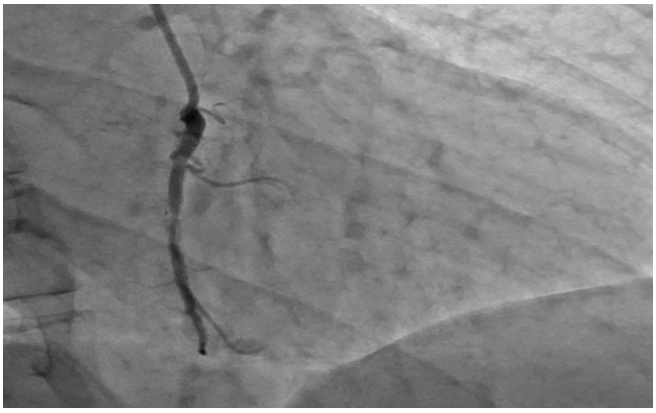

Figure 2 - **Subtotal ISR of RCA in "RAO" position.**

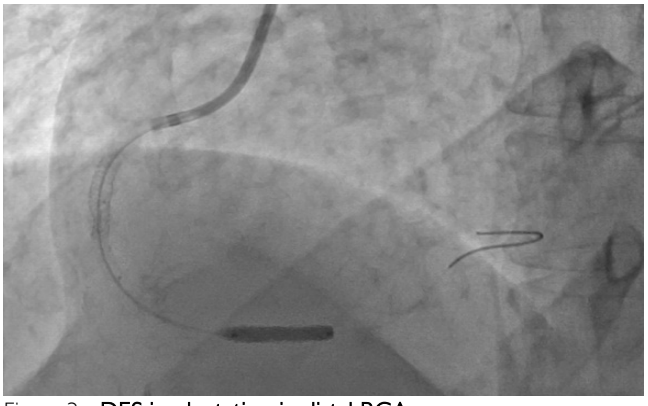

Figure 3 - **DES implantation in distal RCA.**

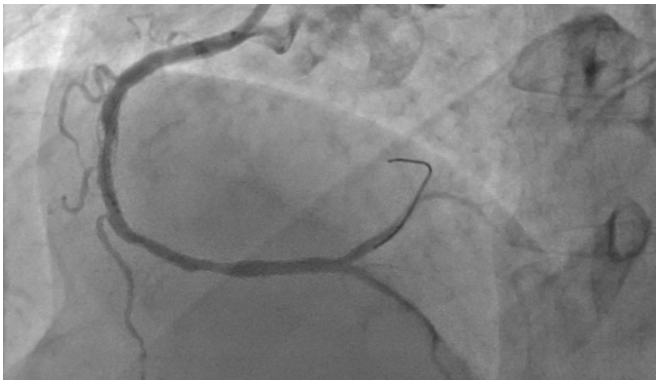

Figure 4 - **Good angiographic result on distal lesion.**

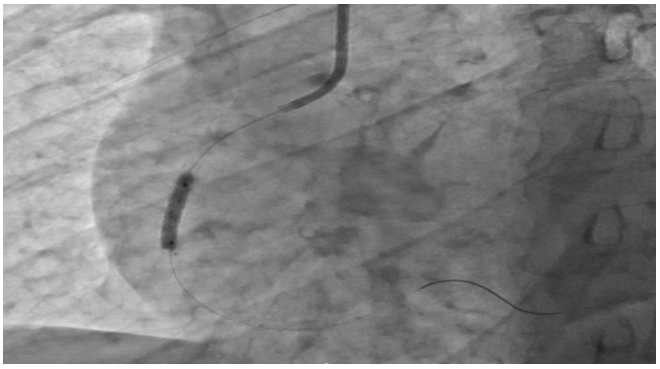

Figure 5 **- Aggressive predilatation of ISR by OPN balloon.**

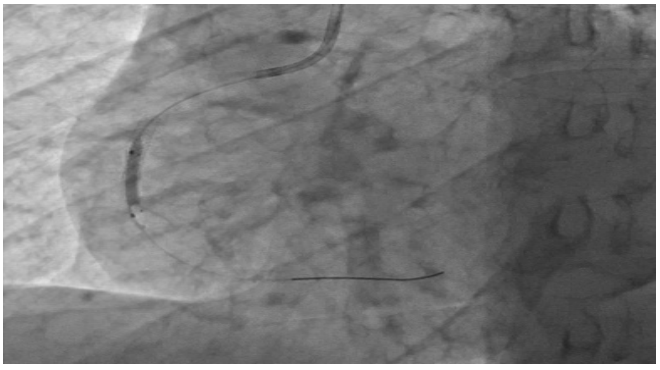

Figure 6 - **Treatment of ISR by DCB.**

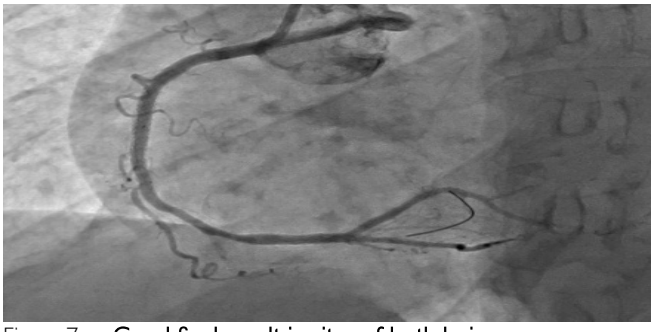

Figure 7 **- Good final result in sites of both lesions.**

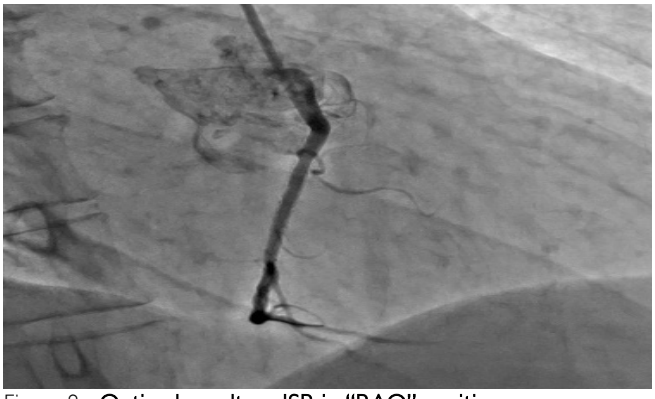

Figure 8 **- Optimal result on ISR in "RAO" position.**

DISCUSSION

Despite technical improvement of stents and manufacturing much better and superior DES generations than first one, ISR remains main long term problem in PCI and leading cause of stent failure.

Use of drug coated balloon (DCB) is increasingly employed in PCI, mostly in case of interventions in small caliber coronary vessels but its usefulness is also remarkable in ISR (4).

However, although are DCBs coated by a drug (usually paclitaxel, but sometimes sirolimus) (5) that inhibits neointimal proliferation and consequent occurrence of ISR, a DCB is made of semicompliant balloon, that is - in majority of case not able to dilate tough ISR in optimal extent. Hence, aggressive predilation of ISR is needed to achieve appropriate luminal area. Predilation is usually made by hard, a non-compliant (NC) balloon, and after optimal predilation-a DCB is applied. In some cases, even despite pressures above 20 atmospheres, an optimal predilation cannot be achieved. Therefore, predilation by dedicated ultra-high pressure balloon with double layer ("OPN balloon", SIS medical-AG, Frauenfeld-Switzerland) offers promising results in that way. This balloon is increasingly used for optimization of result in cases of underexpanded stents (6) and wide spectrum of indications and also in ISR. Official "rated burst pressure" of OPN balloon according to manufacturer is 35 atmospheres, but it is well-known that it can often tolerate much higher pressures, even 50 atmospheres, allowing much better dilatation and appropriate results (7,8).

We demonstrated the case successful treatment by OPN and DCB in patient with severe ISR in right coronary artery (RCA), without need for repeated stent implantation.

CONCLUSION

Although DES implantation represents most frequent treatment of coronary stenoses, an ISR remains common long-term issue. ISR is traditionally treated by implantation of another DES inside the previous stent and some patients are candidates for CABG. In some cases, 3 or more layers of coronary stents are present in some lesions, limiting next interventions and leading to further lumen reduction of coronary vessel. Increasing use of DCB showed efficacious in treatment of ISR in many cases, without implantation of new stents. Dilation of ISR by DCB is typically not sufficient for optimal expansion of tough ISR lesions. Thus, an appropriate, usually

aggressive predilation by ultra-high pressure balloon (OPN), rather than classic NC balloon, and subsequent treatment by DCB seem very effective and proven strategy in number of cases with ISR.

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Reprint requests and correspondence:
Mesud Jamaković, MD, MSc
Clinic of Heart Diseases, Blood Vessels and Rheumatism
Clinical Center University of Sarajevo
Bolnička 25, 71000 Sarajevo
Bosnia and Herzegovina
Email: dr_jamakovic@yahoo.com
ORCID ID: 0000-0001-7260-1815

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Pemphigus vegetans in a patient with psoriasis vulgaris: case report

Vegetantni pemfigus kod pacijentice sa psorijazom: prikaz slučaja

Nina Čamdžić[1]*, Selma Poparić[2], Aida Kapetanović[3], Suada Kuskunović - Vlahovljak[1]

[1]Department of Pathology, Faculty of Medicine, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina
[2]Clinic of Dermatovenerology, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina
[3]Clinical Pathology, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Introduction: pemphigus vegetans (PVeg) is an uncommon variant of Pemphigus vulgaris (PV) with highly variable clinical presentation and many overlapping features with other dermatologic conditions. PVeg has established autoimmune basis, but its association with psoriasis is yet to be fully established. Aim: to report a case of PVeg with unusual clinical presentation in a middle-aged patient previously diagnosed with psoriasis. Case report: a 50-year-old female presented to Dermatology Clinic with skin lesions that affected entire skin surface in form of erythematosquamous papules and plaques. The most severe lesions presented as individual pustules and shallow linear ulcerations evolving into hypertrophic plaques located mostly on pubic and inguinal region. Other severely affected area was scal p with lesions in form of erosive plaques, focally covered by adherent yellowish crusts and multiple areas of hair loss. Years earlier, patient was diagnosed with psoriasis vulgaris but was in a remission for longer period of time. Clinical differential diagnosis included an exacerbation of main disease, pyoderma gangrenosum and erosive pustular dermatosis. Skin biopsy revealed typical morphological findings consistent with pemphigus vegetans. Conclusion: in its typical form, the onset of PVeg commonly corresponds to mucosal lesions and later affects flexural sites in form of vegetating verrucous plaques. But when disease appears on other non-intertriginous areas, and has different clinical course, physicians need high level of suspicion to establish the diagnosis.

Keywords: pemphigus vegetans, psoriasis vulgaris, scalp, intertriginous areas, histopathology

SAŽETAK

Uvod: pemphigus vegetans (PVeg) je rijedak oblik Pemphigus vulgaris-a (PV) sa izrazito varijabilnom kliničkom prezentacijom, te brojnim preklapajućim karakteristikama sa drugim dermatološkim stanjima. Bolest ima jasnu autoimunu osnovu, ali povezanost PVeg sa psorijazom nije još u potpunosti razjašnjena. Cilj: predstaviti slučaj PVeg u atipičnom kliničkom obliku kod pacijentice srednje životne dobi sa već prethodno postavljenom dijagnozom psorijaze. Prikaz slučaja: pacijentica u dobi od 50 godina javila se dermatologu s kožnim lezijama koje su zahvatile cijelu površinu kože u obliku eritemato-skvamoznih papula i plakova. Najteže lezije su bile locirane u pubičnoj i ingvinalnoj regiji, u vidu pojedinačnih pustula i plitkih linearnih ulceracija, postupno se razvijajući u hipertrofične plakove. Vlasište je drugo područje koje je bilo teže pogođeno bolešću u obliku erozivnih plakova, mjestimice prekrivenih žućkastim krustama, sa višestrukim žarištima gubitka kose. Godinama ranije, pacijentici je dijagnosticirana psorijaza, ali je bila u remisiji duže vrijeme. Klinička diferencijalna dijagnoza uključivala je egzacerbaciju glavne bolesti, piodermu gangrenosum i erozivnu pustularnu dermatozu. Biopsija kože otkrila je tipične morfološke promjene konzistentne sa vegetantnim pemfigusom. Zaključak: u svojoj tipičnoj kliničkoj prezentaciji, prvi znak vegetantnog pemfigusa su obično lezije oralne sluznice sa kasnijom pojavom verukoznih vegetacija i plakova u intertriginoznim područjima. No kad se bolest javi u atipičnoj formi, kliničari moraju imati visok nivo sumnje da bi mogli adekvatno dijagnosticirati ovu bolest.

Ključne riječi: pemphigus vegetans, psoriasis vulgaris, poglavina, intertiginozna područja, patohistologija

INTRODUCTION

Pemphigus vegetans (PVeg) is an autoimmune vesiculobullous disorder, representing a rare form of Pemphigus vulgaris (PV), comprising approximately 2-7% of cases worldwide. The disease results from autoantibodies against the keratinocyte cell to cell adhesion molecules, specifically, autoantibodies against desmoglein 3 and desmoglein 1 (1). Clinical presentation of PVeg is highly variable, but common findings include initial stomatitis, followed by cutaneous lesions affecting the trunk, arms, legs, and flexural areas. Cutaneous lesions present as flaccid bullae and pustules which over time rupture and ulcerate, forming hypertrophic plaques and verrucous vegetating

skin lesions. Two main forms of PVeg, the Hallopeau and the Neumann type, have many histologic and immunologic overlapping features but differ in clinical presentation, response to treatment and prognosis (1,2).

Although many studies have confirmed the autoimmune diathesis of pemphigus and its association with many autoimmune diseases (3), its association with psoriasis, as a disease caused by dysregulation of the immune system, is yet to be completely elucidated (4).

We report a case of PVeg with unusual clinical presentation in a middle-aged patient previously diagnosed with psoriasis. To our best knowledge this is the first case of Pemphigus vegetans associated with psoriasis in our country.

AIM

The aim of this study was to report a case of PVeg with unusual clinical presentation in a middle-aged patient previously diagnosed with psoriasis.

CASE REPORT

A 50-year-old female presented to the Dermatology clinic due to worsened condition of skin lesions which appeared one month earlier. Lesions initially appeared on lower back in a form of itchy macules with occasional burning sensation. In a short period of time, entire skin surface was affected, particularly scalp and intertriginous areas. Skin changes evolved into erythematosquamous papules and plaques with the most severe lesions affecting pubic and inguinal region. These lesions presented as individual pustules and shallow linear ulcerations evolving into hypertrophic plaques.

Lesions on scalp manifested as erosive plaques, focally covered by adherent yellowish crusts with multiple areas of hair loss (Figure 1).

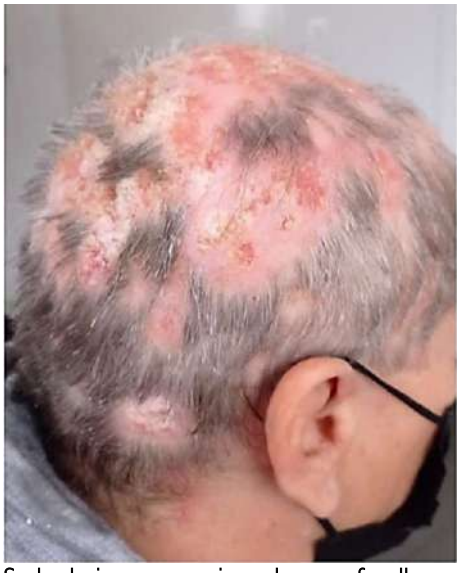

Figure 1 **Scalp lesions - erosive plaques, focally covered by adherent yellowish crusts with multiple areas of hair loss**.

Years earlier, patient was diagnosed with psoriasis vulgaris but was in a remission for longer period of time, with scarce data regarding the main disease. Laboratory analyses revealed increased Ig E in serum (more than 35 times increased above the upper limit of reference values) and positive Antinuclear antibodies (ANA). Otherwise, routine laboratory tests were inconspicuous. Bacterial swabs of the scalp lesion revealed colonization with *Staphylococcus aureus*, while tests for fungal infections were negative.

Since the patient was previously diagnosed with psoriasis vulgaris, clinical differential diagnosis included an exacerbation of main disease, pyoderma gangrenosum and erosive pustular dermatosis (EPD).

Skin biopsy was obtained from scalp and inguinal region. Histopathology revealed focal follicular epithelium involvement together with marked epidermal acanthosis, pseudoepitheliomatous hyperplasia, hyperkeratosis, parakeratosis and papillomatosis. Epidermis showed multiple foci of acantholysis that created discreet suprabasal clefting. There was strong eosinophilic response with eosinophilic spongiosis, intraepidermal eosinophilic micro abscesses, and dense eosinophilic dermal infiltrate accompanied by neutrophils and lymphocytes (Figure 2).

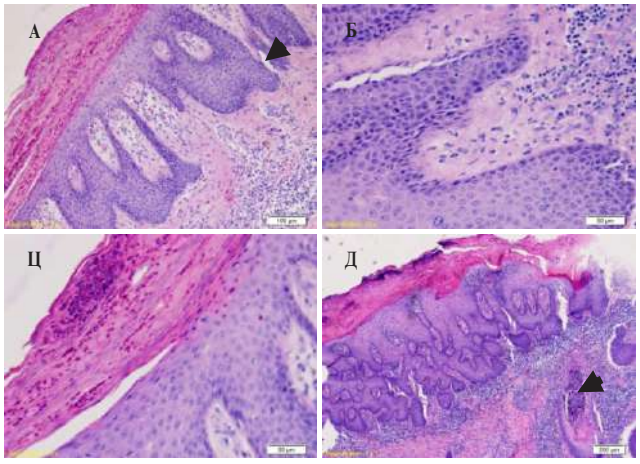

Figure 2 **A) Acanthotic epidermis with accumulation of eosinophils in hyperkeratotic layer. Red arrow showing suprabasal acantholysis. Note dermal inflammatory infiltrate made of eosinophils, lymphocytes and neutrophils (HE, 10x); B) Suprabasal clefting (HE, 20x); C) Eosinophils in corneal layer of epidermis (HE, 20x); D) Pseudoepitheliomatous hyperplasia and follicular involvement (red arrow) with marked inflammatory infiltrate (HE, 4x).**

In multiple periods of disease exacerbation, patient was administered high doses of systemic corticosteroid prednisone (60 mg per day intramuscularly), systemic antibiotic therapy and Methotrexate (10 mg per week) along with pantoprazole, potassium chloride and folic acid. In between periods of slight improvement of skin lesions the patient was also put on isotretinoin in daily doses of 30 and 20 mg. Further on, she continued with Methotrexate 10 mg per week and prednisone which was gradually lowered over a period of two months up to a dose of 10 mg per day.

The patient is currently under control with a low dose of prednisolone, and there is no recurrence of the lesions. As a consequence of the disease patient has cicatricial alopecia (Figure 3) and hyperpigmentation at the site of previous skin lesions (Figure 4).

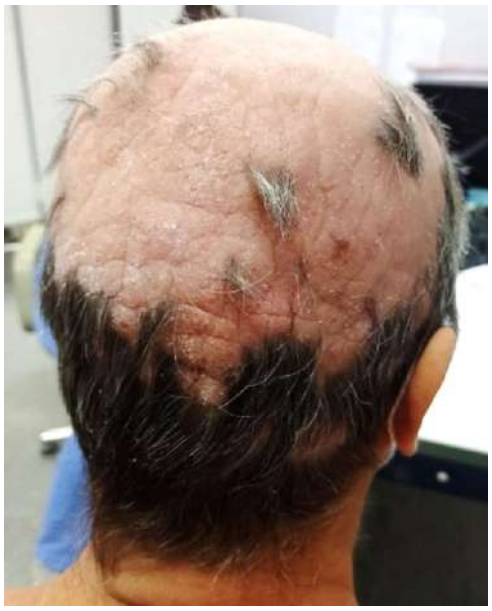

Figure 3 **Scarring alopecia as a consequence of Pemphigus vegetans.**

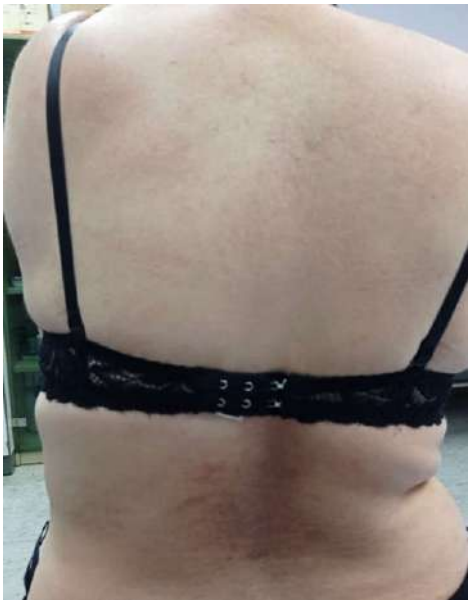

Figure 4 **Hyperpigmentation at the site of previous skin lesions**.

DISCUSSION

Psoriasis is a chronic papulosquamous dermatosis, clinically characterized by scaly erythematous plaques. Besides inflammatory component, psoriasis has related autoimmune component that manifests through autoreactive T cells. That is why psoriasis is commonly associated with thyroiditis, vitiligo and bullous pemphigoid (5). Its association with pemphigus was evaluated in some studies investigating the prevalence of psoriasis among patients with pemphigus. These large cohort studies demonstrate that patients with psoriasis are at three times higher risk of developing pemphigus (4).

Pemphigus vegetans can occur in any age group, but most commonly affects middle-aged women with peak incidence in 40-50 years. Although any skin area may be affected, both clinical forms of PVeg usually affect oral mucosa and intertriginous areas (1). Lesions in oral mucosa may be subtle and are often present at the onset of the disease (2). In the current case, the patient did not experience oral lesions during the entire course of the disease. Cerebriform tongue, as oral manifestation frequently seen in PVeg, was also absent.

Scalp involvement in bullous dermatoses as PV and PVeg, as its variant, is present in variable frequency, according to literature from 16-60% (6). When present, lesions on scalp manifest as erosions and crusted or scaly plaques and can be isolated manifestation of PVeg (7-9). As in our case, many studies reported positive bacterial culture in case of prolonged scalp involvement, with most commonly isolated *Staphylococcus aureus* (10,11). The most common consequence of scalp involvement is scarring alopecia, but it is not completely clear whether bacterial infection is additional factor that enhances this process (6).

Although histopathology alone is insufficient for final diagnosis, especially in case of scarce clinical data, it is critical method to distinguish PVeg from other similar skin conditions. In our case, main clinical differential diagnoses were vegetative pyoderma gangrenosum and erosive pustular dermatosis, both mainly diagnoses of exclusion with possible overlapping features.

Histopathologic features of vegetative pyoderma gangrenosum vary depending on lesion age and site, but neutrophilic infiltrate - neutrophilic folliculitis and intradermal neutrophilic abscess formation, are one of the major criteria to establish diagnosis (12). Erosive pustular dermatosis is rare disease affecting usually scalp of elderly males presenting in form of pustules, erosions and variably thickened grey or yellow-brown crusts, leading at the end stage to scaring alopecia. The difference is that pustules in EPD are sterile, and histologically correspond to neutrophilic dermatosis affecting mainly infundibulum of hair follicles (13).

Since the initial clinical suspicion was not bullous disorder, in this case direct immunofluorescence (DIF) was not performed immediately with histopathology examination. Due to later remission of skin lesions, patient refused further skin biopsy. Indirect immunofluorescence DIF is very important for the final diagnosis in correlation with histopathology, but the detection of anti-Dsg1 and anti-Dsg3 antibodies does not necessarily correlate with the involvement of skin and/or mucous membranes (14).

CONCLUSION

In this case, correlation of clinical presentation with histologic findings was sufficient to exclude other overlapping diagnoses and to diagnose pemphigus vegetans. In addition, great response to pulse doses of systemic corticosteroid and immunosuppressive therapy, together with remission of the disease, point to correct diagnosis. Physicians managing patients with psoriasis should be aware of potential comorbidity with pemphigus, but also should have on mind its possible unusual clinical presentation.

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Reprint requests and correspondence:
Nina Čamdžić, MD, PhD
Department of Pathology
Faculty of Medicine, University of Sarajevo
Čekaluša 90, 71000 Sarajevo
Bosnia and Herzegovina
Phone: 00 387 62 108 006
Email: nina.camdzic@mf.unsa.ba
ORCID ID: 0000-0002-0327-1151

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Hemolysis, Elevated Liver enzymes and Low Platelets syndrome (HELLP): case report

Sindrom hemolize eritrocita, p ovišenih jetrenih enzima i manjka trombocita (HELLP): prikaz slučaja

Emira Škaljić-Delalić*, Admira Mešić

Pediatric Clinic, Clinical Center University of Sarajevo, Patriotske lige 81, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

HELLP syndrome is a disorder that occurs in pregnancy, characterized by hemolytic anemia, elevated liver enzymes and thrombocytopenia. It probably represents a serious form of preeclampsia, but the connection between these two diseases is controversial. The incidence is 0.1-0.8% in all pregnancies and 10-20% in severe preeclampsia and eclampsia. Risk factors are a previous pregnancy with preeclampsia or HELLP sy, close relatives (sisters, mother) who had this syndrome and multiparity. The clinical picture of HELLP syndrome has different presentations. The most common symptoms are: proteinuria, hypertension, pain in the epigastrium and below the DRL, nausea, vomiting, headache, visual disturbances, and jaundice. Signs and symptoms usually develop between 28 and 36 weeks of pregnancy, but onset in the second trimester and postpartum is also common. Postpartum disease is presented in 30% of cases, usually within 48 hours of delivery, but occasionally within 7 days after delivery. In the following, we present the case of a patient treated in the Intensive Care Unit (ICU) of the Clinic of Gynecology and Obstetrics (GAK) who had a very severe form of HELLP syndrome, after which she successfully recovered. Initial patient MN, born in 1985, Master of Pharmacy by profession, was admitted to GAK on October 8, 2021 in the 34th week of pregnancy due to Preeclampsia. She was multipara, in fourth pregnancy and under regular monitoring treated with Methyldopa and Lasix tbl. Shortly after admission, the patient gave birth vaginally due to Partus praetemporarius imm. On 09 October 2021 her general condition deteriorated and she was transferred from the Department to the ICU of the GAK. She was hospitalized in the ICU of GAK in the period from 9 to 29 October 2021. On admission, the patient was slightly dyspnoic, tachycardic, oliguric, with doughy pretibial edema on the extremities. The laboratory findings showed elevated values of transaminases, urea and creatinine, uric acid, D dimer, CRP, mineral and acid-base imbalance with hypoalbuminemia and proteinuria. Immediately after admission, resuscitation treatment was started, an X-ray of the lungs was performed (bilateral chyloperihilar hazy and banded shadows, cardiac shadow enlarged, minor pleural effusions) and the following specialists were consulted: pulmonologist, nephrologist, hematologist, cardiologist, gastroenterologist, infectious disease specialist. The therapy included dual antibiotics (Ceftriaxon+Ciprofloxacin), anticoagulant therapy (Clexan), Mg sulfate, diuretic support with hydration, Allopurinol, with correction of acid-base status, mineralogram and hypoalbuminemia. SSP was also prescribed due to primary fibrinolysis. Due to the increased values of urea and creatinine, it was not possible to perform the chest CT with contrast, so a VP SPECT was performed, which confirmed bilateral PTE. Despite cardiac therapy, elevated diastole and enlarged cardiac shadow persisted, and an ultrasound of the heart was performed, showing an EF of 20-25%, diastolic function altered by the restrictive type, and Insuff. valv. mitralis gr II/III et valvulae tricuspidalis gr II. In the further course of the treatment, the patient's general condition improved, laboratory findings were corrected, acute renal insufficiency was recovered, as well as the regression of lung changes. The patient continued her treatment at the Clinic of Heart Diseases, Blood Vessels and Rheumatism, where she was treated with anticoagulants, PPI, cardiotonics, dual diuretic therapy, Mg, beta blockers and a high-protein diet. The patient was discharged on 11 November 2021, as cardiac compensated. At the first cardiology check-up after hospitalization, the EHO of the heart showed an EF of 40%, and on the second, the EF was 52%. Early recognition of HELLP syndrome and an aggressive multidisciplinary approach to the treatment significantly contribute to a good prognosis for both mother and a child.

Keywords: HELLP syndrome, multidisciplinary treatment

Reprint requests and correspondence:
Emira Škaljić-Delalić, MD
Pediatric Clinic, Clinical Center University of Sarajevo
90 Patriotske lige, 71000 Sarajevo
Bosnia and Herzegovina
Email: emiraskaljic@gmail.com

Sepsis in childhood, novelties in treatment

Sepsa u dječijoj dobi, novine u liječenju

Verica Mišanović*, Duško Anić, Sabina Terzić, Emina Vukas, Selma Dizdar, Amra Džinović, Amila Ključić

Pediatric Clinic, Clinical Center University of Sarajevo, Patriotske lige 81, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Sepsis is the leading cause of death in childhood. The pediatric population has its own characteristics in relation to the adult population. Only a small number of clinical studies have been conducted and therefore the treatment of septic shock in children is one of the greatest therapeutic challenges in intensive care units. It is estimated that over 7 million children die each year from sepsis. Septic shock in children is defined as severe infection accompanied by cardiovascular dysfunction (including hypotension, perfusion disorder, and use of vasoactive drugs), while organ dysfunction associated with sepsis in children is defined as severe infection leading to cardiovascular and/or non-cardiovascular organ dysfunction. Timely recognition, rapid diagnosis, blood culture, rapid application and appropriate choice of antimicrobial drug, fluid therapy, use of vasoactive drugs, mechanical ventilation, enteral nutrition, increase the possibility of a positive treatment outcome. In order to respond to this challenge, it is necessary to continuously monitor the news from this field and apply them in practice by each individual, along with the multidisciplinary engagement of experts from different fields - pediatricians, microbiologists, infectious disease specialists, clinical pharmacologists.

Keywords: sepsis, septic shock, child

Reprint requests and correspondence:
Verica Mišanović, MD, PhD
Pediatric Clinic, Clinical Center University of Sarajevo
Patriotske lige 81, 71000 Sarajevo
Bosnia and Herzegovina
Email: vericamisanovic@gmail.com

Acute renal insufficiency in SARS-CoV-2 -2019 patients

Akutna bubrežna insuficijencija kod SARS-CoV-2 iz 2019. godine

Sadija Lušija*, Suvad Vuk, Erna Salić, Anes Ajanović, Amela Katica -Mulalić, Elma Bečić, Enida Halvadžić

Clinic of Anesthesia and Reanimation, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Acute renal failure is common in SARS CoV2-2019 patients. Acute renal failure defined through KDIGO (Kidney Disease Improving Global Outcomes) criteria relates to 50% increase in creatinine level compared to baseline or 0.3 mg/dl or 26.52 mcmol/L compared to the lowest value within 48 hours. AKI is classified into three stages, with stage III defined as the stage requiring hemodialysis treatment regardless of creatinine values. Studies have shown that renal outcomes are worse in patients with stage III acute renal insufficiency. Studies have also shown an association of acute renal failure with progression of respiratory dysfunction. AKI stage II and III are associated with increased mortality in patients with COVID-19 infection. The pathogenesis of acute renal failure is viral invasion, hypovolemia, systemic inflammation, endothelial dysfunction, coagulopathy, exposure to nephrotoxic drugs, maladaptive organ dysfunction during the disease. Incidence of acute renal failure of insufficiency varies according to different populations and ranges between 7-57% in hospitalized and 19-80% in Intensive Care Unit patients. AKI primarily occurs in patients with respiratory insufficiency, especially in patients on mechanical ventilation due to hypooxygenation and overload of the right heart with consequent ischemia. A disturbed acid-base balance, redistribution of intra- and extracellular fluids, suppression of the immune response as well as disturbed mechanisms of the inflammatory response have a negative impact. The release of cytokines and the activation of complement components infected with the virus can lead to inflammation and cell damage and the creation of blood hypercoagulability resulting in the formation of microthrombi in the lungs and kidneys.

Keywords: acute renal failure, SARS-CoV2 virus

Reprint requests and correspondence:
Sadija Lušija, MD
Clinic of Anesthesia and Reanimation
Clinical Center University of Sarajevo
Bolnička 25, 71000 Sarajevo
Bosnia and Herzegovina
Email:sadijalusija@gmail.com

Treatment of chronic pain caused by herpes zoster virus and treatment of severe peroneal nerve paralysis (case report)

Liječenje hroničnog bola uzrokovanog virusom herpes zoster i liječenja teške pareze peronalnog živca (prikaz slučaja)

Sadija Lušija*, Ilijaz Aslani, Suvad Vuk, Azra Alihodžić, Anes Ajanović, Edin Gadžo, Elma Bečić

Clinic of Anesthesia and Reanimation, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzego vina

*Corresponding author

ABSTRACT

Pain is the most common symptom of the disease that brings most patients to the doctor and is a sign of the existing or threatening organic or functional tissue damage. The first presentation is a case of chronic pain treatment caused as a result of the Herpes zoster virus that affected the intercostal region of the right side of the chest. Namely, the pain appeared immediately after the appearance of the first symptoms in the form of erythematous vesicular smallpox. The intensity of the pain increased and, in addition to organic damage to a part of the body with the formation of scars, created emotional instability in the sense of feelings of depression, insomnia and mood changes, with disbelief in the improvement of ailments as the end result. After three treatments at the Pain Therapy Outpatient Clinic, the patient felt an improvement in terms of reducing the intensity of pain, which also contributed to an emotional improvement in the form of a better mood, along with an improvement in the quality of life. In the second case, diabetic polyneuropathy affected the left calf and paresis of the n. peroneus which resulted in impaired mobility in the entire extremity. After few sessions, which included intra-articular blockages, the pain decreased and the mobility of the left column improved. In both cases, mutual great satisfaction and joy with the achieved results of intacostal and intra-articular blockade application was noticeable, and the motivation for further treatment and improvement of the quality of life with a reduction in the use of oral analgesics was stimulated.

Keywords: pain therapy, blockades

Reprint requests and correspondence:
Sadija Lušija, MD
Clinic of Anesthesia and Reanimation
Clinical Center University of Sarajevo
Bolnička 25, 71000 Sarajevo
Bosnia and Herzegovina
Email: sadijalusija@gmail.com

Cytotoxic effects of etomidate on human cell line (GR-M) and mouse cell line (B16F10)

Citotoksični efekti etomidata na ljudsku staničnu liniju (GR-M) i staničnu liniju miša (B16F10)

Belma Kadić*

Clinic of Anesthesia and Reanimation, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzego vina

*Corresponding author

ABSTRACT

Introduction: etomidate is a non-barbiturate intravenous anesthetic that belongs to the group of fast-acting anesthetics resulting in loss of consciousness within fifteen seconds. This anesthetic is used for minor emergency procedures and operations, for intubation and sedation, given the relatively safe positive effects on hemodynamics, which makes it desirable for the use and maintenance of hemodynamics in patients with cardiac and respiratory problems, as well as in high-risk patients with cerebrovascular diseases. It most likely acts via the GABA A receptor by inhibiting neurotransmitter release by acting on one of the presynaptic mechanisms and possibly affecting the release mechanism itself. It is successfully used in patients of all age groups, but like all medicines it has side effects. Methods: in this study we analyzed the cytotoxic effects of *etomidate* under *in vitro* conditions on human (GR-M) and mouse (B16F10) melanoma cell lines with two assays: Crystal violet proliferation assay and trypan blue exclusion assay, but first anesthetic-treated cell cultures where established over a 24-hour period. Results: ethomidate was found to affect the change in proliferation status of treated cultures and reduce their viability, but the differences observed with the untreated cultures were not statistically significant. Conclusion: it may be considered that etomidate at the tested concentrations did not exhibit significant cytotoxic effect in the B16F10 mouse cell lines and in the human GR-M melanoma.

Keywords: cytotoxic effects, etomidate, human cell line, mouse cell line

Reprint requests and correspondence:
Belma Kadić, MD
Clinic of Anesthesia and Reanimation
Clinical Center University of Sarajevo
Bolnička 25, 71000 Sarajevo
Bosnia and Herzegovina
E-mail: belma.zoljic@gmail.com

Increase in serum lactate dehydrogenase (LDH) in COVID-19 patients between November 2020 and March 2021

Porast serumske laktat dehidrogenaze (LDH) kod pacijenata sa COVID-19 u periodu između novembra 2020. i marta 2021.

Amila Feto*, Elma Bečić, Naida Herenda, Belma Kadić, Amela Muftić, Amela Katica-Mulalić

Clinic of Anesthesia and Reanimation, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzego vina

*Corresponding author

ABSTRACT

Introduction: COVID-19 has reached pandemic proportions and become a global health challenge. Although in most patients with COVID-19 the disease is mildly symptomatic, in some patients it significantly worsens, causing multi-organ failure leading to death. Available biomarkers, especially those that are routinely tested, allow us to figure out development of a severe clinical picture of the disease. Lactate dehydrogenase (LDH) is an intracellular enzyme involved in anaerobic glycolysis that catalyzes the oxidation of pyruvate to lactate. Elevated LDH indicates tissue hypoperfusion and degree of the disease, therefore it can have an effect on the prognosis. LDH could be a potential prognostic biomarker in patients with COVID-19. The aim of this retrospective analysis was to figure the prognostic significance of elevated LDH in patients with COVID-19. Materials and methods: in this retrospective study, we observed 191 patients hospitalized in the Isolation Ward for hospital treatment of patients with COVID-19 in the period from November 2020 to March 2021, of whom 98 survived and 93 died. Patients' age, sex, hospitalization days, intubation day from the hospital admission, serum lactate dehydrogenase levels at admission and discharge with other parameters, initial saturation, comorbidities and the patients' outcome were monitored. Results: in the group of patients who died, the average age was 69.94±10.85 compared to 59.38±14.04 in the group of patients who survived. More frequent use of antibiotics was expressed in the group of those who had died before hospitalization in 60% compared to 40.8% in the group of those who survived. Comorbidities were present in the group of patients who died in 86.0% compared to 57.1% in the group of those who survived. LDH values at admission were not significantly different, while LDH values at discharge/last examination were significantly higher in the group of patients who died. Also, LDH values in the group of survived patients were decreased by -192.73±231.52 of average value, and increased by 133.67±366.23 on average value in the group of patient who died. Conclusion: serum LDH was validated for its potential usefulness as markers for evaluating clinical severity and monitoring treatment response in COVID-19 pneumonia.

Keywords: lactat dehydrogenase (LDH), COVID-19

Reprint requests and correspondence:
Amila Feto, MD
Clinic of Anesthesia and Reanimation,
Clinical Center University of Sarajevo
Bolnička 25, 71000 Sarajevo,
Bosnia and Herzegovina
E-mail: dr.milafeto89@gmail.com

Mediastinal ganglioneuroma in a six-year-old child (case report)

Ganglioneurinom medijastinuma kod šestogodišnjeg djeteta (prikaz slučaja)

Amira Mešić*, Hilmo Kačamaković, Lejla Altumbabić, Ermina Sitnić-Milanović, Ilijaz Pilav, Kenan Karavdić, Ediba Čelić-Spužić

Clinic of Anesthesia and Reanimation, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Ganglioneuroma is a rare and benign tumor of autonomic nerve fibers arising from the sympathogonia of the neural crest, which are completely undifferentiated cells of the system However, ganglioneuromas themselves are fully differentiated neuronal tumors that do not contain immature elements. Ganglioneuromas most often occur in the abdomen, but these tumors can grow anywhere where there is sympathetic nervous tissue. Other common sites include the adrenal gland, paraspinal retroperitoneum, posterior mediastinum, head and neck. Ganglioneuroma is usually asymptomatic and is usually discovered only when being examined or treated for other conditions. For example, a tumor in the chest area can cause breathing difficulties, chest pain and compression of the trachea. They can be diagnosed visually with a CT scan, MRI scan or ultrasound of the head, abdomen or pelvis. Blood and urine tests may be done to determine if the tumor is secreting hormones or other chemicals. A biopsy of the tumor may be required to confirm the diagnosis. If there are symptoms or major physical deformity, treatment usually consists of the tumor removal surgery. Most ganglioneuromas are noncancerous, so the expected outcome is usually good. However ganglioneuroma can become cancerous and spread to other areas or grow back after removal. This case report is about a six-year-old boy, who complained of coughing about twenty days before hospitalization. Based on the clinical picture, physical findings, X-ray, CT, the chest MRI and pathohistological findings of the puncture biopsy he was diagnosed with ganglioneurinoma mediastini lateris dex. In such cases, the mass of the right hemithorax, which almost fills the trachea, causes compression of the trachea in the segment immediately before the bifurcation and the heart moves to the left and forward, compressing the descending thoracic aorta. After complete preoperative preparation the child was scheduled for surgery. Preoperative preparations were made in terms of complete blood count, minerals, ABS, heart ultrasound and multidisciplinary approach to surgery. The introduction and course of anesthesia went smoothly. The child was hemodynamically stable the whole time. Fluids, antibiotics, blood products of the appropriate blood group were prescribed. CVK v.jug.lat.dex. and thoracic drain were placed. The operation lasts for four hours. After extubation, the child was transferred to the Intensive Care Unit of the Pediatric Clinic, accompanied by an anesthesiologist. He stayed in intensive care for two days, and in good general condition was transferred to the Children's Surgery Clinic. On the fifth postoperative day, the thoracic drain was removed, and on the eighth day of hospitalization, he was discharged in a good general condition with a recommendation to continue therapy and regular check-ups. Control X-ray of the lungs was normal.

Keywords: mediastinal ganglioneuroma, anesthesia, team

Reprint requests and correspondence:
Amira Mešić, MD
Clinic of Anesthesia and Reanimation
Clinical Center University of Sarajevo
Bolnička 25, 71000 Sarajevo
Bosnia and Herzegovina
Email: mikica_mesic@hotmail.com

Anesthesiology approach in performing operational treatment of child's diaphragmal herniae using laparoscopy method

Anestezиološki pristup kod operativnog liječenja dijafragmalne kile kod djeteta laparoskopskom metodom

Amira Mešić*, Suvad Vuk, Lejla Altumbabić, Ermina Sitnić-Milanović, Verica Mišanović, Kenan Karavdić, Asmir Jounuzi, Emir Milišić, Samir Muhović

Pediatric Clinic, Clinical Center University of Sarajevo, Patriotske lige 81, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

According to researches, congenital diaphragmatic hernias in children amount to 1:2500 and are most often on the left side. Children do not improve despite intubation and ventilation. The prognosis depends on the degree of pulmonary hypoplasia and is bad if, despite adequate ventilation, C02 values fail to decrease. High pulmonary arterial pressure and hypoxemia favor the reopening of the Ductus Botalli leading to a right-to-left shunt with catastrophic consequences. Factors that could improve the situation include: less aggressive ventilation, NO and choosing the optimal time for operative work. The operation is performed with lung stabilization, FiO2 < 40%, lower dose or without vasoactive drugs. Occasionally, diaphragmatic hernias become apparent only after several weeks or months as an incidental finding. Today's trend includes thoracoscopic surgery and intrauterine tracheal occlusion with peripartum removal and intubation. Surgical treatment will not bring immediate improvement, but continued intensive treatment and adequate ventilation gives good results. Our patient was eleven months old child (SE). On 19 September 2017 the child underwent VSD surgery and following the lungs X-ray check-up, a random finding confirmed the existence of a congenital diaphragmatic hernia on the left. The child was scheduled for surgery sixty days after the heart surgery. Preoperative preparations were made in terms of a complete blood count, mineralogram, ABS, heart ultrasound. Cardiological antibiotic therapy was initiated along with the continued initial therapy (Lasix, Aldactone). The laparoscopic approach was performed on 2 November 2017. After intubation, press mode ventilation was applied. The operation lasted 45 minutes. The child was intubated and moved to the Intensive Care Unit, accompanied by an anesthesiologist. He stayed in intensive care for two days, and in good general condition was transferred to the Children's Surgery Clinic. On the sixth day of hospitalization, he was discharged home in a good general condition with a recommendation to continue therapy and regular monitoring.

Keywords: diaphragmatic hernia, anesthesia, team

Reprint requests and correspondence:
Amira Mešić, MD
Pediatric Clinic, Clinical Center University of Sarajevo
Patriotske lige 81, 71000 Sarajevo
Bosnia and Herzegovina
E-mail: mikica_mesic@hotmail.com

Neurally adjusted ventilatory assist – NAVA

Neuralno prilagođena ventilacijska pomoć – NAVA

Suvad Vuk *, Ermina Sitnić-Milanović, Selma Živojević, Ediba Čelić-Spužić

Clinic of Anesthesia and Reanimation, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Neuroassisted mechanical ventilation - NAVA is one of the newer forms of assisted ventilation mode used in hypoxemic and hypercapnic ventilation disorders, which require ventilatory support, and in patients who are in the phase of separation from the ventilator. It can be used as an invasive, but also as a non-invasive form of assisted mechanical ventilation in patients with maintained activity of the respiratory center and the possibility of spontaneous breathing, regardless of their age. The unique feature of this mode of ventilation is that it is based on monitoring the electrical activity of the diaphragm through a special so-called EAdi catheter that measures diaphragmatic electrical activity signals or EAdi signals. The EAdi catheter is a modified nasogastric tube, on the top of which there are electrodes that record signals of diaphragmatic electrical activity generated by stimulation of the n. phrenicus by signals transmitted from the respiratory center. Compared to other available modes of assisted ventilation, this mode of ventilation enables better synchronization between the patient and the ventilator, providing adequate respiratory support in accordance with nerve impulses and adapting to each individual breath. There is still not enough adequate data on the effectiveness of this type of ventilation. Whether the NAVA mode of ventilation provides better respiratory support, which ultimately gives better treatment results, are questions that are currently awaiting the results of adequate studies.

Keywords: neuroassisted mechanical ventilation, EAdi catheter

Reprint requests and correspondence:
Suvad Vuk, MD
Clinic of Anesthesia and Reanimation
Clinical Center University of Sarajevo
Bolnička 25, 71000 Sarajevo
Bosnia and Herzegovina
E-mail: vuksuvad@bih.net.ba

Chronic foreign body of the bronchus in adults - case report

Hronično strano tijelo bronha kod odraslih - prikaz slučaja

Nermina Duraković-Babić[1]*, Lejla Altumbabić[2], Hilmo Kačamaković[2], Amila Feto[2], Azra Alihodžić -Pašalić[3], Kemal Grbić[3], Ilijaz Pilav[3]

[1]Cantonal Hospital "Dr.Irfan Ljubijankić", Darivalaca krvi 67, 77000 Bihać, Bosnia and Herzegovina
[2]Clinic of Anesthesia and Reanimation, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzego vina
[3]Clinic of Thoracic Surgery, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Introduction: the foreign body of the bronchus is more often right than left because of the anatomical position and greater lumen of the right main bronchus. A chronic foreign body does not show classic characteristic symptoms of foreign body aspiration such as apneic attacks and cough, and often there is no reliable anamnestic data on aspiration. The aim of this work was to present patients with chronic foreign body bronchus who were timely diagnosed and successfully treated with bronchoscopy under general anesthesia. Case report: the patient presented was a younger male involved in a traffic accident one month before the treatment. Since the accident he suffered from severe cough and fever and was referred to the Pulmonary Disease Clinic Podhrastovi, where an attempt of bronchoscopic excision under local anesthesia was unsuccessful. Subsequently, the patient was transferred to the Clinic of Thoracic Surgery of the Clinical Center University of Sarajevo to continue the treatment. Following standard preoperative preparation, bronchoscopy under general anesthesia was performed. During the surgery two connected dental flaps were removed from the right bronchial system. Conclusion: the patients with persistent cough who do not respond to medical therapy or find the cause of standard radiography and laboratory analyzes should undergo further pulmonary treatment in terms of diagnostic bronchoscopy. In most cases, the presence of a foreign body as a cause of coughing, extirpation of the foreign body and complete cure of the patient is possible.

Keywords: bronchial foreign body, bronchoscopy, teeth, gener al anesthesia, thoracic surgery

Reprint requests and correspondence:
Nermina Duraković-Babić, MD
Cantonal Hospital "Dr.Irfan Ljubijankić"
Darivalaca krvi 67
77000 Bihać
Bosnia and Herzegovina

Challenges in COVID-19 ICU

Poteškoće u COVID-19 JIL

Senita Beharić*

Clinic of Anesthesia and Reanimation, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzego vina

*Corresponding author

ABSTRACT

Introduction: COVID-19 disease is an infectious disease caused by the SARS-CoV-2 virus. The virus develops from very mild symptoms, mild colds, to pneumonia that can lead to acute respiratory failure and ultimately to multiorgan failure with a mortality of 2 to 10%. In ICU hospitalized patients, interstitial pneumonia and ARDS usually occur during the second week of treatment, 7-9 days from the onset of the disease. Patients with saturation of 75-80% and PaO_2/FiO_2 <150 require invasive ventilation treatment. Fulminant cardiomyopathy may occur in these patients even in the stages of recovery from the disease. It is not clear yet whether the infection causes viral cardiomyopathy or whether cardiac dysfunction is due to a cytokine storm. ICU protocol: early weaning from mechanical ventilation is one of the key aspects of successful treatment of patients with COVID-19 because respiratory support in general is borderline indicated in most cases. Prolonged ventilation of patients for more than 5-7 days creates conditions for the colonization of other pathogens, often resistant bacteria and fungi that encounter a markedly weakened immune response of the host, which significantly shortens and accelerates the path to sepsis. Conclusion: good organization of intensive care units (ICU) with clearly defined protocols is important for the control and successful treatment of the most severe COVID-19 respiratory infections. Such units must have a sufficient number of medical staff, primarily experienced intensive care physicians, and medical technicians who are essentially the most important personnel.

Keywords: COVID-19, cytokine storm, cardiomyopathy, ICU protocol

Reprint requests and correspondence:
Senita Beharić, MD
Clinic of Anesthesia and Reanimation
Clinical Center University of Sarajevo
Bolnička 25, 71000 Sarajevo
Bosnia and Herzegovina
Email: beharics@gmail.com

Early postoperative nutrition of oncological patients

Rana postoperativna prehrana onkoloških pacijenata

Merima Kruščica[1*], Anes Ajanović[2], Edin Gadžo[2]

[1]Clinic of Abdominal and General Surgery, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herz egovina
[2]Clinic of Anesthesia and Reanimation, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina

*Corresponding author

ABSTRACT

Many studies have shown that in patients who receive enteral nutrition for the first 12 hours after surgery, there is no increased risk of anastomosis dehiscence, despite the flow of nutrients proximal to the intestinal sutures. Enteral nutrition has been shown to improve wound healing and aid in intestinal repair. Does the application of enteral and parenteral nutrition influence the outcome ? In a prospective randomized controlled trial which involved patients who underwent extensive pancreatic resection due to malignant disease, the use of total parenteral nutrition was associated with a higher frequency of severe complications compared to the group without therapy (45% vs. 23%). In a multicenter prospective randomized controlled trial in which two-thirds of subjects were patients with malignant tumors, total parenteral nutrition was not associated with a reduction in postoperative morbidity or mortality. Routine perioperative parenteral nutrition (n=144) in patients undergoing hepatectomy for hepatocellular carcinoma showed a clear beneficial effect.

Keywords: enteral nutrition, parenteral nutrition

Reprint requests and correspondence:
Merima Kruščica, MD
Clinic of Abdominal and General Surgery
Clinical Center University of Sarajevo
Bolnička 25, 71000 Sarajevo
Bosnia and Herzegovina

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Prored: 1,5: lijeva margina: 2,5 cm; desna margina: 2,5 cm; gornja i donja margina: 2,5 cm.
Grafikone, tabele, slike i crteže unijeti/staviti u tekst rada, tamo gdje im je mjesto, bez obzira u kojem programu su rađene. Cijeli rad obavezno napisati na engleskom jeziku, a sažetak i naslov još i na našem jeziku.
Rad se dostavlja na CD-u, i/ili e-mailom, uz dva štampana primjerka (ako je moguće). CD se ne vraća.

RAD SADRŽI:

NASLOV RADA NA ENGLESKOM JEZIKU
NASLOV RADA NA NAŠEM JEZIKU

Ime i prezime autora i koautora

Naziv i puna adresa institucije u kojoj je autor-koautor/i zaposlen/i (jednako za sve autore), na engleskom jeziku, te na kraju rada navedena adresa kontakt-autora.

Sažetak na našem jeziku, kao i na engleskom - max. 200–250 riječi, s najznačajnijim činjenicama i podatcima iz kojih se može dobiti uvid u kompletan rad.
Ključne riječi - Key words, na našem jeziku i na engleskom, ukupno do pet riječi, navode se ispod Sažetka, odnosno Abstracta.

SADRŽAJ

Sadržaj rada mora biti sistematično i strukturno pripremljen i podijeljen u poglavlja i to:
- **UVOD**
- **MATERIJAL I METODE**
- **REZULTATI**
- **DISKUSIJA**
- **ZAKLJUČAK**
- **LITERATURA**

UVOD

Uvod je kratak, koncizan dio rada i u njemu se navodi svrha rada u odnosu na druge objavljene radove sa istom tematikom. Potrebno je navesti glavni problem, cilj istraživanja i/ili glavnu hipotezu koja se provjerava.

MATERIJAL I METODE

Potrebno je da sadrži opis originalnih ili modifikaciju poznatih metoda. Ukoliko se radi o ranije opisanoj metodi dovoljno je dati reference u literaturi. U kliničko-epidemiološkim studijama opisuju se: uzorak, protokol i tip kliničkog istraživanja, mjesto i vrijeme istraživanja. Potrebno je opisati glavne karakteristike istraživanja (npr. randomizacija, dvostruko slijepi pokus, unakrsno testiranje, testiranje s placebom itd.), standardne vrijednosti za testove, vremenski odnos (prospektivna, retrospektivna studija), izbor i broj ispitanika – kriterije za uključivanje i isključivanje u istraživanje.

REZULTATI

Navode se glavni rezultati istraživanja i nivo njihove statističke značajnosti. Rezultati se prikazuju tabelarno, grafički, slikom i direktno se unose u tekst gdje im je mjesto, s rednim brojem i konciznim naslovom. Tabela treba imati najmanje dva stupca s obrazloženjem što prikazuje; slika čista i kontrastna, a grafikon jasan, s vidljivim tekstom i obrazloženjem.

DISKUSIJA

Piše se koncizno i odnosi se prvenstveno na vlastite rezultate, a potom se nastavlja upoređivanje vlastitih rezultata s rezultatima drugih autora, pri čemu se citiranje literature navodi po važećim Vankuverskim pravilima. Diskusija se završava potvrdom zadatog cilja ili hipoteze, odnosno njihovim negiranjem.

ZAKLJUČAK

Treba da bude kratak, da sadrži najbitnije činjenice do kojih se došlo u radu tokom istraživanja i njihovu eventualnu kliničku primjenu, kao i potrebne dodatne studije za potpuniju aplikaciju. Obavezno navesti i afirmativne i negirajuće zaključke.

LITERATURA - Upute za citiranje - pisanje literature

Literatura se obavezno citira po **Vankuverskim pravilima**.

Svaku tvrdnju, saznanje ili misao treba potvrditi referencom. Reference u tekstu treba označiti po redoslijedu unošenja arapskim brojevima u zagradi na kraju rečenice. Ukoliko se kasnije u tekstu pozivamo na istu referencu, navodimo broj koji je referenca dobila prilikom prvog unošenja/pominjanja u tekstu. Literatura se popisuje na kraju rada, rednim brojevima pod kojim su reference unesene u tekst (ulazni broj reference), a naslov časopisa se skraćuje po pravilima koje određuje Index Medicus. Ukoliko je citirani rad napisalo više autora, navodi se prvih šest i doda "et al.".

Vrlo je važno ispravno oblikovati reference prema uputama koje se mogu preuzeti na adresama National Library of Medicine Citing Medicine http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=citmed.TOC&depth=2 , ili International Committee of Medical Journal Editors Uniform Requirements for Manuscripts Submitted to Biomedical Journals:

Sample References http://www.nlm.nih.gov/bsd/uniform_requirements.html.