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SPASIMO ŽIVOT

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Bosnia and Herzegovina was the fourth country in Europe that developed National version of HeartScore program !

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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
Previous stroke
TIA or systemic embolism
Age ≥75 years

Clinically relevant non-major risk factors
CHF or moderate to severe LV systolic dysfunction [e.g. LV EF ≤ 40%]
Hypertension
Diabetes mellitus
Age 65-74 years
Female sex
Vascular disease

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

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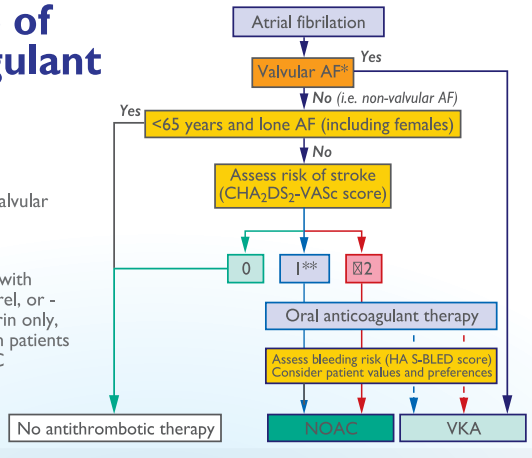
Algoritam antikoagulantne terapije nakon procjene CHA_2DS_2VASc i major risk faktora!

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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Multisystem inflammatory syndrome associated with COVID-19 in previously healthy children

Multisistemski inflamatorni odgovor udružen sa COVID-19 kod prethodno zdrave djece

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ABSTRACT

Introduction: Multisystem Inflammatory Syndrome in Children MIS-C is a hyperinflammatory condition associated with Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Making a prompt diagnosis is crucial with the goal of early introduction of immunosuppressive therapy and adequate inflammation control. Diagnostic criteria include clinical examination, laboratory tests, and additional, radiological and cardiac examinations. Objective: to establish the demographic, clinical and laboratory characteristics of patients diagnosed with MIS-C; to evaluate therapy response and outcome; to draw pediatricians attention to MIS-C in order to diagnose the disease without delay and treat it adequately. Materials and methods: a retrospective study that analyses all patients treated at the Pediatric Clinic of the Clinical Center University in Sarajevo with a diagnosis of MIS-C. Data were processed using descriptive statistics methodology. Results: a total of 19 children, with a diagnosis of MIS-C, were treated of which 10 boys and 9 girls. The oldest child was 17 years old and the youngest 2 years old. All patients had positive IgG antibodies to SARS -CoV-19, while only one had a positive PCR SARS-Co-V test. On admission 100% of patients were febrile for more than 3 days and all presented with mucocutaneous symptoms, 73% had gastrointestinal symptoms, 58% hypotension, 79% signs of cardiac dysfunction, 63% had neurological symptoms. Elevated CRP values were present in all subjects, while lymphopenia was recorded in 63% of patients. Elevated proBNP was presented in 95%, troponin in 79%, Interelukin-6 in 94.7% and D-dimers in 95% of subjects. Majority of patients (95%) were treated with intravenous immunoglobulins and systemic corticosteroids, two (10%) required use of biologic therapy-anakinra, 42% inotropes, and one child (5%) was mechanically ventilated. There were no lethal outcomes. Conclusion: this is the first case series study of children with MIS-c in Bosnia and Herzegovina. The disease is life-threatening and requires emergency treatment, but we had not lethal outcomes. The largest percentage of patients were presented with signs of shock, gastrointestinal and mucocutaneous symptoms and signs of cardiac dysfunction with elevated markers of acute inflammation and positive IgG class antibodies to SARS-CoV-2.

Keywords: multisystem inflammatory response, children, COVID -19

SAŽETAK

Uvod: multisistemski inflamatorni sindrom kod djece MIS-C je hiperinflamatorno stanje koje je povezano sa bolesti koju izaziva Teški akutni respiratorni sindrom koronavirus 2 (SARS-CoV-2). Postavljanje pravovremene dijagnoze je ključno radi ranog uključivanja terapije i adekvatne kontrole inflamacije. Dijagnostički kriterijumi uključuju klinički pregled, laboratorijske analize, dodatne radiološke i kardiološke preglede. Cilj rada: ustanoviti demografske, kliničke i laboratorijske karakteristike pacijenata sa dijagnozom MIS-C, odgovor na terapiju i ishod bolesti. Materijali i metode: retrospektivna studija koja analizira sve pacijente koji su tretirani na Pedijatrijskoj klinici Kliničkog centra Univerziteta u Sarajevu sa dijagnozom MIS-C u periodu od godine. Izvor podataka je medicinska dokumentacija, a podaci su obrađeni koristeći deskriptivnu statističku metodologiju. Rezultati: ukupno je liječeno 19-oro djece sa dijagnozom MIS-C, od čega 10 dječaka i 9 djevojčica. Najstarije dijete je imalo 17 godina, a najmlađe 2 godine. Svi pacijenti su imali pozitivna IgG antitijela na SARS CoV-19, dok je samo jedno dijete imalo pozitivan PCR SARS-CoV-2 test. 100% pacijenata je na prijemu bilo febrilno duže od 3 dana i svi su se prezentirali sa mukukutanim simptomima. 73% je imalo gastrointestinalne tegobe, 58% hipotenziju, 79% znake kardijalne disfunkcije, 63% znake afekcije centralnog nervnog sistema. Povišene vrijednosti CRP-a su imali svi ispitanici, dok je limfopenija evidentirana kod 63% pacijenata. Povišen proBNP kod 95% a troponin kod 79%, Interelukin- 6 kod 94,7%, D-dimeri kod 95%. 95% pacijenata je tretirano intravenskim imunoglobulinima i sistemskim kortikosteroidima, 10% biološkom terapijom-anakinrom, 42% je zahtijevalo upotrebu inotropa i jedno dijete (5%) je bilo mehanički ventilirano. Nije bilo letalanih ishoda. Zaključak: ovo je prva studija u Bosni i Hercegovini koja je pratila kliničke i laboratorijske karakteristike te ishod liječenja pacijenata sa MIS-C, koji je životno ugrožavajuće i urgentno stanje u pedijatriji. Zahvaljući ranoj dijagnozi, agresivnoj imunosupresivnoj terapiji uz multidisciplinarni pristup, svi pacijenti su preživjeli. Najveći procenat pacijenata prezentiran je sa znacima šoka, gastrointestinalnim i mukokutanim simptomima te znacima kardijalne disfunkcije uz povišene markere akutne inflamacije i pozitivna antitijela IgG klase na SARS-CoV-2.

Ključne riječi: multisistemski inflamatorni odgovor, djeca, COVID-19

INTRODUCTION

Most children and adolescents with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection have mild COVID-19 (Corona virus disease-19) disease that does not lead to medical intervention. During the progression of pandemic there were reports of hyperinflammatory syndrome in children with symptoms similar to Kawasaki disease (KD) and toxic shock that require emergency treatment. The first case report was published in April 2020 but the first major call to attention was on April 26, 2020 when National Health Service in the United Kingdom (NHS) issued an alert to highlight a rise in cases of critically ill children with overlapping features of toxic shock syndrome, atypical KD and severe COVID-19 infection (1,2). These patients frequently had evidence of exposure to SARS-CoV-2, two to four weeks prior to the development of hyperinflammation, which was named as Multisystem Inflammatory Syndrome associated with SARS-CoV-2 (MIS-C) (3,4). Although the etiology is unknown, MIS-C is presumed to reflect a postinfectious cytokine-mediated hyperinflammatory process, triggered by COVID-19 infection. The pathophysiology of MIS-C is still not clear but there are evidence of exaggerate or maladaptive immune response of the host to the virus. Cytokines play an important role during infection but, when in excess and when is not properly regulated, it can cause a severe and life threatening co ndition known as cytokine storm (5). MIS-C shares characteristics with other inflammatory conditions (KD, sepsis, macrophage activation syndrome and secondary hemophagocytic lymphohistiocytosis) where large quantity of cytokine induces dysfunction of several organs. Cytokines have very profound negative effects on vascular bed causing hypotension and the leakage of fluids in the lung, heart and other organs. Heart is one of the most affected organs whether it is myocardial dysfunction, pericarditis, valve dysfunction or coronary abnormality (6). Children affected by MIS-C associated with COVID-19 typically present with persistent high fever, weakness and systemic hyperinflammation frequently manifesting with abdominal pain, vomiting, diarrhea, maculopapular rash, respiratory distress demanding oxygen supplementation, hypotension, oliguria, bilateral non-purulent conjunctivitis, signs of mucocutaneous inflammation (red and cracked lips, hand and feet edema) (7,8,9). Main laboratory findings shows elevated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), brain natriuretic peptide (BNP), interleukin -6 (IL-6), D-dimer, troponin and neutrophils and reduction in lymphocyte count and albumins (10).

MATERIALS AND METHODS

Retrospective analysis included 19 patients hospitalized at Pediatric Clinic of the Clinical Center University of Sarajevo. Defined criteria for inclusion of patients in the study were case definition published by World Health Organization (Table 1).

Table 1 **World Health Organisation case definiton of MIS-C.**

WHO case definition (11)
All 6 criteria must be met:
1. Age 0 to 19 years
2. Fever for ≥3 days
3. Clinical signs of multisystem involvement (at least 2 of the following):
▪ Rash, bilateral nonpurulent conjunctivitis, or mucocutaneous inflammation signs (oral, hands, or feet)
▪ Hypotension or shock
▪ Cardiac dysfunction, pericarditis, valvulitis, or coronary abnormalities (including echocardiographic findings or elevated troponin/BNP)
▪ Evidence of coagulopathy (prolonged PT or PTT; elevated D-dimer)
▪ Acute gastrointestinal symptoms (diarrhea, vomiting, or abdominal pain)
4. Elevated markers of inflammation (eg, ESR, CRP, or procalcitonin)
5. No other obvious microbial cause of inflammation, including bacterial sepsis and staphylococcal/streptococcal toxic shock syndromes
6. Evidence of SARS-CoV-2 infection
▪ Any of the following: Positive SARS-CoV-2 RT-PCR Positive serology Positive antigen test Contact with an individual with COVID-19

Demographic information, clinical characteristics, laboratory values, hospital course, treatment, and outcomes were abstracted from medical history. Presenting signs and symptoms were classified as constitutional (fever and chills), cardiac dysfunction, gastrointestinal (abdominal pain, nausea, vomiting, or diarrhea), mucocutaneous (rash, bilateral nonpurulent conjunctivitis, mucosal changes-swollen hands and feet), neurologic (headache, altered mental status). Cardiac dysfunction was defined as any ventricular dysfunction, hypokinesia, decreased contractility or ejection fraction or arrhythmia on electrocardiography, elevated proBNP. Coronary artery aneurysm was reported on the basis of echocardiographic findings. Coagulopathy was defined as elevated D-dimer and abnormal prothrombin time (PT) and activated partial thromboplastin time (aPTT). We defined values for hypotension as systolic blood pressure of less than 70 + (2x age in years) mmHg for 1 to 10 years and less than 90 mmHg for older than 10 years. Lymphopenia was defined as less than 2% for 2 to younger than 4 years, less than 1.5% for 4 to younger than 10 years, less than 1.2 % for 10 to younger than 16 years, and less than 1 % for 16 years and older. Thrombocytopenia was defined as platelet less than 100.000. Lymphopenia, hypoalbuminemia with albumin < 30 g/l, elevated levels proBNP, troponin, Elevated d-dimer >0.55 mg/l, fibrinogen, ESR, CRP, IL-6, ferritin and LDH (all during the first 24 hours of admission) on the basis of age standards (12).

RESULTS

The total of 19 patients diagnosed with MIS-C wastreated at our department. Out of 19 patients with MIS-C, 10 (52%) were male. Median age was 7.7 years, the youngest child was 2 year old and the oldest was 17 (Table 2).

Table 2 **Patient demographic data.**

Gender and age	
Male no./total no. (%)	10/19 (52)
Female no./total no. (%)	9/19(48)
Age median (years)	7.7 (2-17)

None of the children had preexisting condition, but they all had fever or chills at admission that had lasted more than 3 days before

admission to our department and they all had mucocutaneous symptoms. Other common presenting symptoms were gastrointestinal (73%). Neurologic symptoms, predominantly headache and altered mental status were present in 63% children. Cardiac dysfunction was common (79%), in 8 patients (42%) receiving vasoactive support (Table 3).

Table 3 **Symptoms at admission.**

Symptoms at admission	no./total no. (%)
Constitutional: fever and chills	19/19 (100)
Gastrointestinal symptoms	14/19 (73)
Mucocutaneous	19/19 (100)
Hypotension	11/19 (58)
Cardiac dysfunction	15/19 (79)
Neurologic (headache, altered mental status)	12/19 (63)
Evidence of coagulopathy	18/19 (95)

Table 4 **Laboratory findings (proBNP-brain natriuretic peptide, ESR-Erythrocyte sedimentation rate).**

Laboratory values	
Median white-cell count-10*9	15.3
Lymphopenia no./total no.(%)	12/19 (63)
Platelet count 10*9 < 100 no./total no. (%)	6/19 (31)
Elevated pro BNP level pg/ml no./total no.(%) median pro BNP level pg/ml	18/19 (95) 13524
Elevated troponin level ng/l no./total no.(%) median troponin level ng/l	15/19 (79) 50.6
Median C-reactive protein level mg/l Elevated C-reactive protein level mh/l no./total no.(%)	197 19/19 (100)
Fibrinogen level >4 g/l -no./total no.(%)	12/19 (63)
D-dimer level>0.55mg/l-no./total no. (%) Median d-dimer level mg/l	18/19 (95) 4.06
Ferritin level>300 ng/ml-no./total no.(%) Median ferritin level ng/ml	10/19 (52) 409
Albumin level < 30 g/l no./total no.(%) Median albumin level g/l	16/19 (84) 25.6
Median LDH level U/l	274
Interleukin-6 level > 5,0 pg/ml -no./total no. (%) Median interleukin-6 level pg/ml	18/19 (95) 554
Median ESR-mm/hr ESR mm/h > 40 no./total no. (%)	54 14/19 (73)
Positivity for SARS-CoV-2	**no/total no. (%)**
PCR assay no./total/no. (%)	1/19 (5)
Serologic assay for IgG antibodies no./total/no. (%)	19/19 (100)

Elevated CRP values were present in all subjects, while lymphopenia was recorded in 63% of patients. Elevated proBNP were presented in 95% and troponin in 79% of subjects. Majority of children (95%) had elevated Interelukin-6 and d-dimers. On PCR assay one (5%) patient had positive result, while 100% tested positive for serologic assay for IgG antibodies against SARS-CoV-2. Half of the patents had increased ferritin and ESR (Table 4).

We treated 95% of patients with intravenous immunoglobulins and systemic corticosteroids, two (10%) received interleukin-1Ra inhibitor (anakinra), 42% inotropes, and one child was mechanically ventilated. Most patients were cared for in a standard care ward, while 6 patients (31%) were treated in pediatric intensive care unit. One patient (5%) received invasive mechanical ventilation. The median length of hospitalization was 11 days. There were no lethal outcomes (Table 5 and 6).

Table 5 **Therapy options (BiPAP- Bilevel Positive Airway Pressure, CPAP-Continuous Positive Airway Pressure, IVIG-intravenous immunoglobulins, LMWH-low molecular weight heparin, ASA-acetylsalicylic acid).**

Therapy – no./total no. (%)	
BiPAP or CPAP	0/19(0)
High flow nasal cannula	0/19 (0)
Mechanical ventilation	1/19 (5)
Vasopressor support	8/19 (42)
Systemic glucocorticosteroids	18/19 (95)
IVIG	18/19 (95)
interleukin-1Ra inhibitor (anakinra)	2/19 (10.5)
LMWH	18/19 (95)
ASA	19/19 (100)

Table 6 **Clinical course and outcome (PICU-pediatric intensive care unit).**

Clinical course no./total no. (%)	
PICU admission-no.(%)	6/19 (31)
Median length of stay on ICU –days (18 children)	4 (2-8)
Median length of stay overall-days (17 children)	11 (6-20)
Death	0/19

DISCUSSION

Our study described 19 pediatric patients who met the criteria for MIS-C associated with SARS-CoV-2 infection. All patients (100%) had laboratory-confirmed previous SARS-CoV-2 infection, and none of them had documented underlying conditions. Cardiovascular involvement was common (79%), with almost half (42%) receiving vasopressor. Six patients (31%) were cared for in Pediatric Intensive Care Unit (PICU) and one child (5%) received invasive mechanical ventilator support. All patients were treated with IVIG, systemic corticosteroids and majority with vasopressors and anticoagulant therapy. Two patients (10%) required introduction of biologic therapy (ankinra). All patients survived without current signs of permanent organ damage. Case study published by Belhadjer, et al. in May 2020 reported 35 patients, median age 10 years; 100% of patients had fever and 80% gastrointestinal symptoms, 100% left ventricular dysfunction, 65% respiratory distress, RT-PCR was detected in 40%, serology IgG in 86% of patients. All children were treated in PICU; 62% was mechanically ventilated, 28% required extracorporeal membrane oxygenation (ECMO), 71% received IVIG, 34% corticosteroid, 65% heparin, 3% anakinra. There were no deaths. Authors suggest that treatment with IVIG may be associated

with ventricular recovery (13). In the study of Grimaud, et al., a case series of 20 MIS-C patients from France with median age of 10 years, 100% patients were admitted to PICU, 100% were treated with high doses of IVIG, 10% with systemic corticosteroids and 5% received anakinra, 95% vasoactive support and 40% mechanical ventilation (14).

With number of COVID-19 cases continue to increase in general population, it is of vital interest for all health care providers to vigorously monitor patients and identify children with hyperinflammatory syndrome with shock and cardiac involvement. It is important to distinguish patient with MIS-C from those with acute COVID-19 infection, sepsis and toxic shock syndrome, in order to make prompt and accurate diagnosis and start appropriate treatment. MIS-c is life-threatening hyperinflammatory syndrome that involves damage to multiple organ systems in predominantly previously healthy children and adolescents during the Covid-19 pandemic. Patient care should be provided in highly specialized pediatric clinic where multidisciplinary team is available - pediatric rheumatologist, cardiologist, intensivist, infectious disease specialist and hemathologist. Patients with clinical instability should be transferred or admitted to the PICU. Long-term cardiac sequelae of MIS-C are still unknown, Kawasaki's disease guidelines for follow-up should be applied which recommend repeat echocardiographic imaging at 1 to 2 weeks and 4 to 6 weeks after treatment for patients whose disease course is uncomplicated and more frequent echocardiography for patients with coronary-artery aneurism (15). More studies should be indicated in order to define the clinical and laboratory characteristics of MIS-c including identification of parameters that will help distinguish the illness from other similar conditions.

CONCLUSION

We describe the first case series of children with MIS-C in Bosnia and Herzegovina. The largest percentage of patients presented with signs of shock, gastrointestinal and mucocutaneous symptoms and signs of cardiac dysfunction with elevated markers of acute inflammation and positive IgG class antibodies to SARS-CoV-2. It is necessary to spread awareness of MIS-C as a life-threatening condition in the pediatric population where early diagnosis and treatment can positively affect the patient's outcome. Further studies are urgently needed for better definition of MIS-C and its impact on child's health, the best clinical and therapeutic approach and prognosis.

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Correlation of preoperative cytological findings and types of surgical treatment in nodal thyroid gland disorders

Korelacija preoperativnog citološkog nalaza i vrste operativnog tretmana kod nodoznih promjena štitne žlijezde

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ABSTRACT

Introduction: solitary or multiple nodal disorders of the thyroid gland are an indication for surgical treatment. The type and extent of surgical treatment of nodal disorders depends on the preoperative finding of ultrasound-controlled fine needle aspiration (FNA). The aim of our study was to correlate the results of preoperative FNA, definitive pathohistological findings, type of surgical treatment and complications after thyroid surgery in 133 patients operated at Clinic of General and Abdominal Surgery of the Clinical Center University of Sarajevo (CCUS). Materials and methods: all patients underwent preoperative FNA according to the decision of the multidisciplinary thyroid disease team. According to the findings of FNA, patients were divided into 4 groups: malignant, benign, intermediate or suspected of malignancy and non-diagnostic, and accordingly operated. Statistical analysis was made in the IBM SPSS Statistics v. 21.0 for Windows. The methods of descriptive and analytical statistics were used in the analysis. Results: in our study, FNA showed a sensitivity of 76.1% compared to a definitive pathohistological diagnosis, a positive predictive value of 94.6%, a false negative rate of 19.6%, and a false positive rate of 2.3%. Complications were more common in the group of patients with malignant pathohistological findings, but the chi-squared independence test didn't show a significant relationship between the definitive pathohistological finding and the occurrence of postoperative complications (x^2=6.106, p=0.296). All postoperative complications were more common in patients who underwent total thyroidectomy, but the hi-squared independence test also showed no statistically significant association between the type of surgery and postoperative complications (x^2=2.502 p=0.776). Conclusion: although FNA is still the "gold standard" in setting the indication for appropriate surgical treatment of nodal thyroid gland disorders, we believe that when selecting the type and extent of surgical treatment, clinical and radiological characteristics of nodal disorders should be taken into consideration to improve outcomes of treatment.

Keywords: nodular changes, histo - pathology, thyroid surgery, postoperative complications

SAŽETAK

Uvod: solitarne ili multiple nodozne promjene štitne žlijezde predstavljaju indikaciju za operativni tretman. Vrsta i opseg operativnog tretmana nodoznih promjena zavisi od preoperativnog nalaza aspiracione punkcije pod kontrolom ultrazvuka (enlg. fine needle aspiration, FNA). Cilj našeg rada bio je korelacija rezultata preoperativne FNA, definitivnog pato-histološkog nalaza, vrste operativnog tretmana i komplikacija nakon operacije štitne žlijezde kod 133 pacijenta operisanih na Klinici za opštu i abdominalnu hirurgiju Kliničkog centra Univerziteta u Sarajevu (KCUS). Materijal i metode: svi pacijenti su prema odluci multidisciplinarnog tima za bolesti štitne žlijezde podvrgnuti preoperativnoj FNA. Prema nalazu FNA pacijenti su podijeljeni u 4 skupine, a to su maligni, benigni, intermedijarni ili sumnjivi na malignitet i nedijagnostički, a shodno tome i operisani. Statistička analiza je urađena IBM SPSS Statistics v. 21,0 for Windows. Prilikom analize korištene su metode deskriptivne i analitičke statistike, a rezultati su prikazani pomoću tabela i grafikona. Rezultati: u našoj studiji FNA je u komparaciji sa definitivnm pato-histološkom dijagnozom pokazala senzitivnost u iznosu od 76.1 %, pozitivna prediktivna vrijednost iznosila je 94,6%, lažno negativna stopa 19,6% i lažno pozitivna stopa iznosila je 2,3%. Komplikacije su se češće pojavljivale u skupini pacijenata sa malignim patohistološkim nalazom, ali X^2 test nezavisnosti nije pokazao značajnu vezu između definitivnog pato-histološkog nalaza i nastanka postoperativnih komplikacija (x^2=6,106, p=0,296). Sve postoperativne komplikacije su se češće pojavljivale kod pacijenata kod kojih je rađena totalna tireoidektomija, ali X^2 test nezavisnosti također nije pokazao statistički značajnu vezu između vrste operativnog zahvata i postoperativnih komplikacija (x^2=2,502 p=0,776). Zaključak: iako FNA još uvijek predstavlja „zlatni standard" u postavljanju indikacije za odgovorajući operativni tretman nodoznih promjena štitne žlijezde, smatramo da se prilikom odabira vrste i ekstenzivnosti hirurškog tretmana u obzir se trebaju uzeti kliničke i radiološke karakteristike nodoznih promjena, a u cilju poboljšanja ishoda liječenja.

Ključne riječi: nodularne promjene, histopatologija, hirurgija štitne žlijezde, postoperativne komplikacije

INTRODUCTION

The incidence of nodal disorders, as one of the most common indications for surgical treatment of the thyroid gland, is about 4% in the entire population, of which 5 to 10% is malignant. Distinguishing benign from malignant nodal disorders is very important because it determines the type and extent of surgical treatment (1,2).

In the preoperative differentiation of benign from malignant thyroid nodes, the most important is fine needle aspiration (FNA), whose results are divided into four groups: malignant, benign, intermediate or suspected of malignancy and undiagnostic (3). In case of malignant or suspected malignant findings, surgical treatment is indicated. However, due to the fact that FNA has a certain percentage of false positive and false negative results that differ from the definitive pathohistological finding (PH), in determining the type of surgical treatment of the thyroid gland, especially in benign and undiagnostic nodal disorders, other parameters such as the size and localization of nodal disorders, their radiological characteristics, the status of the lymph nodes of the neck, etc. must be taken into consideration (4-7).

In our study, we retrospectively correlated the results of preoperative FNA, definitive pathohistological findings, type of surgical treatment, and percentage of postoperative thyroid complications in 133 patients.

MATERIALS AND METHODS

The study was retrospective, descriptive-analytical and included 133 patients of Clinic of General and Abdominal Surgery of the Clinical Center University of Sarajevo (CCUS), surgically treated in the period from January 2017 to January 2018 due to ultrasound and FNA verified nodal disorders of the thyroid gland.

All patients included in the study had a history, clinical examination, complete hormonal treatment performed by the Clinic of Endocrinology and Nuclear Medicine of CCUS and preoperative cytological findings in the form of FNA performed under ultrasound control. All patients underwent preoperative ultrasound of the neck region, and optionally, computed tomography of the neck region (CT) and examination of external laryngoscopy performed by an otorhinolaryngology specialist (ENT).

Surgical treatment of nodular thyroid changes is conciliatory indicated by a multidisciplinary team consisting of a general or ENT surgeon, nuclear medicine specialist, endocrinologist and oncologist. Surgical procedures were performed at the Clinic for General and Abdominal Surgery of CCUS in the form of hemithyroidectomy (left or right lobectomy of the thyroid gland with or without isthmectomy) or total thyroidectomy with vacuum drainage.

Postoperative patho-histological analysis of thyroid samples was performed at the Clinic for Clinical Pathology, Cytology and Human Genetics of CCUS according to appropriate protocols.

Statistical analysis was made in the IBM SPSS Statistics v. 21.0 for Windows, and the most important results are presented in the form of tables and graphs. The data were processed using the descriptive and analytical statistics methods, and we used the Hi-square test to prove the correlation between the variables. The statistical significance threshold was set at the conventional level ($p<0.05$).

RESULTS

Table 1 **Gender structure of patients.**

	Gender	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Female	113	85.0	85.0	85.0
	Male	20	15.0	15.0	100.0
	Total	133	100.0	100.0	

The study included 133 patients, 113 (85%) female and 20 (20%) male patients (Table 1).

Table 2 **Age groups of patients.**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	20-29	3	2.3	2.3	2.3
	30-39	20	15.0	15.0	17.3
	40-49	42	31.6	31.6	48.9
	50-59	27	20.3	20.3	69.2
	60-69	22	16.5	16.5	85.7
	>70	19	14.3	14.3	100.0
	Total	133	100.0	100.0	

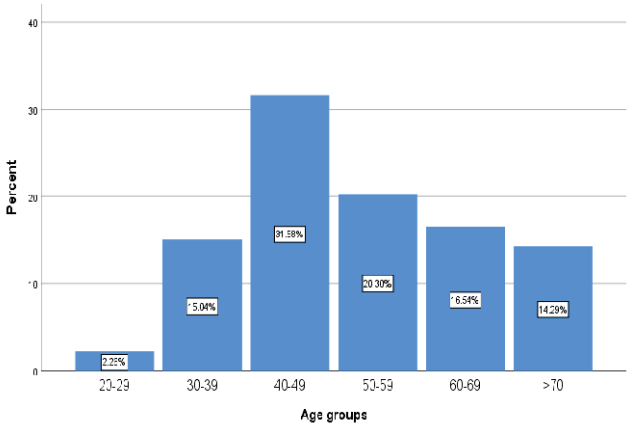

Figure 1 **Age groups of patients.**

In relation to age, all patients in the study were divided into six groups. Most patients belong to the group of patients in the range of 40-49 years, 42 of them (31.6%), and the least in the age group in the range of 20-29 years, 3 of them (2.3%) (Table 2, Figure 1). The youngest patient was 18 and the oldest was 71. The average age was 48.17 years.

Table 3 **Fine needle aspiration (FNA) finding.**

	FNA finding	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Benign	75	56.4	56.4	56.4
	Suspect	18	13.5	13.5	69.9
	Malignant	37	27.8	27.8	97.7
	Insufficient sample	3	2.3	2.3	100.0
	Total	133	100.0	100.0	

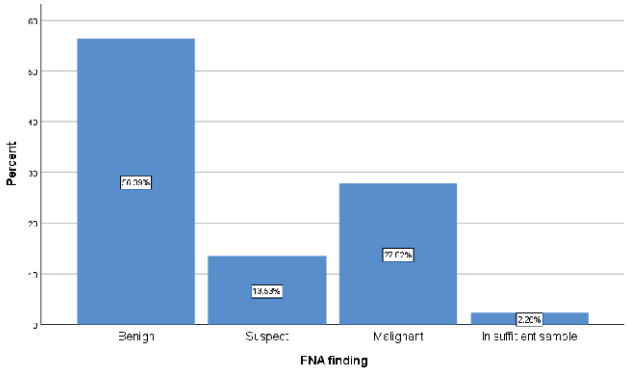

Figure 2 **Fine needle aspiration (FNA) finding.**

According to the results of FNA analysis, most patients had benign findings, 74 (56.4%) patients, followed by malignant 37 (27.8%), 19 (13.5%) patients were suspected, and the least patients with insufficient sample was 3 (2.3%) (Figure 2, Table 3).

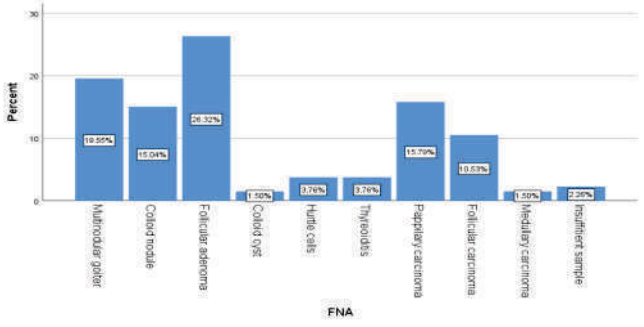

Figure 3 **Cytological finding of fine needle aspiration (FNA).**

The most common cytological finding of FNA was follicular adenoma 35 (26.3%) and the rarest colloid cyst was 2 (1.5%). The most common findings in the group of malignancies were papillary carcinoma 21 (15.8%), followed by follicular carcinoma 14 (10.5%) and medullary carcinoma 2 (1.5%) (Figure 3).

Table 4 **Prevalence of benign and malignant findings in definitive pathohistological findings.**

	Patho-histological finding	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Malign	46	34.6	34.6	34.6
	Benign	87	65.4	65.4	100.0
	Total	133	100.0	100.0	

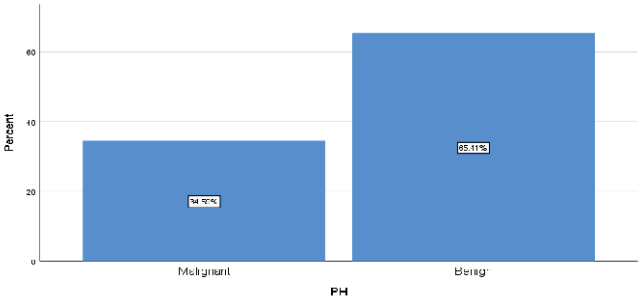

Figure 4 **Percentage of benign and malignant disorders in definitive pathohistological finding.**

In the definitive pathohistological finding, benign disorders were present in 87 (65.4%) patients, while malignant disorders were found in 46 (34.6%) patients.

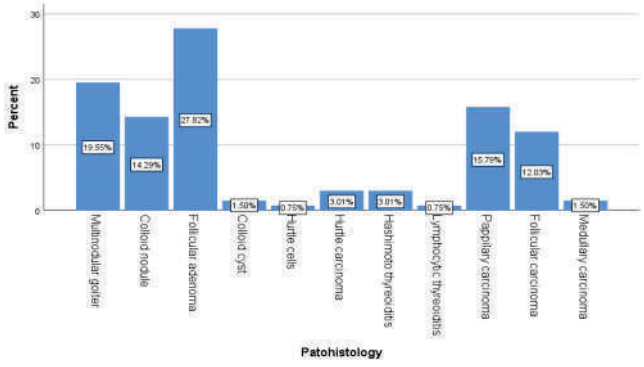

Figure 5 **Definitive pathohistological finding.**

According to the definitive patho-histological finding, the most common disorder was follicular adenoma 37 (27.8%), and the rarest disorder was Hurtle cells and lymphocytic thyroiditis 1 (8%). The most common malignancies were papillary carcinoma 21 (15.8%), followed by follicular adenoma 16 (12%) and medullary carcinoma 2 (1.5%) (Figure 5).

Table 5 **Diagnostic accuracy of fine needle aspiration (FNA) in relation to the definitive pathohistological finding (PH).**

FNA finding		PH		
		Malignant	Benign	Total
Benign	Count	9	66	75
	% within FNA finding	12.0%	88.0%	100.0%
	% within PH	19.6%	75.9%	56.4%
Suspect	Count	1	17	18
	% within FNA finding	5.6%	94.4%	100.0%
	% within PH	2.2%	19.5%	13.5%
Malignant	Count	35	2	37
	% within FNA finding	94.6%	5.4%	100.0%
	% within PH	76.1%	2.3%	27.8%
Insuffitient sample	Count	1	2	3
	% within FNA finding	33.3%	66.7%	100.0%
	% within PH	2.2%	2.3%	2.3%
Total	Count	46	87	133
	% within FNA finding	34.6%	65.4%	100.0%
	% within PH	100.0%	100.0%	100.0%

Table 6 **Sensitivity, specificity, positive and negative predictive value and false- positive and negative rate of FNA compared to the PH finding.**

FEATURE	%	DEFINITION
Sensitivity	76.1 %	Likelihood that patient who has disease has positive test results
Specificity	75,9%	Likelihoood that patient who hasn't disease has negative test result
Positive predictive value	94,6%	Fraction of patients who have positive test (who have disease)
Negative predictive value	88,0%	Fraction of patients who have negative test (who haven't disease)
False-negative rate	19,6%	FNA negative; histology positive for cancer
False-positive rate	2,3%	FNA positive; histology negative for cancer

According to the findings of fine needle aspiration (FNA), 9 (12%) patients had a negative cytological finding for malignancy, which was marked as malignant on the definitive patho-histological finding (Table 5). The sensitivity and specificity test, positive and negative predictive values, and false-negative and false-positive rates are presented in a table with appropriate explanations (Table 6).

Table 7 **Type of thyroid gland surgery.**

Type of thyroid gland surgery		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Total thyroidectomy	68	51.1	51.1	51.1
	Hemithyroidectomy	65	48.9	48.9	100.0
	Total	133	100.0	100.0	

Total thyroidectomy was performed in 68 (51.1%), and hemithyroidectomy was performed in 65 (48.9%) patients (Table 7).

Table 8 **Frequenc of postoperative complications after surgical treatment of the thyroid gland**

Types of postoperative complications	Frequency		Percent	Valid Percent	Cumulative Percent
Postoperative complications	Recurrent nerve paresis	7	5.3	30.4	30.4
	Recurrent nerve paralysis	2	1.5	8.7	39.1
	Postoperative bleeding	2	1.5	8.7	47.8
	Postoperative seroma	3	2.3	13.0	60.9
	Hypoparathyreoidism	7	5.3	30.4	91.3
	Postoperative wound infection	2	1.5	8.7	100.0
	Total	23	17.3	100.0	
	Total	13	100.0		
		3			

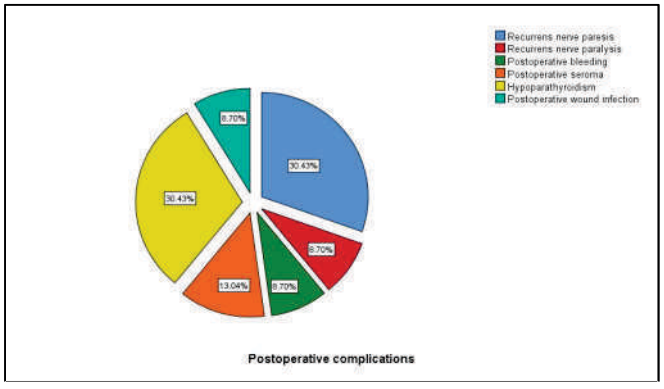

Figure 6 **Types and percentage of complications after surgical treatment of the thyroid gland.**

Postoperative complications were noted in 23 (17.3%) patients in the total sample. The most common postoperative complication was hypoparathyroidism, which was present in 7 (30.4%) patients, and the rarest postoperative wound infection, present in 2 (8.7%) patients (Table 8, Figure 6).

Table 9 **Frequency of postoperative complications in relation to definitive benign and malignant patho-histological findings.**

Pathohistology		Complications						Total
		Recurrens nerve paresis X=0,483 P=0,50	Recurrens nerve paralysis X=0,628 P=0,23	Postop. bleeding X=0,799 P=0,07	Postop. seroma X=0,587 P=0,26	Hyper parathyrod. X=0,483 P=0,49	Postop. wound infection X=0,389 P=0,72	
M Malignant	Count	5	2	0	1	5	1	14
	% within PH	35.7%	14.3%	0.0%	7.1%	35.7%	7.1%	100.0%
Benign	C	2	0	2	2	2	1	9
	Count % within PH	22.2%	0.0%	22.2%	22.2%	22.2%	11.1%	100.0%
Total	Count	7	2	2	3	7	2	23
	% within PH	30.4%	8.7%	8.7%	13.0%	30.4%	8.7%	100.0%

X^2=6,106 p=0,296

Complications were more common in the group of patients with malignant pathohistological findings, but the hi-squared independence test didn't show a significant relationship between the findings of a definite pathohistological analaysis and the occurrence of postoperative complications (x^2=6.106, p=0.296). Recurrent nerve paralysis occurred in 2 (14.3%) patients with malignant pathohistological findings, but without statistical significance in relation to patients with benign pathohistological findings. Postoperative bleeding occurred in 2 (22.2%) patients with benign findings, but without statistical significance compared to patients with malignant pathohistological findings. Recurrent nerve paresis and hyperparathyroidism occurred in 5 (35.7%) patients with malignant findings, and only in 2 (22.2%) patients with benign findings, but no statistically significant difference was also found. Other complications occurred equally in both groups of patients (Table 9).

Table 10 **Frequency of postoperative complications in relation to the type of thyroid gland surgery.**

Extent of operative treatment		Complications						Total
		Recurrens nerve paresis X=0,53 P=0,394	Recurrens nerve paralysis X=0,54 P=0,0,379	Postoperative bleeding X=0,52 P=0,420	Postoperative seroma X=0,38 P=0,759	Hypopara-thyroidism X=0,35 P=0,858	Postop. wound infection X=0,52 P=0,420	
Total thyroidectomy	Count	6	2	1	2	5	1	17
	% within Type of surgery	35.3%	11.8%	5.9%	11.8%	29.4%	5.9%	100.0%
Hemi thyroidectomy	Count	1	0	1	1	2	1	6
	% within Type of surgery	16.7%	0.0%	16.7%	16.7%	33.3%	16.7%	100.0%
Total	Count	7	2	2	3	7	2	23
	% within Type of surgery	30.4%	8.7%	8.7%	13.0%	30.4%	8.7%	100.0%

X^2=2,502 p=0,776

All complications were more common in patients who underwent total thyroidectomy, but the hi-squared test of independence didn't show a significant relationship between the extent of surgery and postoperative complications (x^2=2.502 p=0.776). Paresis of recurrent nerve occurred in 6 (35.3%) patients undergoing total thyroidectomy, but without statistical significance in relation to patients who underwent hemithyroidectomy. Recurrence nerve paralysis occurred in 2 (11.8%) patients undergoing total thyroidectomy, but without statistical significance compared to the control group. Hypoparathyroidism occurred in 5 (29.4%) patients

who underwent total thyroidectomy, in 2 (33.3%) patients with hemithyroidectomy, but without a statistically significant difference. Other complications occurred equally in both groups (Table 10).

DISCUSSION

In setting the indication for surgical treatment as well as choosing the type and extent of surgical treatment, cytological fine needle aspiration (FNA) under ultrasound control is a very important diagnostic method (3,4,8,9,10).

Our study analyzed data of 133 patients, 113 (85%) female and 20 (15.0%) male, in a female-to-male ratio of about 6:1 (5.65:1). Patients were divided into six age groups. Most patients belong to the range of 40-49 years, 42 of them (31.6%), and the least number of patients was in range from 20-29 years, 3 (2.3%) patients. The youngest patient was 18 and the oldest was 71. The average age was 48.17 years. Compared with the data from the available medical literature, there is no significant difference in terms of patient age in our study, nor in terms of female-male patient ratios (11-14).

After FNA diagnosis of nodular thyroid changes, 75 (56.4%) patients in our study had a benign finding, we had a malignant finding in 37 (27.8%) patients, cytologically suspected finding was present in 18 (13.5%) patients, while we had an insufficient sample in 3 (2.3%) patients.

The finding of an insufficient or inadequate sample represents the greatest limitation of FNA whose percentage in the available literature varies from 3 to 32%. In our study, we had a slightly lower percentage of insufficient samples which may be due to the small sample of the study (7,10,15).

In our study, we had a sensitivity of 76.1%, a specificity of 75.9%, a positive predictive value of 94.6%, a negative predictive value of 88%, while the percentage of false-negative results was 19.6%, and the percentage false positive findings was 2.3%.

A false-negative finding is the most undesirable variant of FNA analysis, given the possibility that these thyroid nodal disorders may be surgically insufficiently treated or may be an indication for reoperation, which carries an increased risk of postoperative complications compared to primary surgery. Data obtained from a number of clinical studies indicate that the percentage of false-negative results varies from 1 to 11%, while the percentage of false-positive results ranges from 1 to 8%.

In comparison with the data from the available literature regarding the percentage of false-negative results obtained by FNA puncture of nodular thyroid changes, it is evident that the values in our study are slightly higher. The reason for this difference lies in the fact that we compared the findings of all four categories of FNA with the definitive PH finding in terms of false-negative and false-positive findings. However, in our study, we had a "true" false negative result in only two cases of preoperative benign FNA findings, which is important for the type and extent of surgical treatment, and is an indication for more extensive secondary surgical treatment (16-19).

Data from a number of clinical studies indicate that the percentage of sensitivity is 65 to 98% and the specificity is 72 to 100%, while the percentage of positive predictive value is 94.9% to 100% and 91.8% to 96% for negative predictive value, which coincides with the results of our study (18,19,20,21,).

In the group of patients who underwent total thyroidectomy, postoperative complications were recorded in 17 (12.8%) patients, while in the group of patients with hemithyroidectomy, postoperative complications were found in 6 (4.51%) patients. The hi-square independence test did not show a statistically significant relationship between the type of surgery, the definitive pathohistological finding, and postoperative complications.

Regarding the type of postoperative complications, the most common was hypoparathyroidism, in 7 (5.3%) patients, while the rarest complication was wound infection recorded in 2 (1.5%) patients. Data from the literature indicate that the percentage of postoperative hypoparathyroidism ranges from 1 to 30%, especially after total thyroidectomy. Our results are comparable with data obtained from the available literature (22).

Recurrent nerve injuries range from 0.5 to 2.5% whether it is paresis or recurrent nerve paralysis (23,24). In our study, we had recurrent nerve injury (paresis and paralysis) in 8 (6.0%) patients, which can be considered conditionally comparable with the available data from the literature, since we performed our thyroid surgeries without intraoperative monitoring of the recurrent nerve.

Other postoperative complications such as neck hematoma, postoperative seroma and surgical wound infection occur in a percentage of 0.5 to 5%. The data obtained from the analysis of the data of our study indicate that the percentages of the mentioned postoperative complications correlate with the data from the available literature (25,26,27).

CONCLUSION

In setting the indication for appropriate surgical treatment of nodular thyroid disorders, FNA is still the "gold standard". However, given the possibility of different percentages of false-negative FNA findings, clinical and radiological characteristics of nodal disorders must be taken into consideration in selecting patients for appropriate surgical treatment to accurately determine the extent of surgical treatment and reduce the percentage of reoperations and postoperative complications.

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Diagnostic significance of T-cell subpopulations and their activation markers in infectious mononucleosis

Dijagnostički značaj T-ćelijskih subpopulacija i njihovih aktivacijskih markera u infektivnoj mononukleozi

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ABSTRACT

Introduction: primary Epstein Barr virus (EBV) induced Infectious mononucleosis (IM) is followed by a latent lifelong infection that can lead to neoplastic formations and malignancies. The diagnosis of latent EBV infection is possible by flow cytometry that analyses different lymphocyte subpopulations forming the immunophenotypic profile. Aim: to determine immunophenotypic profile of Bosnian subjects exposed to EBV and to identify residual infection and the damage of the immune system. Materials and methods: 150 subjects (age 3 months-55 years) diagnosed with EBV-induced Infectious mononucleosis (acute primary forms or earlier infection) were included in this study and divided into two experimental and one control group. Immunophenotypic profile was analyzed for all subjects. Results were analyzed by IBM SPSS Statistics version 23. Results: patients suffering from acute form of IM have significantly higher values of T-cell populations, $CD3^+$, $CD8^+$ subpopulations and $CD8^+HLA\text{-}DR^+$ specific marker, as well as significantly lower $CD4^+$ subpopulation and CD4/CD8 ratio when compared to healthy controls and patients with history of IM ($p<0.001$, for all parameters). Subjects with history of IM have significantly higher values of $CD8^+HLA\text{-}DR^+$ specific marker when compared to healthy controls ($p<0.001$), while values of $CD3^+$, $CD4^+$, $CD8^+$ and CD4:CD8 ratio do not differ between these two groups. Conclusion: immunophenotypic profile of patients suffering from acute IM shows higher values of $CD3^+$, $CD8^+$ and $CD8^+HLA\text{-}DR^+$ and lower values of $CD4^+$ and CD4/CD8 ratio. Immunophenotypic profile differs significantly between healthy, subjects with history and patients with acute IM.

Keywords: T-cell subpopulation, Infectious mononucleosis, immunophenotypic profile

SAŽETAK

Uvod: primarnu infektivnu mononukleozu (IM) izazvanu Epstein Barr virusom (EBV) prati latentna doživotna infekcija koja može dovesti do neoplastičnih formacija i maligniteta. Dijagnozu latentne EBV infekcije moguće je postaviti protočnom fluocitometrijom koja analizira različite subpopulacije limfocita formirajući imunofenotipski profil. Cilj ovog istraživanja je bio utvrditi imunofenotipski profil ispitanika izloženih EBV-u, te identificirati rezidualne infekcije i oštećenja imunološkog sistema. Materijali i metode: ovo istraživanje obuhvatilo je 150 ispitanika (3 mjeseca - 55 godina) sa dijagnozom infektivne mononukleoze uzrokovane EBV (akutni primarni oblici ili prethodna infekcija), podijeljenih u dvije eksperimentalne i jednu kontrolnu grupu. Imunofenotipski profil je analiziran za sve ispitanike. Rezultati su analizirani uz pomoć IBM SPSS Statistics ver. 23. Rezultati: pacijenti koji pate od akutnog oblika IM imaju značajno veće vrijednosti T-ćelijskih populacija, CD3+, CD8+ subpopulacija i CD8+HLA-DR+ specifičnog markera, kao i značajno nižu subpopulaciju CD4+ i CD4/CD8 omjer u poređenju sa zdravim kontrolama i pacijentima koji su prebolovali IM ($p<0,001$, za sve parametre). Ispitanici koji su prebolovali IM imaju značajno veće vrijednosti CD8+HLA-DR+ specifičnog markera u poređenju sa zdravim kontrolama ($p<0,001$), dok se vrijednosti CD3+, CD4+, CD8+ i CD4:CD8 ratia ne razlikuju između ove dvije grupe. Zaključak: imunofenotipski profil pacijenata oboljelih od akutne IM pokazuje veće vrijednosti CD3+, CD8+ i CD8+HLA-DR+, te niže vrijednosti odnosa CD4/CD8 i CD4+. Imunofenotipski profil značajno se razlikuje između zdravih, ispitanika koji su prebolovali i pacijenata sa akutnom IM.

Ključne riječi: T-ćelijske subpopulacije, infektivna mononukleoza, imunofenotipski profil

INTRODUCTION

Infectious mononucleosis (IM) is an acute, benign disease of the reticuloendothelial and lymphatic system caused by Epstein Barr virus (EBV), and in most immunocompetent hosts it is a self-limiting infection. EBV's primary tropism is for epithelial cells and B lymphocytes. The human immune response to EBV includes activation and proliferation of natural killer (NK) cells, B and T cells, in particular cytotoxic T cells. IM is characterized as a "lymphoproliferative" disease calling into question its benignity (1).

An acute lytic phase of the EBV infection is characterized by a self-limiting proliferation of the cytotoxic CD8+ T lymphocytes and helper CD4+ T lymphocytes (1,2). After productive primary infection, the EBV virus establishes a latent infection, persisting in memory B-cells.

Diagnosis of EBV infection is possible due to the characteristic response of specific antibodies (serological tests). EBV infection is also accompanied by changes in the cellular immunity, where different subpopulations of lymphocytes express an abundance of specific markers on their surface and intracellularly, allowing them to be identified by flow cytometry. An absolute number and percentage of cellular subpopulations and the recognition of pathological changes in cellular immunity, make the immunophenotypic profile (1,3). Clinical management of IM considers CD8+ T-lymphocytes, CD8+HLA-DR+ T-lymphocytes, and CD4+/CD8+ ratio to monitor disease activity. Activation of the reticuloendothelial system can have severe consequences. EBV is an oncogenic virus linked to a variety of malignancies (4).

Many lymphocytes have altered expression during acute EBV infection and in healthy people already recovered from IM. Flow cytometry identifies residual changes after an EBV infection and detects damage to the immune system (5).

AIM

The aim of the research was to determine the immunophenotypic profile of subjects with the acute form of IM and those who previously recovered from the disease using flow cytometry.

MATERIALS AND METHODS

Patient population and study design

The trial was a controlled, prospective, descriptive clinical study, including a total of 150 patients of both sexes, aged 3 months to 55 years, divided into 3 groups. The complete survey was conducted at Clinical Center University of Sarajevo.

Targeted laboratory tests were performed on 50 peripheral blood samples from patients processed at the Clinic of Infectious Diseases, and all of them had a serologically confirmed diagnosis of acute IM (first group-acute IM). 50 blood samples were also taken from patients whose anamnestic history revealed that they had suffered from IM before (second group -previously recovered from IM), as well as 50 blood samples from healthy individuals as controls (third group). Blood collection and analysis was performed over a period of two years (2014-2016).

Laboratory tests

Blood samples were taken according to the standard operating procedures. Lymphocyte immunophenotyping was performed on multiparametric flow cytometer FACS-Canto-II (Becton Dickinson) by using several different monoclonal antibodies. Percentages of individual lymphocyte subpopulations of peripheral blood, determined to all subjects, included: T-lymphocytes (CD3), CD4+ T-lymphocytes, CD8+ T-lymphocytes, activated CD8+CD69+, CD8+CD25+, CD8+HLA-DR+, CD4+CD69+, CD4+CD25+ and CD4+HLA-DR+ T-cells.

Statistical analysis

Statistical evaluation of results was performed by IBM SPSS Statistics version 23 (Chicago, IL, USA). Parametric and nonparametric tests were used. Median, interquartile range (IQR), ratio, and frequency values were used in the descriptive statistics. The difference in the values of the analyzed parameters between groups was determined by one-way analysis of variance (ANOVA) and Kruskal-Wallis tests. $p < 0.05$ was considered statistically significant.

RESULTS

The study group (N=150) consisted of 50 (33.33%) healthy subjects, 50 (33.33%) subjects who had previously suffered from IM and 50 (33.33%) patients with acute IM. The group of patients with acute IM included 19 (38%) males and 31 (62%) females; the group of subjects with history of IM included 24 (48%) males and 26 (52%) females, while the group of healthy subjects included 29 (58%) males and 21 (42%) females. Our study included 44 (29.3%) children from 3 months to 12.5 years; 18 (12%) teenagers aged 13-18.5, 81 (54%) adults aged 19-35 and 7 (4.7%) mature adults aged 36 to 55. Age distribution according to study groups is shown in Table 1; whereas the age and gender differences between the groups were not analyzed.

Table 1 **Age distribution according to study groups.**

	3 months-12.5 years	13-18 years	19-35 years	36-55 years	Total
	N (%)	*N (%)*	*N (%)*	*N (%)*	
Acute form of IM	39 (78%)	7 (14%)	3 (6%)	1 (2%)	50
Recovered from IM	1 (2%)	4 (8%)	40 (80%)	5 (10%)	50
Healthy subjects	4 (8%)	7 (14%)	38 (76%)	1 (2%)	50
Total	44	18	81	7	150

Values represent number of subjects/frequency (N) and percentage (%).

Peripheral blood cell population, total lymphocyte count and subpopulations (CD3+ T-lymphocytes, CD3+CD4+ helper T-cells, CD3+CD8+ cytotoxic T-cells and CD4+CD25+, CD8+CD25+, CD4+HLA+DR, CD8+HLA+DR regulatory T-cells) as well as CD4:CD8 ratio were determined in the study population as shown in Table 2.

There is a significant difference between healthy subjects and patients with acute IM in all observed parameters (lymphocyte count, CD3+, CD3+ CD4+, CD3+ CD8+, CD4:CD8 ratio, CD8+ CD25+, CD4+HLA+DR and CD8+HLA+DR) except CD4+ CD25+. Between healthy and subjects who previously recovered from IM there is no significant difference in any of the observed parameters except CD8+HLA+DR.

Similarly as to healthy subjects, patients with acute IM showed significant difference in all of the observed parameters (including CD4+CD25+) when compared to subjects who had previously recovered from IM.

Table 2 **Cell counts and values of immunologic markers between the study groups**.

Observed parameter	Group (N)	Mean ±SD	p*	p**	p***
Lymphocyte count	Healthy subjects (50) Recovered from IM (50) Acute form of IM (50)	36.6208±3.12904 38.7296±2.88233 55.5652±1.98327	**<0.001**	0.872	**<0.001**
CD3+	Healthy subjects (50) Recovered from IM (50) Acute form of IM (50)	73.1830±1.39616 73.0063±1.39616 81.0690±1.44568	**0.001**	0.996	**<0.001**
		Median (25-75%)			
CD3+ CD4+	Healthy subjects (50) Recovered from IM (50) Acute form of IM (50)	49.14 (24.24-71.57) 40.28 (27.19-65.05) 14.63 (6.8.5-25.56)	**0.001**	0.221	**0.001**
CD3+ CD8+	Healthy subjects (50) Recovered from IM (50) Acute form of IM (50)	26.00 (20.20 -30.20) 24.41 (20.60-27.80) 66.20 (47.60-70.60)	**<0.001**	0.980	**<0.001**
CD4:CD8 ratio	Healthy subjects (50) Recovered from IM (50) Acute form of IM (50)	1.89 (1.20-2.37) 1.65 (1.32-2.34) 0.221 (0.144-0.362)	**<0.001**	0.967	**<0.001**
CD4+ CD25+	Healthy subjects (50) Recovered from IM (50) Acute form of IM (50)	6.81 (3.63-8.80) 7.45 (2.99-13.86) 5.00 (3.50-7.00)	0.847	0.064	**0.015**
CD8+ CD25+	Healthy subjects (50) Recovered from IM (50) Acute form of IM (50)	1.92 (0.800-3.00) 1.74 (0.600-3.20) 0.300 (0.200-0.700)	**0.005**	0.964	**0.002**
CD4+HLA-DR+	Healthy subjects (50) Recovered from IM (50) Acute form of IM (50)	3.05 (2.10-4.80) 7.20 (4.70-9.80) 26.15 (17.60-38.90)	**<0.001**	0.268	**<0.001**
CD8+HLA-DR+	Healthy subjects (50) Recovered from IM (50) Acute form of IM (50)	6.99 (4.10-8.70) 16.59 (12.81-21.30) 86.60 (76.10-91.00)	**<0.001**	**<0.001**	**<0.001**

Differences were tested using the one-way analysis of variance- ANOVA (results presented as mean and ±SD- standard deviation) and Kruskal-Wallis test (results presented as median with interquartile range, 25-75 percentile). p - significance between healthy subjects and patients with acute infectious mononucleosis. p** - significance between healthy and subjects who previously recovered from infectious mononucleosis. p*** - significance between subjects who previously recovered from and patients with acute infectious mononucleosis. p< 0.05 was considered statistically significant. IM- infectious mononucleosis.*

Average values of CD3+ (reference values 59 -85%) did not differ between healthy and subjects who previously recovered from IM (73.18 vs 73.01%, respectively). Patients with acute IM, although within reference range, had significantly higher average values of CD3+ (81.07%) when compared both to healthy and subjects who recovered previously (p=0.001 and p<0.001, respectively).

Average values of CD3+CD4+ subpopulations were within reference values (30-59%) in both healthy and subjects who previously recovered from IM (44.19 and 42.8%, respectively). In contrast, patients with acute IM, had significantly lower average values of CD3+ CD4+ (16.16%), well below reference ranges and also significantly lower when compared both to healthy and subjects who recovered previously (p<0.001 for both) (Figure 1).

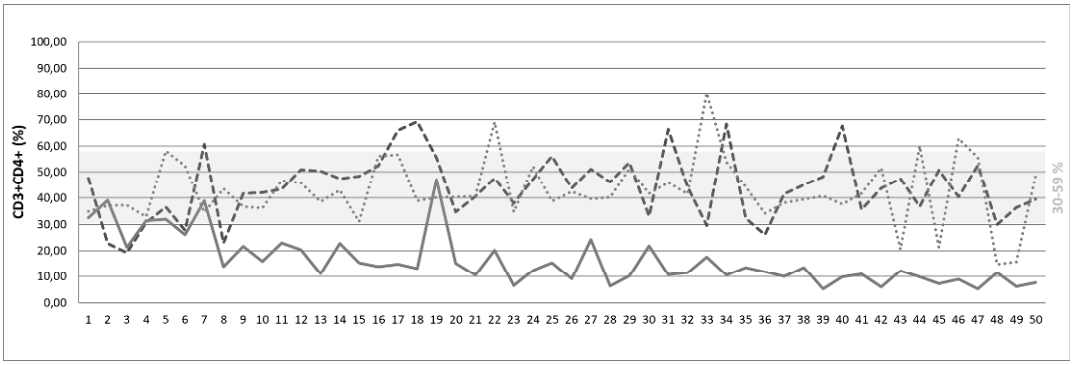

Figure 1 **CD3+CD4+ values according to study groups in relation to reference values.**

Y-axis shows CD3+CD4+ values in percentages (%). Individual subjects are represented on X-axis, healthy subjects (dashed line), subjects who previously recovered from infectious mononucleosis (dotted line) and patients with acute infectious mononucleosis (solid line). Light gray shade represents the reference range for CD3+CD4+ (30-59%).

Average values of CD3+CD8+ subpopulations were within reference values (11-38%) in both healthy and subjects who previously recovered from IM (26.38 and 25.94%, respectively). In contrast, patients with acute IM, had significantly higher average values of CD3+CD8+ (60.54%), well above reference ranges and also when significantly higher compared both to healthy and subjects who recovered previously ($p<0.001$ for both) (Figure 2).

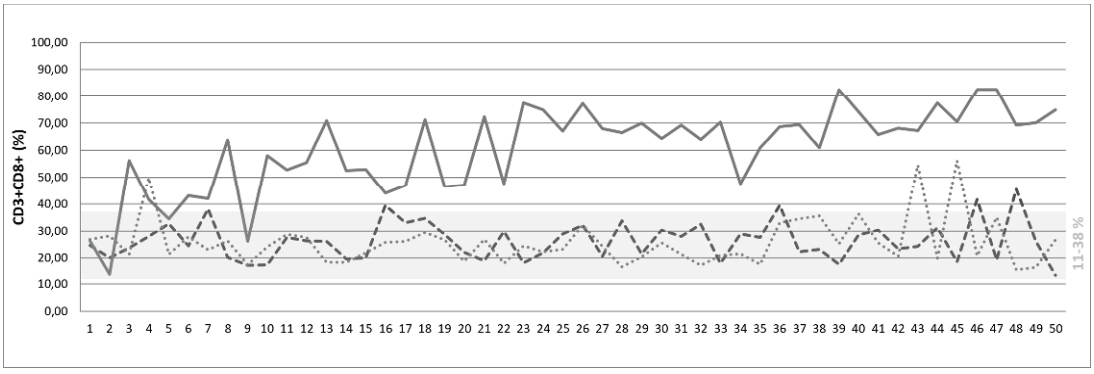

Figure 2 **CD3+CD8+ values according to study groups in relation to reference values.**

Y-axis shows CD3+CD8+ values in percentages (%). Individual subjects are represented on X-axis, healthy subjects (dashed line), subjects who previously recovered from infectious mononucleosis (dotted line) and patients with acute infectious mononucleosis (solid line). Light gray shade represents the reference range for CD3+CD8+ (11-38%).

Following CD3+CD4+ and CD3+CD8+ values, CD4:CD8 ratio also did not differ significantly between healthy and subjects who previously recovered from IM (1.78 and 1.81%, respectively) and it was within reference ranges (0.9-3.6%) for both groups. Patients with acute IM, had significantly lower CD4:CD8 ratio (0.36), below the reference ranges and significantly lower when compared both to healthy and subjects who recovered previously ($p<0.001$ for both) (Figure 3).

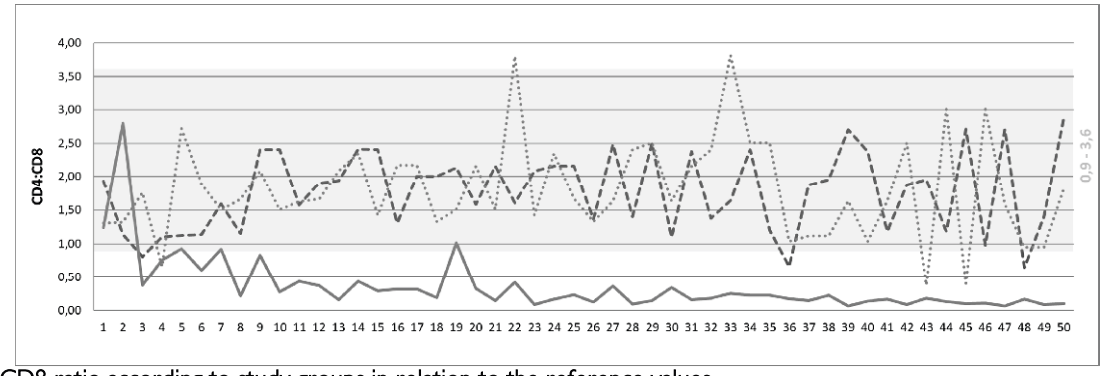

Figure 3 **CD4:CD8 ratio according to study groups in relation to the reference values.**

Y-axis shows values of CD4:CD8 ratio. Individual subjects are represented on X-axis, healthy subjects (dashed line), subjects who previously recovered from infectious mononucleosis (dotted line and patients with acute infectious mononucleosis (solid line). Light gray shade represents the reference range for CD4:CD8 (0.9-3.6).

Average values of CD4+HLA+DR+ subpopulations were similar in healthy and subjects who previously recovered from IM (3.77 and 7.18%, respectively). Patients with acute IM, had significantly higher average values of CD4+HLA+DR+ (29.65%), when compared both to healthy and subjects who recovered previously ($p<0.001$ for both) (Figure 4).

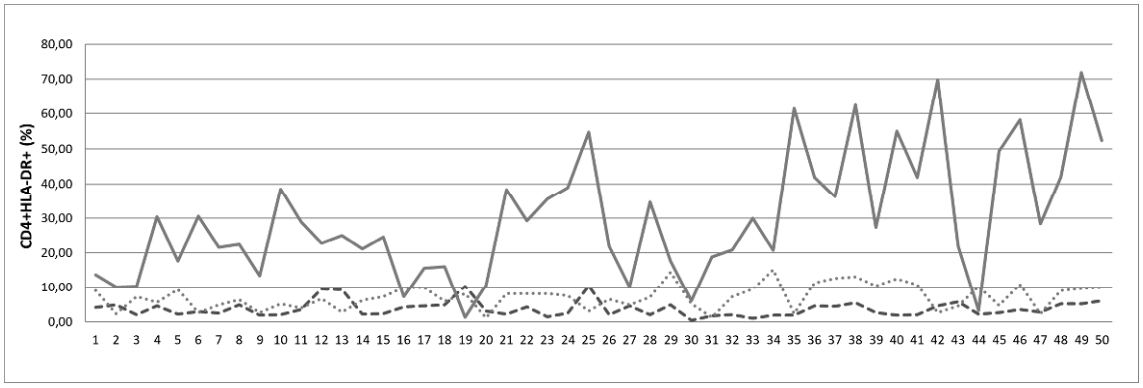

Figure 4 **CD4+HLA-DR+ values according to study groups**.

Y-axis shows CD4+HLA-DR+ values in percentages (%). Individual subjects are represented on X-axis, healthy subjects (dashed line), subjects who previously recovered from infectious mononucleosis (dotted line) and patients with acute infectious mononucleosis (solid line).

Average values of CD8+HLA+DR+ subpopulations differed significantly between healthy and subjects who previously recovered from IM (6.41 and 18.65%, respectively with $p<0.001$). Average values of CD8+HLA+DR+ in patients with acute IM (82.26%) were significantly higher, compared both to healthy and subjects who recovered previously ($p<0.001$ for both) (Figure 5).

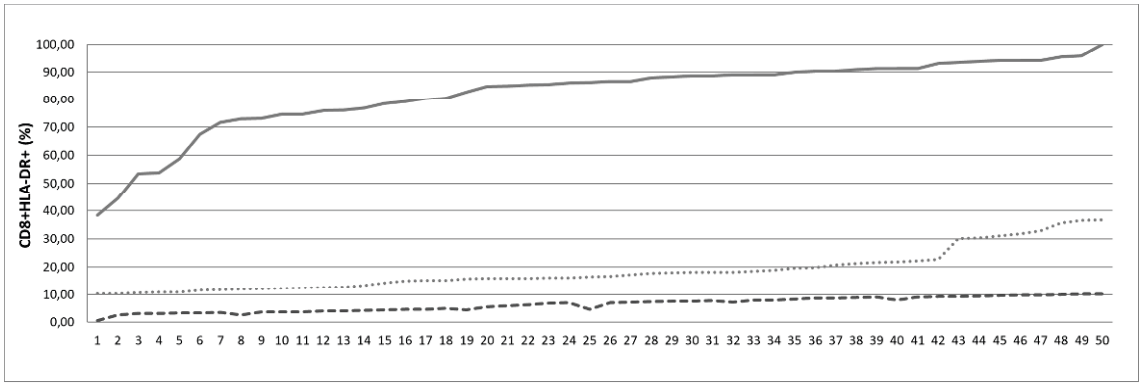

Figure 5 **CD8+HLA-DR+ values according to study groups.**

Y-axis shows CD8+HLA-DR+ values in percentages (%). Individual subjects are represented on X-axis, healthy subjects (dashed line), subjects who previously recovered from infectious mononucleosis (dotted line) and patients with acute infect ious mononucleosis (solid line).

DISCUSSION

Patients with IM in our study were admitted for treatment from the 1st to the 23rd day of their acute illness. EBV infection is almost ubiquitous in humans, men and women are equally susceptible (6). Female to male ratio in our study was 1.08. Women are somewhat more represented both when it comes to acute and when it comes to respondents with a history of IM. Other studies (7) did not find association between serostatus and sex.

By the age of 40 years, about 95% of the population had already been in contact with EBV. In our study, we find the biggest prevalence of primary EBV IM in childhood, i.e. in the group of subjects from 3 months to 12.5 years, which speaks in favor of very early exposure to EBV. Infantile IM is usually asymptomatic and associated with poorer socioeconomic living conditions, while adolescence IM occurs with severe symptoms in 25% of cases (6).

In an attempt to investigate the diagnostic utility of immunophenotyping in IM we analyzed the distribution of several lymphocyte subpopulations in patients with EBV-induced IM and compared it to subjects who previously recovered from EBV IM as well with healthy controls.

Immunophenotyping gains significance as studies (8) pointed out the virus susceptibility to certain malignancies and development of known entities such as Burkitt's lymphoma, nasopharyngeal cancer, Hodgkin's disease, T and B cell lymphomas and some other diseases.

Our study analyzed indicators of T lymphocyte status (CD3+ T-lymphocytes, CD3+CD4+ helper T-cells, CD3+CD8+ cytotoxic T-cells and CD4+CD25+, CD8+CD25+, CD4+HLA-DR+, CD8+HLA-DR+ regulatory T-cells) as well as CD4:CD8 ratio.

The state of B lymphocytes was not considered which could be a shortcoming of our study and a limiting factor in setting the complete picture of immunophenotyping profile.

The data show that acute IM is primarily characterized by exact activation of CD8+ T cell populations and significant expression of HLA-DR+ activation markers, as well as changes in the CD4/CD8 ratio. Other studies (9) noted an increase in the number of NK cells and CD8+ T cells, but not in the number of CD4+ T cells during acute infection.

Measuring the proportion of CD4/3/8 T-lymphocytes by flow cytofluorimetry creates the phenotyping profile of patients with acute IM and is a significant diagnostic parameter. It is also important to mention that the characteristic phenotyping profile of subjects who previously recovered from IM differs from the healthy population profile.

Group I subjects (subjects with acute primary IM EBV etiology) had: elevated CD3+ (mean 81.07%), decreased CD3+CD4+ (mean 16.16%, ref. values 30-59%), elevated CD3+CD8+ (mean 60.54%, ref. value 11-38%), decreased CD4:CD8 (mean 0.36), increased CD4+HLA-DR+ (mean 29.65%), increased CD8+HLA-DR+ (mean 82.26%).

Patients with acute presentation of EBV-induced IM showed significantly increased percentage of total T cells, cytotoxic-suppressor CD8+ T lymphocytes and activated HLA-DR+ T lymphocytes when compared to healthy controls and subjects previously recovered from IM.

The percentage of CD4+ T lymphocytes, as well as the value of CD4/CD8 ratio, were significantly lower in the group of subjects with acute EBV IM compared to healthy controls and subjects who already recovered from EBV IM.

Similar results from other researchers (10) recognize that the immunophenotypic pattern in patients with IM shows dramatic increase of extensively activated CD8+ T cells and HLA-DR on CD8+ T cells when compared to healthy donors.

In group II, subject who had previously recovered from EBV IM, we found the following: CD3+ within the reference range (mean 73.01%), CD3+CD4+ within the reference range (mean 42.8%), CD3+ CD8+ within the reference range (mean 25.94%), normal ratio of CD4:CD8 (mean 1.81), average values of CD4+ HLA-DR+ of 7.18% and average values of CD8+HLA-DR+ of 18.65%. The immunophenotypic profile of subjects previously recovered from IM differs from that of healthy subjects only in values of CD8+HLA-DR+ (8.65 vs. 6.41% with $p < 0.001$).

Group III, healthy subjects, had: CD3+ within the reference range (mean 73.18%), CD3+CD4+ within the reference range (mean 44.19%), CD3+CD8+ within the reference range (mean 26.38%), normal CD4:CD8 ratio (mean 1.78), average values of CD4+ HLA-DR of 3.77% and average values of CD8+HLA-DR of 6.41%.

The analyzed data show that the characteristic immunophenotypic profile of subjects with acute IM differs from the ones of healthy and subjects who previously recovered from IM in average values of CD3+, CD3+CD4+, CD3+CD8+, ratio CD4/CD8, CD4+HLA-DR+, CD8+H-DR+, all with statistical significance $p<0.001$. The CD8+HLA-DR+ cell fraction was markedly elevated in all 50 analyzed patients with acute IM EBV, yielding a mean value of this parameter 82.26%, almost tenfold that of healthy and individuals who recovered from IM.

From the results shown, it is reasonable to assume that changes in the number and proportion of different lymphocyte subpopulations may be diagnostically useful. Extended immunophenotyping of lymphocytes, including cell activation markers, can define disease-related patterns thus providing valuable diagnostic information for these entities and in general for patients with unusual inflammatory symptoms (11). Immunophenotyping distinguishes acute EBV infections from the patterns of previously recovered from IM.

Other studies (11-14) provided similar results, indicating that the response to EBV is dominated by an increase in the number and activation of CD8+ T lymphocytes. Prolonged CD8+ T cell stimulation during IM causes EBV to enter latency and is under lifelong immune control in most individuals that experience this disease (14).

Studies (15,16) also state that response to EBV involve NK cells and CD4+ T cells, controlling the primary infection and limiting periodic reactivation.

Apart from CD8 T-cytotoxic cell levels increased, studies (17) emphasize that T cells in all cases express HLA-DR + antigen activation, and that the CD4/CD8 ratio is significantly reduced in all patients with acute IM (11,17), which coincides with our results. These results are also confirmed for the pediatric population (18,19), showing that children with EBV-induced IM have a reduced CD4/CD8 ratio and increased proportions of activated HLA-DR+ CD4+ and CD8+ T-lymphocytes in the peripheral blood.

Patients with EBV-induced IM, compared to healthy controls, show an increased percentage of total T cells, cytotoxic-suppressor CD8+ T lymphocytes, activated HLA-DR+ T lymphocytes and reduced CD4+ T lymphocytes, as well as the CD4/CD8 ratio. This has been known for decades and it is confirmed in our and other studies (20-23).

Some scientists (24) believe that all future research on EBV IM should be focused on the development of vaccines and drugs against EBV, on prevention of complications (multiple sclerosis, Hodgkin's and Burkitt's lymphoma, nasopharyngeal carcinoma among other entities) and identification of genetic, immunological and environmental factors. To achieve the listed goals, we believe that it is first necessary to clearly identify the target population, where, again, the best information is offered by immunophenotyping profiles.

In our research, we also examined the early activation markers CD4+CD25+, CD8+CD25+, CD4+CD69+ and CD8+CD69+ (data not shown), assuming that we will not get positive results, because patients with IM diagnosis almost never come to the clinic at an early stage of the disease. With symptom onset, the response of early activation markers already subsides and a hemophagocytosis like syndrome is activated due to the rapid expansion of CD8+ T-cells. Thus, analysis of activating CD25+ and CD69+ receptors is a less informative pattern probably due to the large inter-individual variation of disease reporting.

Finally, it is reasonable to assume that changes in the number and proportion of lymphocyte subpopulations may be a diagnostically useful indicator. Increased CD8+ cytotoxic-suppressor T-cells and changes in other subpopulations have also been observed in other viremia, including HIV, cytomegalovirus infections, he patitis C, and the most current Sars CoV-2 in the Covid-19 pandemic. In contrast to EBV, the expression of HLA-DR+ activation markers on CD8+ T lymphocytes was not present in these viruses, except in the case of Sars CoV-2 (25-28).

CONCLUSION

EBV infection, clinically presented as IM, significantly alters the quantitative relationships of major subpopulations of T lymphocytes: helper CD4+, cytotoxic CD8+, activated CD4+HLA-DR+ and CD8+HLA-DR+ T-cells in the peripheral blood. The immunophenotyping profile of patients with acute IM has the following characteristics: elevated CD3+, elevated CD3+CD8+, decreased CD3+CD4+ and CD4:CD8 ratio, slightly elevated CD4+HLA-DR+ and markedly elevated CD8+HLA- DR+. Subjects who previously recovered from IM have elevated CD8+HLA-DR+ values. Flow cytometry in IM defines informative disease-specific patterns, enabling early immune system damage detection and risk assessment for onset of complications and malignancies.

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Comparative incidence of psychiatric disorders under the pandemical conditions of the SARS-CoV-19

Komparativna incidenca psihijatrijskih poremećaja u uslovima pandemije SARS-CoV-19

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ABSTRACT

Introduction: the state of stress leads to a disturbance of homeostatic balance, which results in an imbalance of neurohumoral, neurovegetative, somatic systems with changes in the psychological plane. This represents a defensive reaction of the organism, in response to the influence and action of a number of unfavorable exogenous stressors, which was especially manifested in the conditions of a global pandemic caused by a new strain of SARS-COV-2 virus. Aim: to assess the incidence of mental disorders/suicide attempts in the context of the COVID-19 pandemic. Materials and methods: the research was retrospective, comparative, based on data from the medical documentation of Psychiatric Clinic Clinical Center University of Sarajevo in the eight months period (March 2019/2020 - November 2019/2020). Results: there was a statistically significant difference ($p<0.01$) diagnosed with X61 (increase of 12.4%) and F23 (increase of 15%) during 2020 compared to 2019. Also if we observe the total prevalence of X61 and F23 diagnoses, we notice an increase of 26.9% during 2020 compared to 2019. Conclusion: our research showed an increase in the incidence of psychotic disorders/suicide attempts by taking medication during the pandemic 2020 compared to 2019, which was largely due to socioeconomic limitations.

Keywords: SARS-CoV-2, psychotic disorder, suicide

SAŽETAK

Uvod: stanje stresa dovodi do narušavanja homeostatske ravnoteže, što za posljedicu ima disbalans neurohumoralnih, neurovegetativnih, somatskih sistema uz promjene na psihičkom planu. Navedeno predstavlja odbrambenu reakciju organizma, kao odgovor na utjecaj i djelovanje niza nepovoljnih egzogenih stresogenih činilaca što se posebno manifestiralo u uslovima globalne pandemije uzrokovane novim sojem virusa SARS-CoV-2. Cilj istraživanja: procjena incidence psihičkih poremećaja/pokušaja suicida u uslovima pandemije COVID-19. Ispitanici i metode: istraživanje je bilo retrospektivno, komparativno, na osnovu podataka iz medicinske dokumentacije protokola Psihijatrijske klinike Kliničkog centra Univerziteta u Sarajevu u periodu od osam mjeseci (mart 2019/2020. godine - novembar 2019/2020. godine). Rezultati: zabilježena je statistički značajna razlika ($p<0,01$) s dijagnozom X61 (povećanje od 12,4%) i F23 (porast od 15%) tokom 2020. godine u odnosu na 2019. godinu. Također, ako promatramo ukupnu prevalenciju dijagnoza X61 i F23, primjećujemo povećanje od 26,9% tokom 2020. u odnosu na 2019. godinu. Zaključak: naše istraživanje dokazalo je porast incidence psihotičnih poremećaja/pokušaja suicida uzimanjem lijekova tokom pandemijske 2020. godine u odnosu na 2019. godinu, a što je u velikoj mjeri posljedično vezano za socioekonomsku limitiranost.

Ključne riječi: SARS-CoV-2, psihotični poremećaj, suicid

INTRODUCTION

The state of stress disturbs the homeostatic balance of the organism, neurohumoral and neurovegetative regulation and is actually a defensive reaction to the influence and action of unfavorable stressors. Psychophysiological manifestations and emotional states that manifest themselves, having in mind the phenomenon of anxiety, are actually a syndrome of adaptation that has its own characteristic stages of development. The coronavirus pandemic is a "new and unknown" that, in addition to affecting people's physical health, also has a significant impact on the mental condition (1). People are increasingly lonely and uncertainly "listening" to the near future (2). People with COVID-19 are afraid of the more severe consequences of the disease, while those suspected of COVID-19 feel fear of the test results. As a result, loneliness, denial, anxiety, depression, insomnia and despair can occur, and the risk of aggression and suicide may even increase (3,4,5). Isolated individuals, in anticipation of whether or not to develop symptoms, may suffer from anxiety due to the uncertainty of their health and develop obsessive-compulsive symptoms, such as frequent control of body temperature and excessive disinfection (6). Regardless of the unfavorable circumstances that surrounding us during the pandemic, the risk of suicidal behavior can be significantly reduced, because the crisis is collective, not individual, and no one should go through it alone. Social cohesion is one of the crucial factors in suicide prevention measures. Substantial social isolation, along with all the other challenges this situation poses, can potentially become a trigger for the development of suicidal thoughts. In the context of scientific

knowledge about the intensity, forms and specifics of human reactions under the influence of psychological stress in pandemic conditions, certain experiences and knowledge about the behavior of the population are of special interest (7,8).

AIM

The aim of this study was to determine the possible increase in the number of patients diagnosed with acute polymorphic psychotic disorder in correlation with suicide attempts with regard to gender, age and socioeconomic status.

MATERIALS AND METHODS

The research was retrospective and comparative based on data obtained from medical documentation of Psychiatric Clinic of the Clinical Center University of Sarajevo (CCUS) in the eight months period (March 2019/ 2020 - November 2019/ 2020). The study included the total of 136 respondents; 39 in 2019 (F=25, M=14) and 97 in 2020 (F=57, M=40), over 18 years of age, admitted at the Psychiatric Clinic, with no histories of previous psychiatric illness and treatment. Admission where realized consecutively with the diagnosis of acute polymorphic psychotic disorder (F23) and a deliberate poisoning with and exposure to antiepileptic, sedative-hypnotics, antiparkinsonian and psychotropic drugs, nonaligned elsewhere (X61), arranged on the basis of the ICD-10. The average age of respondents was 40.45±1 (the mean value ± SD), ranging from 39.5 up to 41.4 years.

All participants signed the informed consent approved by the Ethics Committee of the Clinical Center University of Sarajevo. Additionally, based on their gender, the respondents were divided in three groups depending on their professional qualifications: a) unskilled (US) without professional qualifications, b) medium professional qualifications (MPQ), with completed secondary education and c) high professional education (HPE), with university degree. The division was also made based on their employment status. Results were presented as mean value ± standard deviation (SD). Due to the strict inclusion criteria and the short period of data collection, based solely on the Psychiatric Clinic data, there was a real possibility that the results did not reflect the actual situations given that the CCUS provided four out of ten Cantons in the Federation of Bosnia and Herzegovina (FB&H).

RESULTS

Figure 1 shows the demographic data of both respondent groups compared based on gender distribution. The study was conducted on a group consisting of 61.3% of women (64% in 2019 and 59% in 2020) and 38.7% of men (36% in 2019 and 41% in 2020). The prevalence of women was higher, but no statistically significant difference (p>0.05) was recorded within the compared groups as the gender ratio within the groups was almost the same.

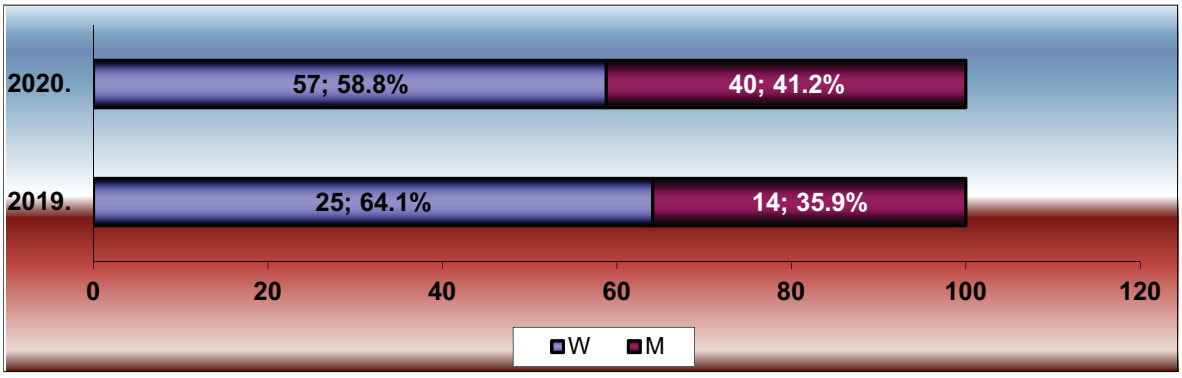

Figure 1 **Demografic data based on gender distribution.**

The study included the total of 136 respondents over 18 years of age. Statistical processing of demographic data showed that the average age of respondents in 2019 was 39.5±5.24 years, and in 2020 it was 41.4±6.28 years. There were no significant differences (p>0.05) with respect to age in both study groups.

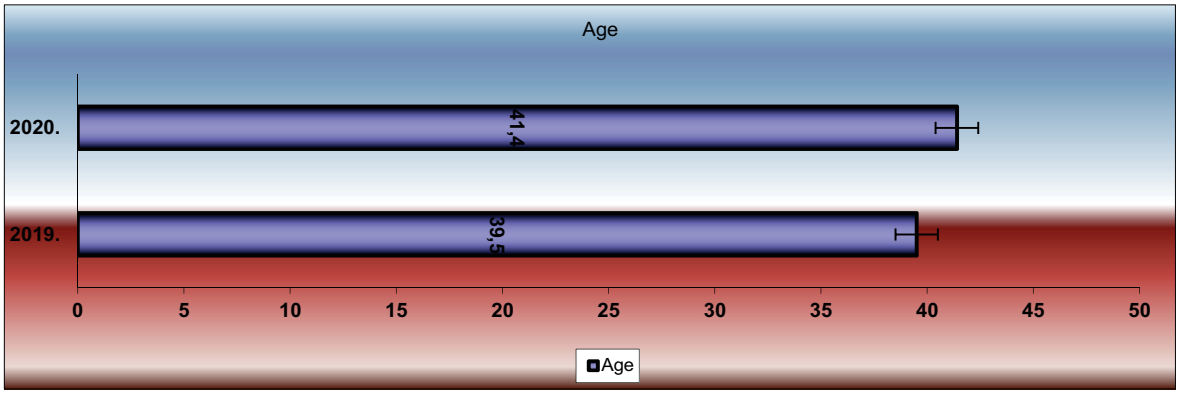

Figure 2 **Demographic data based on age.**

Figure 3 shows the socio-economic status of respondents in the 2019/2020 group, where there was no statistically significant difference in socio-economic representation, but percentage of the unemployed increased from 39.2% to 56.4% during 2020 (an increase of 17.2%) which can be explained by the change in employment status during the pandemic. Due to the imposed socio-economic measures, many shops were closed or due to the imposed measures the workers were prevented from doing their job.

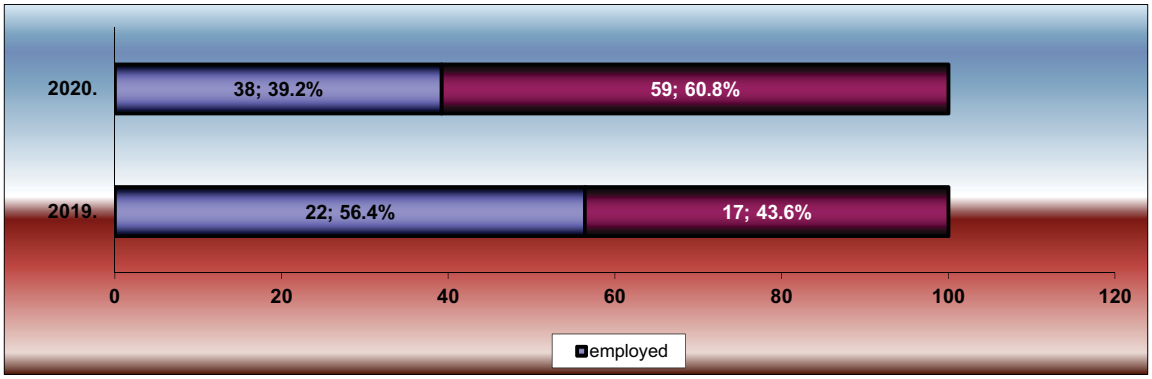

Figure 3 **Socio-economic status of respondents.**

Figure 4 shows the level of qualification, the most common was secondary school, specifically 59% (23 of 39) of the respondents belong to the above category in 2019 and 58% (56 of 97) in 2020. There were 25% of respondents with primary school education in 2019 (10 out of 39), and 21% in 2020 (21 out of 97). The lowest representation was recorded in the category of highly educated respondents, which in 2019 was 15% (6 out of 39), and in 2020 it was 20% (20 out of 97). There was no statistically significant difference (Chi-squared=0.600). p=0,741)

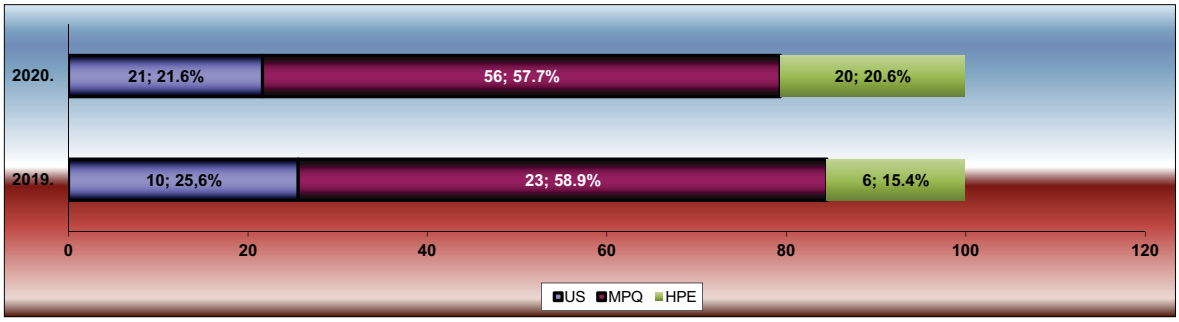

Figure 4 **Educational status of the respondents.**

Figure 5 shows an equal gender ratio with respect to educational status. Thus, the comparison between 2019 and 2020 year showed that the ratio of female and male respondents with primary education was 15/16, with secondary education 42/37, and with higher education that ratio was 14/12. According to the summarized results, no statistically significant deviation was recorded (Chi-squared=1.418; p=0.965).

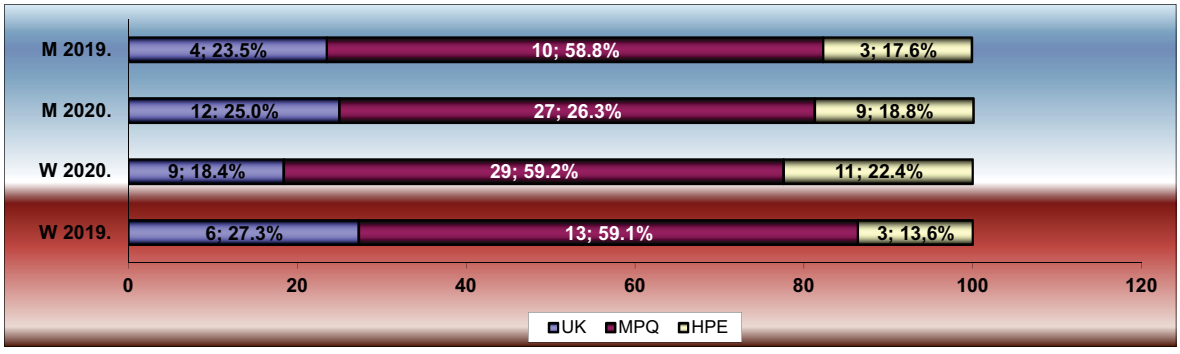

Figure 5 **Educational status of respondents.**

Figure 6 shows a comparison of data related to employment status. The data shows that the percentage of employees in 2019 was higher among women, while in 2020 the unemployment rate was the same for both sexes in the ratio of 1:1.5 (Chi-squared = 4.997; p=0.172).

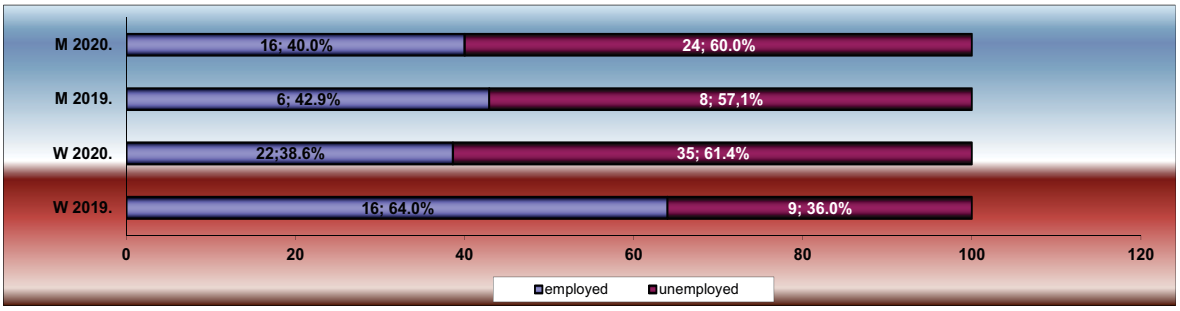

Figure 6 **Employment ratio.**

Figure 7 shows the division of subjects within the comparative groups based on admission diagnoses. Out of the total number of patients in 2019 (n=198) and 2020 (n=206) there was a statistically significant difference (p<0.01) diagnosed with X61 (increase of 12.4%) and F23 (increase of 15%) in 2020 as compared to 2019. Also with regard to the total prevalence of X61 and F23 diagnoses, an increase of 26.9% was recorded in 2020 as compared to 2019.

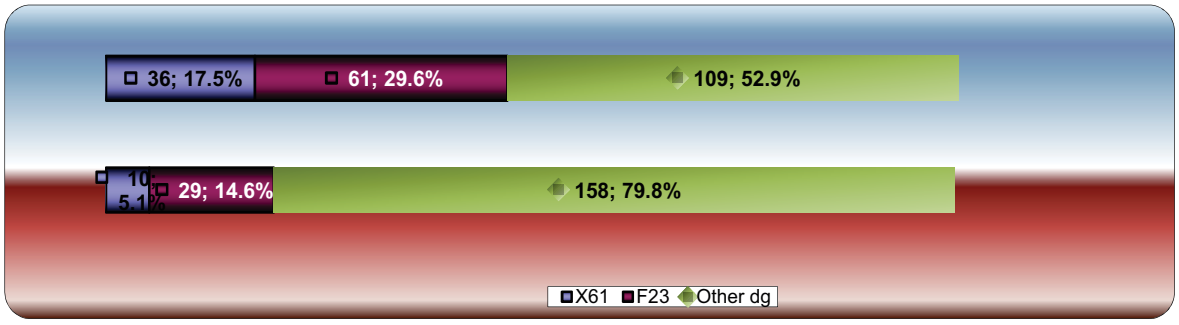

Figure 7 **Division of respondents based on the admission diagnoses.**

Figure 8 shows diagnostic gender differences with a statistically significant increase in psychotic symptoms and suicide attempts during 2020 in both groups. Statistically, the prevalence of suicide attempts was almost identical in both sexes (Chi-squared = 0.682; p=0.877) while psychological decompensation was somewhat more prevalent in male subjects, which could be rationalized to some extent by the protective effect of estrogen in female subjects.

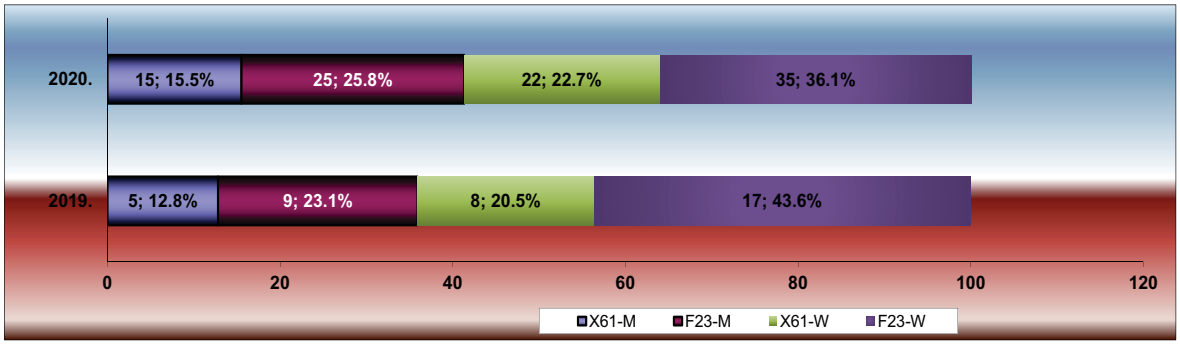

Figure 8 **Diagnostic distinctions in relation to gender.**

In the case of diagnostic entity X61, the share of the unemployed increased in 2020 to 72.2% compared to 60.0% of the unemployed in 2019 (total increase of 12.2%). In the case of F23, the share of the unemployed decreased from 65.5% in 2019 to 60.7% in 2020 (total decrease of 4.8%). No statistically significant difference was observed (Chi-squared = 3.107; p=0.3755).

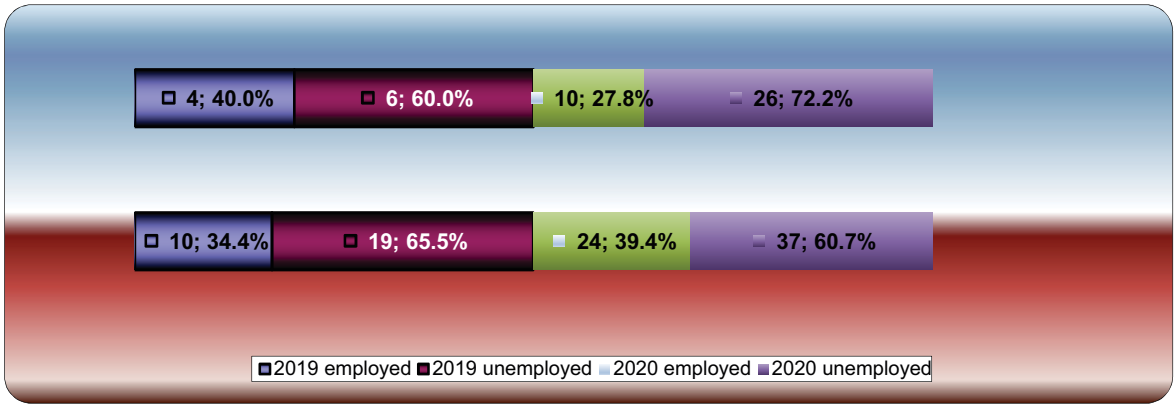

Figure 9 **Diagnostic distinctions in relation to socioeconomic status.**

DISCUSSION

Covid-19 pandemic and the consequent social isolation, resulted in impaired mental health of population, i.e. the emergence of psychotic disorders and suicide attempts (9). Our study involved 136 subjects who, according to admission diagnosis, were divided into a group of those admitted under the symptomatology of acute psychosis and a group of those who attempted suicide by drug intoxication. A meta-analysis of 54 studies conducted during 2020 indicated an increase incidence of psychotic disorders and suicide attempts (5). Our findings correlate with the above-mentioned meta-analysis with an evident increase in the observed entities. Out of the total number of patients during 2019 (n=198) and 2020 (n=206) there was a statistically significant difference ($p<0.01$) diagnosed with X61 (increase of 12.4%) and F23 (increase of 15%) during 2020 compared to 2019. Also if we observe the total prevalence of X61 and F23 diagnoses, we notice an increase of 26.9% during 2020 compared to 2019. Study has shown that women, young and middle-aged people are the most vulnerable group during COVID-19 pandemic (10). In this study, statistically, the prevalence of suicide attempts is almost identical in both sexes (chi-squared= 0,682; p=0,877) while psychological decompensation is somewhat more prevalent in male subjects, which can be rationalized to some extent by the protective effect of estrogen in female subjects. The results of this study did not show a significant deviation when it comes to the average age, and in both comparison groups it was approximately 40 years.

Data from this study show that the socio-economic status of respondents in the 2019/2020 group, where there is no statistically significant difference in socio-economic representation, but that the % of the unemployed increased from 39.2% to 56.4% during 2020 (an increase of 17.2%) which can be explained by the change in employment status during the pandemic, and global recession (11). In studies, poor socio-economic security has been cited as the leading reason for suicide attempts, as one is left without secure sources of income in an uncertain atmosphere (12). According to the B&H Central Bureau of Statistics, the largest job losses during the pandemic occurred in the manufacturing and services sector, which in most cases employs people with secondary education. If we take into account the statistical data that according to the census in B&H, the largest number of people with secondary education, then it is objective to expect that it is more likely that among the respondents will be the most of them with secondary education. The low representation of highly educated respondents can be rationalized by the fact that members of this target group, thanks to broader education, were able to better understand the real situation and develop more adequate compensation mechanisms, given all the benefits of better socioeconomic status (13).

CONCLUSION

Our research indicates an increase in the incidence of psychotic disorders/suicide attempts by taking medication during the pandemic 2020 compared to 2019. Informing the wider community about problems, especially vulnerable groups, can preserve mental health and prevent similar problems with poor outcomes. It is necessary to promote hygiene, mental health, health behavior, as well as the distribution of psychoeducational material. We need to think about problems in the field of mental disorders, and talk "out loud", because that's the only way we can help each other. However, the consideration of multidimensional causes of mental disorders is further investigated in order to find a preventive and therapeutic model and as a mechanism of psychological protection of the population in the conditions of possible future pandemics.

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Authors' Contributions: GS and AM-S gave substantial contribution to the conception or design of the article and in the acquisition, analysis and interpretation of data for the work. Author had role in article drafting and in process of revision. The author gave final approval of the version to be published and agrees 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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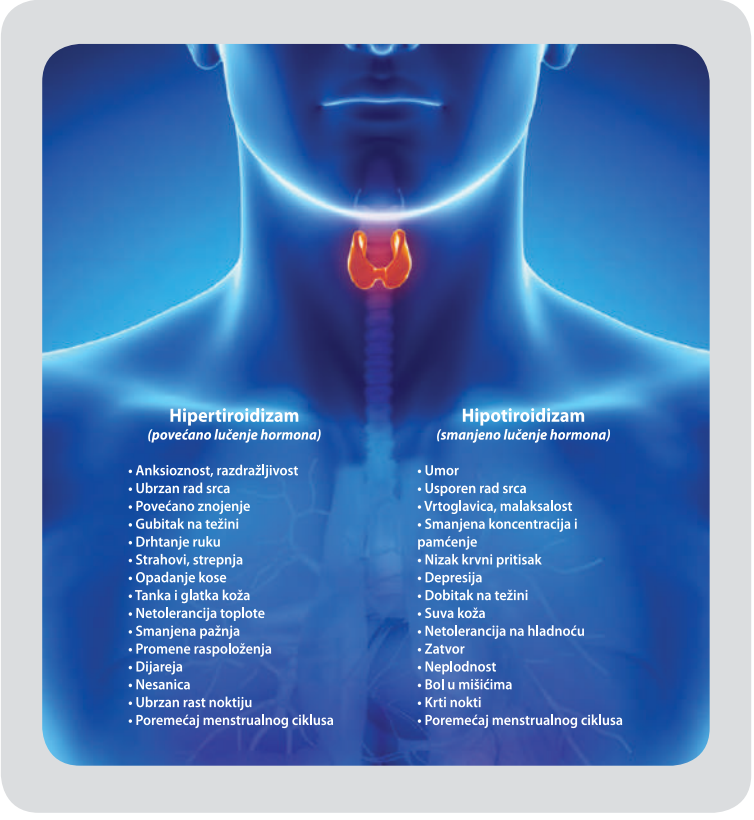

Influence of innovative biliopancreatic diversion and sleeve gastrectomy methods on body weight in experimental animals

Uticaj inovativne metode biliopankreatične diverzije i sleeve gastrektomije na tjelesnu masu kod eksperimentalnih životinja

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ABSTRACT

Introduction: obesity is an important risk factor for the development of cardiovascular disease, type 2 diabetes, metabolic syndrome, hypertension and cancer. Biliopancreatic diversion (BPD) and sleeve gastrectomy (SG) are some of the most successful bariatric methods for treating severe obesity that lead to better glucose regulation in patients with type 2 diabetes but are associated with protein and vitamin deficiency and complications that can be fatal. Bariatric surgery seeks to find new methods and ways of losing weight in obese patients, and the aim of this study is to try to experimentally introduce a innovative modification of BPD and compare its effectiveness in weight loss compared to sleeve gastrectomy. Aim: the aim of this paper is to examine the effect of an innovative method of modified biliopancreatic diversion (MBD) and its comparison with sleeve gastrectomy (SG) as one of the successful methods leading to weight reduction. Materials and methods: the study was conducted at the Medical and Veterinary Faculty of the University of Sarajevo as an experimental, randomized study on an animal model of rats that included 36 adult, healthy albino rats, the Wistar strain. The experimental part of the study was conducted in 3 phases: inducing obesity (4 weeks), operative procedure and postoperative follow-up (4 weeks). In the preoperative period, body weight (in grams-gm) was increased by a high-fat diet for 4 weeks (n=36). Experimental animals were divided into 3 groups immediately before surgery: sleeve gastrectomy (SG; n=12); modified biliopancreatic diversion (MBD; n=12) and abdominal opening without bariatric intervention (positive control) (SHAM; n=12) and weight of the animals was followed up for 28 days postoperatively. Results: the body weight in the control group was significantly higher than the body weight in MBD group and SG group 18 and 28 days after surgery. Weight loss on day 28 compared to preoperative period was 29.5% in MBD group and 32.0% in SG group, however body weight did not differ significantly between MBD and SG group either preoperatively or 9, 18 and 28 days postoperatively. Conclusion: sleeve gastrectomy as a standard method and the innovative MBD method have shown similar results on weight reduction suggesting that the innovative modified BPD can be used as an operative procedure for long term weight loss maintenance.

Keywords: body weight, innovative method biliopancreatic diversion, sleeve gastrectomy

SAŽETAK

Uvod: pretilost je važan faktor rizika za razvoj kardiovaskularnih bolesti, dijabetesa tipa 2, metaboličkog sindroma, hipertenzije i raka. Biliopankreatična diverzija (BPD) i sleeve gastrektomija (SG) su neke od najuspješnijih barijatrijskih metoda za liječenje teške pretilosti koje dovode do bolje regulacije glukoze u bolesnika s dijabetesom tipa 2, ali su povezane s nedostatkom proteina i vitamina i komplikacijama koje mogu biti smrtonosne. Barijatrijska hirurgija nastoji pronaći nove metode i načine mršavljenja u pretilih pacijenata, a cilj ovog istraživanja je pokušati eksperimentalno uvesti inovativnu modificiranu BPD-u i usporediti njenu učinkovitost u gubitku tjelesne mase u odnosu na Sleeve gastrektomiju. Cilj: ispitati učinak inovativne metode modificirane biliopankreatične diverzije (MBD) i njena komparacija sa sleeve gastrektomijom (SG) kao jednoj od uspješnih metoda koja dovodi do redukcije tjelesne mase. Materijal i metode: istraživanje je provedeno na Medicinskom i Veterinarsk om fakultetu Univerziteta u Sarajevu kao eksperimentalna, randomizirana studija na životinjskom modelu štakora koji je uključivao 36 odraslih, zdravih, albino štakora, soja Wistar. Eksperimentalni dio istraživanja proveden je u 3 faze: izazivanje pretilosti (4 sedmice), operativni zahvat i postoperativno praćenje (4 sedmice). U preoperativnom periodu tjelesna težina (u gramima-gm) je povećana dijetom s visokim udjelom masti tokom 4 sedmice (n=36). Pokusne životinje podijeljene su u 3 grupe neposredno prije operacije: sleeve gastrektomija (SG; n=12); modificirana biliopankreatična diverzija (MBD; n=12) i otvaranje abdomena bez barijatrijske intervencije (pozitivna kontrola) (SHAM;

n=12) i težina životinja praćena je 28 dana nakon operacije. Rezultati: tjelesna masa u kontrolnoj grupi bila je značajno viša od tjelesne mase u grupi MBD i grupi SG 18 i 28 dana nakon operacije. Gubitak tjelesne mase 28. dana u usporedbi s preoperacijskim periodom bio je 29,5% u grupi MBD i 32,0% u grupi SG, međutim tjelesna masa se nije značajno razlikovala između grupa MBD i SG ni prije operacije ni 9, 18 i 28 dana nakon operacije. Zaključak: sleeve gastrektomija kao standardna metoda i inovativna MBD metoda pokazale su slične rezultate u smanjenju tjelesne mase što sugerira da se inovativna modificirana BPD može koristiti kao operativni zahvat za dugoročno održavanje gubitka težine.

Ključne riječi: tjelesna težina, inovativna metoda biliopankreatične diverzije, sleeve gastrektomija

INTRODUCTION

Data from the World Health Organization show that in 2016, about 39% of the adult population was overweight (1). It is estimated that the health impact of the obesity epidemic could be even greater in the future, given the increasing incidence of obesity in children and young adults (2). Obesity is an important risk factor for the development of cardiovascular disease, type 2 diabetes, metabolic syndrome, hypertension and cancer. Unfortunately, as obesity increases, life expectancy decreases. The life expectancy of severely obese people is reduced by approximately 5–20 years (3). The effects of bariatric surgery in the treatment of obesity have shown considerable effectiveness (4). In addition, there are more and more papers in the literature that support the inclusion of bariatric surgery to treat T2DM and obese patients (5). Biliopancreatic diversion (BPD) and Sleeve gastrectomy (SG) are some of the most successful bariatric methods for treating severe obesity that lead to better glucose regulation in patients with type 2 diabetes but are associated with protein and vitamin deficiency and complications that can be fatal. Bariatric surgery seeks to find new methods and ways of losing weight in obese patients, and the aim of this study is to try to experimentally introduce an innovative modification of BPD that would have similar results, but fewer complications. The innovative modified BPD technique could reduce the number of complications and side effects while preserving proven metabolic efficiency. The aim of this paper is to examine the effect of an innovative method of biliopancreatic diversion and its comparison with sleeve gastrectomy as one of the successful methods leading to weight reduction.

AIM

The aim of this paper is to examine the effect of an innovative method of modified biliopancreatic diversion (MBD) and its comparison with sleeve gastrectomy (SG) as one of the successful methods leading to weight reduction.

MATERIALS AND METHODS

Animals

The study was conducted at the Medical and Veterinary Faculty of the University of Sarajevo as an experimental, randomized study on an animal model of rats that included 36 adult, healthy albino rats, the Wistar strain. The experimental part of the study was conducted in 3 phases: inducing obesity (4 weeks), operative procedure and postoperative follow-up (4 weeks). In the preoperative period, body weight was increased by a high-fat diet for 4 weeks in the experimental groups (n=24) and in the control group (n=12). Experimental animals were divided into 2 groups immediately before surgery: sleeve gastrectomy (SG; n=12); and innovative modified biliopancreatic diversion group (MBD; n=12). The control group was a group of animals in which no bariatric procedure was performed, but underwent abdominal opening without bariatric intervention (positive control) (n=12). After general anesthesia laparotomy was performed in all animals for 20 minutes (the time period required to perform an innovative method of BPD and sleeve gastrectomy for each individual). Weight loss was monitored in all individuals during the 28-day postoperative period.

Procedures

Innovative method of BPD that is being tested involves placing a ligature of non-resorptive material on the stomach just above the pylorus, which would prevent the passage of food, and creating a gastro-ileo anastomosis and juncture of the distal jejunum with ileum by Roux-vortex type. In this way, the duodenum and jejunum are excluded from the food flow, and bile and pancreatic enzymes have less time to break down food, creating only two anastomoses that very rarely dehiscate. Sleeve gastrectomy was performed in the standard way by resecting 2/3 of the stomach along a large curve in order to form a tube from the stomach that connects the esophagus and stomach.

Statistical analysis

Statistical analysis was performed using the SPSS 16.0 software. The Shapiro–Wilk test tested the distribution of variables. The values are presented as the mean ±standard deviation (SD) or median and interquartile range. One-way analysis of variance (ANOVA) was used to evaluate the changes in the weight. The differences between the groups were analysed with ANOVA followed by the Tuckey posthoc test for the variables with normal distribution or with the Kruskal Wallis test followed by the Mann-Whitney test for the variables with the non-normal distributions. P-value <0.05 was considered statistically significant.

RESULTS

The body weight of the control group [290.0 gm (241.25-322.0)] was significantly higher than the body weight of the MBD group [227.5 gm (198.25-247.0); p=0.007], and significantly higher than the body weight of the SG group (226.0 gm (186.75-253.75); p=0.008] 18 days after surgery. The body weight of the control group (286.66±47.91 gm) was significantly higher than the body weight of the MBD group (208.33±29.25 gm; p=0.001), and significantly higher than the body weight of the SG group (205.0±31.58 gm; p<0.001) 28 days after surgery. Body weight did not differ significantly between MBD and SG groups either preoperatively or 9, 18 and 28 days postoperatively (Table 1).

Table I **Preoperative and postoperative body weight in animals in modified biliopancreatic diversion, sleeve gastrectomy and control group.**

Weight (gm)	MBD Group (n=12)	SG Group (n=12)	Control group (n=12)	p
Preoperative	299.0 (259.0-322.75)	301.5 (256.25-342.0)	288.5 (247.0-327,0)	0,607
Postoperative 9 days	248.5 (218,5-270.25)	257.0 (312.5-287.0)	281.0 (233.35-317.75)	0.177
Postoperative 18 days	227.5 (198.25-247.0)	226.0 (186.75-253.75)	290.0 (241.25-322.0) **,¶	0.008
Postoperative 28 days	208.33±29.25	205.0±31.58	286.66±47.91**,¶	<0.001

***Significant difference between control group and MBD group; ¶-Significant difference between control group and SG group; MBD group - animals that have undergone bariatric treatment of modified biliopancreatic diversion; SG group - animals that underwent bariatric sleeve gastrectomy.*

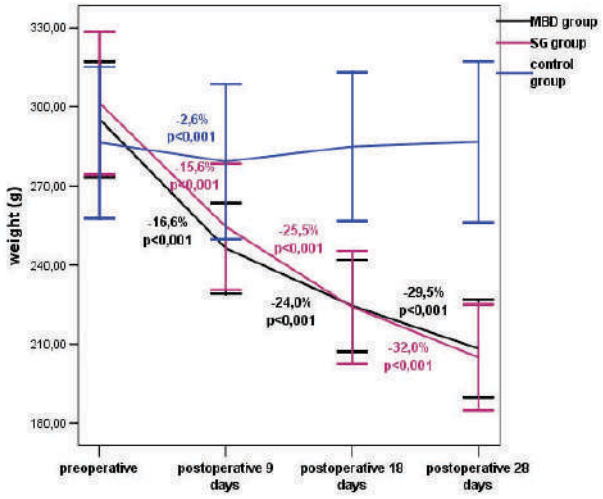

Figure I **Weight changes in animals subjected to modified biliopancreatic diversion and sleeve gastrectomy 28 days after bariatric surgery**.

In the group of animals that underwent modified biliopancreatic diversion bariatric surgery, body weight before surgery was 295.25±34.35 gm, and on the 9th day after surgery decreased significantly by 16.6% when body weight was 246.33±27.06 gm (p<0.001). Body weight continued to fall, and on the 18th day postoperatively it was 224.58±27.42 gm, which was a significant loss by 24.0% (p<0.001). Continued weight loss was observed 28 days after surgery when it was 208.33±29.25 gm, which was a significant loss of 29.5% (p <0.001) (Figure 1).

In the group of animals that underwent bariatric sleeve gastrectomy, body weight before the procedure was 301.33±42.50 gm, and decreased significantly by 15.6% 9 days after the procedure when body weight was 254.50±7,64 gm (p<0.001). Body weight continued to fall, and on day 18 postoperatively it was 224.08±33.57 gm, which was a significant loss of 25.5% (p<0.001). Continued weight loss was observed 28 days after surgery when it was 205.0±31.58 gm, which was a significant loss of 32.0% (p<0.001) (Figure 1).

In the control group, body weight before the procedure was 286.41±45.25 gm, and 9 days after the procedure decreased significantly by 2.6%. However, on the 18th day post operative body weight increased to 284.91±44.42 gm, and on the 28th day after the procedure, it continued to grow slightly to 286.66±47.91 gm, so that in the last two measurements it didn't significantly differ compared to body weight before the procedure (Figure 1).

DISCUSSION

In our study, we had a significant reduction in body weight on the 18th postoperative day (p <0.008) and on the 28th postoperative day (p <0.001) compared to the control group of animals. Stefater, et al. (6) had similar results in their research, which showed that SG leads to a significant reduction in food intake during the first 3 weeks, which was reflected in a significant reduction in body weight compared to the control group of animals.

The SG study by Wilson-Perez, et al. (7) also showed a significant reduction in body weight and reduction in caloric intake over a 4-week period postoperatively compared to the control group, as we had in our study where there was a significant reduction body weight of 32% (p <0.001) after 4 weeks.

Mukorako, et al. (8) examined the effects of BPD on body weight and showed significantly lower body weight at week 8 postoperatively compared to the control group of rats and compared to SG-treated rats. In our study with MBD after 4 weeks we had a significant reduction in body weight of 29.5% (p <0.001) compared to the control group, while we did not have a significant reduction in body weight compared to SG (32%) over a period of 4 weeks.

Baraboi, et al. (9) examined the effects of BPD and SG on body weight over 9 weeks. During the first two weeks postoperatively, the BPD and SG methods showed the same effects in weight reduction compared to the control group of rats. While at the end of the study, total body weight loss was significantly lower in BPD-treated rats compared to the control group. In our study, there was a significant weight loss in both MBD and SG (p <0.001) compared to the control group, but there was no significant weight loss between MBD and SG groups.

With this experimental study we can conclude that the sleeve gastrectomy resulted in a 32% reduction in body weight over a 28-day period, whereas the innovative modified BPD led to a weight loss of 29.6% over a period of 28 days. Control group of experimental animals did not have a significant weight loss after 28 days. Sleeve gastrectomy as a standard method and the innovative MBD method

have shown similar results on weight reduction, and based on this we can conclude that the innovative modified BPD can be used as an operative procedure for weight loss. The analysis of our research indicates that sleeve gastrectomy and innovative method of MBD lead to a decrease in body weight compared to the control group, but given the results known so far from the literature we can assume that MBD compared to sleeve gastrectomy will maintain weight loss for longer time compared to sleeve gastrectomy.

Innovative MBD as a method could be introduced in the treatment of obesity and diabetes in humans, and its introduction in the treatment of obesity would reduce the number of postoperative complications and recurrence of obesity in humans that otherwise occur after existing bariatric surgical procedures. This method could use a laparoscopic approach due to its easy performance and thus would enable fast and efficient recovery of patients.

CONCLUSION

Sleeve gastrectomy as a standard method and the innovative MBD method have shown similar results on weight reduction suggesting that the innovative modified BPD can be used as an operative procedure for long term weight loss maintenance.

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Imunoserological and clinical parameters in the diagnosis of poststreptococcal focalosis

Imunološki i klinički parametri u dijagnostici poststreptokoknih fokaloza

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ABSTRACT

Introduction: focalosis is the name used when pathogenic germs and toxins spread from a chronic, latent, infected focus into the blood, less often into the lymph, and cause the appearance of a disease on another organ. The most common diseases from a focus are: rheumatic inflammation of the joints, inflammation of the heart valves, kidneys, optic nerve, etc. In tonsillitis, focalosis is also possible, which means the retention of bacteria or its toxins in the tonsils, and causing diseases in distant organs (rheumatic fever, dermatitis, monoarthritis, alopecia areata). The affect on distant organs is possible by the action of toxins or through the development of a autoimmune disease. The serum of patients with acute rheumatic fever contains high values of antibody titers against streptolysin O, while pyogenic streptococcus is usually no longer present in the pharyngeal swab. Materials and methods: the study included subjects, patients treated at Clinic of Heart, Blood Vessel and Rheumatic Diseases Diagnostics and Polyclinic ambulance (DIP) of the Clinical Center and the University of Sarajevo. The conducted research is a prospective-retrospective, targeted study, in a six-month period. In all patients, the frequency of positive isolates of Streptococcus pyogenes in throat swabs, anamnestic data, laboratory-biochemical parameters, immunoserological parameters, clinical parameters were monitored. The study included 40 subjects, patients with positive finding of antistreptolysin antibodies O. Aim: to diagnose and monitor poststreptococcal focaloses based on the analysis of immunoserological and clinical parameters; confirm earlier streptococcal infection by the detection of antistreptolysin antibodies; determine the importance of ASTO test in the diagnosis and monitoring of post-streptococcal focaloses; analyse the prevalence of positive isolates of Streptococcus pyogenes in the development of focaloses; analyse the details of the medical history of subjects, analyse biochemical laboratory parameters of subjects, analyse immunoserological parameters of subjects, analyse clinical parameters of subjects, analyse the therapeutic protocol. Results: the average age of the respondents in the sample was 39.95 +/- 14.2 years with a median of 37 years, where the youngest respondent was 17 and the oldest was 70. Among the determined diagnoses, in addition to Focalosis, the most dominant diagnosis was Status post streptococcosis in 20 (50,0%) patients, followed by Arthritis reactiva in 16 (40%) patients, and Erythema nodosum with 3 (7.5%) cases. As noted, all patients had elevated ASO values compared to the references, and among other laboratory parameters as indicators with pathological values stand out fibrinogen (87.5%), globulins (80,0%), sedimentation (75.0%), and albumins (42.5%). Statistical analysis by chi-square test showed a statistically significant deviation from the expected prevalence of pathological values (x2=15.201; p=0.0001). Conclusion: this study shows that immunoserological and clinical parameters have diagnostic significance in streptococcal focalosis; reactive arthritis is still a challenge today, precisely because it occurs a few weeks after the primary infection and may remain unrecognized; the development of new diagnostic methods (especially molecular diagnostics) and new therapeutic procedures and understanding of immunogenetics, and the application of new generations of drugs, opens a new chapter in the effective treatment of reactive arthritis.

Keywords: poststreptococcal focalosis, Streptococcus pyogenes, immunological and clinical parameters

SAŽETAK

Uvod: fokaloza je naziv koji se upotrebljava kada se iz nekog hroničnog, latentnog, inficiranog žarišta patogene klice i toksini rasipaju u krv, rjeđe u limfu, i na nekom drugom organu uzrokuju pojavu bolesti. Najčešće bolesti od nekog žarišta su: reumatska upala zglobova, upala srčanih zalistaka, bubrega, vidnog živca i dr. Pri bolesti tonzila moguće su i fokaloze, pri čemu se podrazumijeva zadržavanje bakterija ili njegovih toksina u tonzilama, te uzrokovanje bolesti u udaljenim organima (reumatska groznica, dermatitis, monoartritis, alopecija areata). Djelovanje na udaljene organe moguće je djelovanjem toksina ili putem razvoja autoimune bolesti. U serumu bolesnika sa akutnom reumatskom groznicom nalaze se visoke vrijednosti titra antitijela protiv streptolizina O, dok se u brisu ždrijela obično više ne nalazi piogeni streptokok. Materijali i metode: u studiju su uključeni ispitanici, pacijenti Klinike za bolesti srca, krvnih žila i reumatizma Kliničkog centra Univerziteta u Sarajevu i ambulante DIP-a. Sprovedeno istraživanje je prospektivno-retrospektivna, ciljana studija, u šestomjesečnom periodu. Kod svih pacijenata pratila se učestalost pozitivnih izolata Streptococcus pyogenes u brisu grla, anamnestički podaci, laboratorijsko-biohemijski parametri, imunološki parametri, klinički parametri. Istraživanjem je obuhvaćeno 40 ispitanika, pacijenti sa pozitivnim nalazom antistreptolizinskih antitijela O. Cilj: dijagnosticirati i pratiti poststreptokokne fokaloze na osnovu analize imunoseroloških i kliničkih parametara; potvrditi raniju

streptokoknu infekciju detekcijom antistreptolizinskih antitijela; utvrditi značaj ASTO testa u dijagnostici i praćenju poststreptokoknih fokaloza; analizirati zastupljenost pozitivnih izolata Streptococcus pyogenes u nastanku fokaloza, analizirati anamnestičke podatke ispitanika, analizirati laboratorijsko-biohemijske parametre ispitanika, analizirati imunoserološke parametre ispitanika, analizirati kliničke parametre ispitanika, analizirati terapijski protokol. Rezultati: prosječna dob ispitanika u uzorku je iznosila 39,95+/-14,2 godine uz medijanu od 37 godina, te najmlađeg ispitanika u dobi od 17 godina i najstarijeg u dobi od 70 godina. Među utvrđenim dijagnozama pored Focalosis, najdominantnija dijagnoza je Status post streptococcosis kod 20 (50,0%) pacijenata, zatim slijedi Arthritis reactiva kod 16 (40%) pacijenata, a na trećem mjestu po učestalosti Erythema nodosum sa 3 (7,5%) slučaja. Kao što je navedeno, svi pacijenti su imali povišene vrijednosti ASTO u odnosu na referentne, a među ostalim laboratorijskim parametrima kao indikatorima se izdvajaju sa patološkim vrijednostima fibrinogen (87,5%), globulini (80,0%), sedimentacija (75,0%), te albumini (42,5%). Statistička analiza putem hi-kvadrat testa pokazuje statistički signifikantno odstupanje od očekivane zastupljenosti patoloških vrijednosti (x2=15,201; p=0,0001). Zaključak: ova studija pokazuje da imunološki i klinički parametri imaju dijagnostički značaj kod streptokoknih fokaloza; reaktivni artritis i danas je izazov, upravo stoga jer se javlja nekoliko sedmica nakon primarnog infekta i može ostati neprepoznat; razvojem novih dijagnostičkih metoda (posebice molekularne dijagnostike) i novih terapijskih postupaka i razumijevanja imunogenetike, te primjenom novih generacija lijekova, otvara se novo poglavlje u učinkovitom liječenju reaktivnog artritisa.

Ključne riječi: poststreptokokne fokaloze, Streptococcus pyogenes, imunološki i klinički parametri

INTRODUCTION

Focal disease is the name used when pathogenic germs and toxins are spread from a chronic, latent, infected focus into the blood, less often into the lymph, and cause the appearance of disease on another organ. The most common diseases from a hot spot are: rheumatic inflammation of the joints, inflammation of the heart valves, kidneys, optic nerve, etc. Streptococcal diseases of the upper respiratory tract have the greatest social and medical significance, because they often occur and can lead to permanent disability. In developing countries, acute rheumatic fever is a major health problem, as it is the leading cause of heart disease in young people. Penicillin is a drug of choice in the treatment of group A streptococcal infections. If a patient is allergic to penicillin, the drug of choice is erythromycin. Antimicrobial agents have no effect on pre-existing glomerulonephritis and rheumatic fever. Antimicrobial drugs are also very effective in preventing reinfection with group A beta-hemolytic streptococci in rheumatic individuals (1,2).

In tonsillitis, focal infections are also possible, which means that there is retention of bacteria or its toxins in the tonsils, causing diseases in distant organs (rheumatic fever, dermatitis, monoarthritis, alopecia areata). The effect on distant organs is possible by the action of toxins or through the development of autoimmune disease. The serum of patients with acute rheumatic fever has high levels of antibody titers against streptolysin O, while the pharyngeal swabs usually no longer contains pyogenic streptococcus. Most pathological changes in patients with acute rheumatic fever are in the heart, then in the joints and skin, and sometimes in the central nervous system (chorea minor) (3,4).

Streptococcus beta-haemolyticus group A (Streptococcus pyogenes) is a typical representative of aggressive microorganisms. It is capable of combining the action of cellular and extracellular products to cause numerous diseases with different localization, which can be divided into three basic groups: invasive, inflammatory-purulent; toxemic, local infections with signs of general intoxication and poststreptococcal sequelae, complications of the previous two forms of streptococcal infections in predisposed individuals. Depending on endogenous and exogenous factors, sequelae manifest in two clinical forms: rheumatic fever and glomerulonephritis (5,6,7).

AIM

The aim of the study is to diagnose and monitor poststreptococcal focaloses based on the analysis of immunoserological and clinical parameters; confirm earlier streptococcal infection by the detection of antistreptolysin antibodies; determine the importance of ASTO test in the diagnosis and monitoring of post-streptococcal focaloses; analyse the prevalence of positive isolates of Streptococcus pyogenes in the development of focaloses; analyse the details of the medical history of subjects, biochemical laboratory parameters of subjects, immunoserological parameters of subjects, clinical parameters of subjects and therapeutic protocol.

MATERIALS AND METHODS

The study included patients from Clinic of Heart, Blood Vessel and Rheumatism and Diagnostics and Polyclinic Ambulance (DIP) of the Clinical Center University of Sarajevo. It was a prospective-retrospective, targeted study, conducted over the period of six month. The frequency of positive isolates of Streptococcus pyogenes in throat swabs, anamnestic data, laboratory-biochemical parameters, immunoserological parameters, clinical parameters were monitored in all patients. The study included 40 subjects, patients with a positive test of antistreptolysin antibodies O. For all patients the same anamnestic data were processed: body temperature, loss of appetite, weight loss, weakness and fatigue, joint pain, sweating, headache, nosebleeds, abdominal pain, vomiting, severe pain and limited mobility.

The clinical part of the study was followed by auscultation of the heart and lungs, ECG, examination of joint pain and mobility, neurological examination and skin examination. The value of laboratory-biochemical parameters was monitored for all patients: sedimentation (SE), C-reactive protein (CRP), differential blood count (DKS), fibrinogen, alpha-2 globulin, proteinogram, aspartate aminotransferase (AST), alanine aminotransferase (ALT)), creatine kinase (CK), CK-MB, lactate dehydrogenase (LDH), urea, creatinine. Microbiological analysis was performed by standard microbiological methods of cultivation and identification, throat swab was processed by standard microbiological methods in order to isolate and identify Streptococcus pyogenes. ASO immunoserological tests were performed in each patient.

RESULTS

The study included a total of 40 patients with positive ASO values, of which 8 (20.0%) were male and 32 (80.0%) female (Table 1).

Table 1 **Presentation of the subjects gender structure in the total sample.**

Gender		
	N	%
Male	8	20.0
Female	32	80.0
Total	40	100.0

Table 2 **Age of subjects in the total sample.**

Age	
Average	39.95
SEM	2.242
Median	37.00
SD	14.182
Minimum	17
Maximum	70

The average age of patients in the sample was 39.95 +/- 14.2 years with a median of 37 years, with the youngest patient being 17 and the oldest one 70.

Table 3 **Presentation of diagnoses in the total sample.**

Dg		
	N	%
Arthritis juvenilis idiopathica in obs.	1	2.5
Exanthema papulosum	1	2.5
Exanthema maculopapulosum	1	2.5
Hypothyreosis	1	2.5
M. Bechterew	1	2.5
Prolapsus valvule mitralis	1	2.5
St. post tonsilectomiam	1	2.5
St. post febris rheumatica	1	2.5
Vituim aortale comp	1	2.5
Tonsilitis chr.	2	5
Erythema nodosum	3	7.5
Cariesdentalis	5	12.5
Arthritis reactiva	16	40
St. post streptococosis	20	50
Total	40	100.0

Among the determined diagnoses, in addition to Focalosis, the most dominant diagnosis was Status post streptococcosis in 20 (50.0%) patients, followed by Arthritis in 16 (40.0%) patients, and Erythema nodosum in 3 (7.5%) patients.

Table 4 **Presentation of laboratory parameters in the total sample.**

Pathological values of laboratory parameters		
	N	%
ASO	40	100.0
SE	30	75.0
CRP	10	25.0
LE	10	25.0
ER	4	10.0
HGB	14	35.0
HCT	17	42.5
MCV	13	32.5
TR	12	30.0
Urea	1	2.5
Kreatinin	3	7.5
Fibrinogen	35	87.5
Alfa 2 globulin	9	22.5
AST	5	12.5
ALT	4	10.0
CK	6	15.0
CKMB	1	2.5
LDH	15	37.5
Total proteins	11	27.5
Albumins	25	62.5
Globulins	36	80.0
C3	1	2.5
C4	4	10.0

As stated, all patients had elevated ASO values compared to the reference, and among other laboratory parameters, fibrinogen (87.5%), globulins (80.0%), sedimentation (75.0%), and albumins (42.5%) stand out as indicators. Statistical analysis by Chi-square test showed a statistically significant deviation from the expected representation of pathological values ($x2=15.201$; $p=0.0001$).

DISCUSSION

Streptococcal infections are one of the most important bacterial infections in humans. The dominant place of a Streptococcus pyogenes infection is the pharynx. With the development of immunology and the wider application of new immunological tests, the influence of focal focus in the organism, is being actualized again. Arthritis / arthralgia caused by a previous or still active latent infection is now included in the group of "reactive arthritis" (ReA). Ahvonen et al. defined in 1969, ReA as a "acute, sterile synovitis associated with localized infection anywhere in the body" (8). Since the infectious focus cannot always be determined, the additional term "undefined seronegative arthritis" has been introduced (9). In recent years, the range of incriminated causative agents has been expanding, and today more than 20 are known, including streptococcs (9). Reactive

arthritis is defined as aseptic inflammatory arthritis, which occurs as an immune-mediated response to infection elsewhere in the body. It usually occurs after urogenital (post-venous form) or intestinal infection (postenteric form), and is caused by arthritic bacteria (4,10). In the etiopathogenesis of ReA the role of antigen modulation, interaction with host immunogenic features, the hypothesis of "molecular mimicry" - specific immune tolerance and "cross reactivity" are just some of the theories that explain the etiopathogenesis of ReA (11). There are three main aspects in the pathogenesis of ReA: the presence of the bacterium or part of it in the joint, the bacterial-host interaction, and the local autoimmune-mediated response against the microorganism (11). A significant proportion of patients with focalosis are likely to be encountered in dental practice. The results of research by domestic authors warn that the immune response to granulomas (focus) has a complex nature (12,13,14). Granuloma induces a humoral and cellular response to antigens of infectious and other factors that exist during granuloma development (13). In some patients, circulating immunocomplexes are increased, which are deposited in the synovium of the joints and cause ReA (8,9,15).

Although today, in developed countries, most streptococcal infections are milder in nature and prognostically favorable, in recent years, severe invasive forms have become increasingly common (16). With widely available oral antimicrobial drugs, strains resistant to various antimicrobial agents are increasing in parallel. Resistance to macrolide antibiotics is on the rise (17). Within the significant genotypic heterogeneity of S. pyogenes, the tendency of macrolide-resistant strains to cause more severe clinical pictures and invasive forms of diseases is increasingly observed.

CONCLUSION

This study shows that immunoserological and clinical parameters are of diagnostic importance in streptococcal focal diseases. Reactive arthritis is still a challenge today, precisely because it occurs a few weeks after the primary infection and may remain unrecognized. The development of new diagnostic methods (especially molecular diagnostics) and new therapeutic procedures and understanding of immunogenetics, and the application of new generations of drugs, opens a new chapter in the effective treatment of reactive arthritis. All patients had elevated ASO values compared to the reference ones, and among other laboratory parameters, fibrinogen (87.5%), globulins (80.0%), sedimentation (75.0%), and albumins (42.5%) stand out as indicators.

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The significance of severe chest injuries in emergency treatment of polytraumatized patients

Značaj teških povreda grudnog koša u urgentnom zbrinjavanju politraumatiziranih pacijenata

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ABSTRACT

Introduction: early treatment of polytraumatized patients is great challenge for emergency doctors especially in cases with severe chest injuries. Those injuries often lead to acute respiratory symptoms and possibility of fast death. Aim: to show the prevalence of severe life-threatening chest injuries among the polytraumatized patients and point their significance in expected mortality and to find the endangered group of people for this kind of injuries. Materials and methods: this retrospective study included 146 polytraumatised patients with chest injuries AIS ≥3 admitted to Clinic for Emergency Medicine of the Clinical Center University of Sarajevo during the 18-month period (01.07.2019 - 31.12.2020). Descriptive analyses, Chi-square, Fischer and One-way ANOVA tests were performed. Results: sociodemographic analysis showed that mean age of observed patients was 49.75±20.18. There were 84.93% men and 15.07% women. Mean ISS score of observed polytraumas was 35.73±13.14 and there was no statically significant difference between age groups. Tube thoracostomy was performed to 50.68% of all observed patients. Analysis of age groups showed statistically significant difference between groups according to number of thoracostomies compared with number of all patients in each group. Patients of age 26-45 had highest prevalence of chest tube insertion (70.97%) and patients of age >65 had lowest prevalence (25.00%). Results: although expected polytrauma mortality according to ISS-score did not statistically differ between the groups there was a difference in prevalence of life-threatening chest injuries indicating emergency thoracostomy. Conclusion: chest injuries have to be specially observed during the emergency treatment of polytraumatised patients even before the final diagnostic procedures ISS scoring. ISS scoring is not appropriate for expressing the real severity and potential consequences of chest injuries. Male sex aged 25-46 could be considered as risk factors for thoracostomy, so we can conclude that males of this age are endangered category of people for getting severe respiratory compromising chest injuries, although further researches are needed.

Keywords : polytrauma, chest injury, expected mortality

SAŽETAK

Uvod: rani tretman politraumatiziranih pacijenata predstavlja veliki izazov za ljekare urgentne medicine posebno ako se radi o slučajevima sa teškim povredama grudnog koša koje često uzrokuju respiratorne simptome i mogućnost ranog smrtnog ishoda. Cilj studije je prikazati zastupljenost teških životno ugrožavajućih povreda grudnog koša među politraumatiziranim pacijentima i pokazati njihov doprinos očekivanom mortalitetu kao i pronaći ugrožene grupe za zadobivanje ovih vrsta povreda. Materijali i metode: retrospektivna studija je uključila 146 politraumatiziranih pacijenata sa povredama grudnog koša AIS-vrijednosti ≥3 koji su zbrinuti na Klinici urgentne medicine Kliničkog Centra Univerziteta u Sarajevu u 18-mjesečnom periodu (01.07.2019-31.12.2020). Prilikom statističke obrade podataka korištene su deskriptivne analize, Chi square, Fischer i One-way ANOVA testovi. Rezultati: sociodemografska analiza je pokazala da je srednja dob pacijenata bila 49.75±20.18, 84.93% muškaraca i 15.07% žena. Srednja vrijednost ISS skora obrađenih politrauma je bio 35.73±13.14 i nije bilo statistički signifikantne razlike među dobnim skupinama. Torakalnoj drenaži je bilo podvrgnuto 50.68% pacijenata. Analiza dobnih skupina pokazala je statistički signifikantnu razliku među grupama u odnosu na udio dreniranih pacijenata u svakoj od njih. Pacijenti dobi 26-45 godina su imali najveću prevalencu pacijenata sa torakalnom drenažom, 70.97%. Pacijenti starosti >65 su imali najnižu prevalencu, 25.00%. Rezultati studije su pokazali da iako nije bilo statistički značajne razlike između grupa prema očekivanom mortalitetu procijenjenom na osnovu ISS skora razlikovale su se prema udjelu životno-ugrožavajućih povreda grudnog koša koje su zahtijevale hitnu torakodrenažu. Zaključak: potrebno je posebno obratiti pažnju na povrede grudnog koša prilikom urgentnog tretmana politraumatiziranih pacijenata još prije finaliziranja dijagnostičkih procedura i konačnog izračuna ISS skora, budući da ISS kategorizacija nije prikladna za procjenu stvarne težine i mogućih posljedica ovakvih povreda. Osobe muškog spola starosti 25-46 godina se mogu smatrati ugroženom skupinom za zadobivanje ovakvih povreda, mada su potrebna dodatna istraživanja.

Ključne riječi: politrauma, povreda grudnog koša, očekivani mortalitet

INTRODUCTION

Polytraumatized patients belong to the most serious cases for emergency physicians specially if suffer from severe chest injuries. Final outcome of these patients largely depends on quality of initial assessment and early care provided in (pre)hospital emergency units. Chest injuries are especially interesting because of consequent respiratory symptoms that often lead to immediate life threatening conditions.

According to New Berlin Definition polytrauma is injury of two or more different body regions with an AIS value ≥ 3 and disorder of one or more of five physiologic parameters (hypotension [systolic blood pressure ≤ 90 mmHg], unconsciousness [Glasgow Coma Scale score ≤ 8], acidosis [base excess ≤ -6.0], coagulopathy [partial thromboplastin time ≥ 40 s or international normalized ratio ≥ 1.4], and age [≥70 years]) (1). AIS (Abbreviated Injury Scale) and its derivation ISS (Injury Severity Score) are anatomically based scores. Except as a tool for defining polytrauma ISS score is predominantly used as injury severity and mortality predictor for which purpose it was originally created. Inclusion of physiologic parameters in polytrauma definition partially compensated predomination of anatomically related criteria. But mortality prediction is still based on assessment of anatomical damage expressed by AIS/ISS values (2,3).

This seems to be mathematically perfect system but in practice numbers lose their weight. For emergency doctor there is a difference between injuries of each body region not matter the numerical value. Severity of symptoms and possibilities of urgent active treatment are points of interest of emergency doctors specially having in mind the fact that they provide initial treatment even before diagnostic procedures and final assessment of damages. They have to care for the patients even without knowing the „numbers".

Chest injuries play important role in early management of polytraumatized patients due to severe respiratory symptoms and possibility of fast death. They are leading cause of trauma mortality during the early period of initial care (4).

AIM

The aim of the study was to show the prevalence of severe life-threatening chest injuries among the polytraumatized patients and point to their significance in expected mortality, and also to find the endangered group of people for this kind of injuries.

MATERIALS AND METHODS

This study included patients with polytrauma according to New Berlin Definition who were admitted at Clinic of Emergency Medicine of the Clinical Center University of Sarajevo during the 18-month period (01.07.2019 to 31.12.2020).

Inclusion criteria were, beside defined criteria for polytrauma, injury of chest area with AIS value ≥3 but only chest wall, lung, heart and thoracic vascular injuries. Those injures can cause respiratory disorder. Patients without chest injury or with chest injury <3 were excluded. Patients whose chest region injuries related only to thoracic spine injuries were also excluded regardless of the AIS value due to not possible respiratory repercussions.

Data collection

Patients' data were collected and analyzed retrospectively according to medical database of the Clinic. All patients with incomplete records were excluded. That referred to records of patients who died before completing diagnostic procedures. Patients whose sex and age remained unknown due to their unconsciousness combined with a lack of identification documents were also excluded.

Statistical analysis

Collected data were analyzed using SPSS for Windows. Results of descriptive analyses were presented as numbers, means, standard deviations, minimal and maximal values and percentages. Categorical variables were compared using Chi-squared test with the aim to evaluate significance of the data. Significance of data was also checked with Fischer's test because of small-number samples. P-values <0.05 were considered to be statistically significant. Continuous numerical variables were analyzed using One-way ANOVA test where p-values <0.05 were considered statistically significant.

RESULTS

The study comprised 146 patients. Sociodemographic analysis showed that mean age of the observed patients was 49.75±20.18. There were predominantly men 84.93%, women only 15.07% (Table 1).

Table1 **Demographic characteristics of patients.**

SEX		
Characteristics	Number	Percentage
Male	124	84.93%
Female	22	15.07
Total	146	100%

AGE			
Mean	St. Dev.	Min.	Max.
49.75	20.18	6	94

Mean ISS score of the observed polytraumas was 35.73±13.14 which gives information about high severity. Table 2 showed that there was no statistically significant difference in severity of polytrauma between the age groups. That was concluded based on the finding that means of ISS values of the four age groups were not statistically different from each other (p>0.05).

Table 2 **Severity of injuries expressed by ISS-score according to age.**

Age	Number	Mean (ISS-score)	St. Dev.
≤ 25	28	35.50	10.74
26-45	31	39.23	14.93
46-65	51	35.18	15.08
>65	36	33.67	9.74
Total	146	35.73	13.14

Tube thoracostomy was performed in 50.68% of all observed patients. Analysis of age groups showed various frequency of this procedure. Comparison of numbers of thoracostomies among groups was not credible indicator due to number of patients in the groups. Thus, percentages of patients who underwent this procedure in each group were compared.

Analysis of age groups showed statistically significant difference among groups according to number of thoracostomies compared to number of all patients in each group (p<0.05). Results were shown in Table 3. It is visible that patients aged 26-45 had the highest prevalence of chest tube insertion, 70.97%. On the other hand,

patients aged >65 had lowest prevalence, 25.00%. Patients under the age of 26 had 46.43% and those between 46-65 years of age had 58.82% incidence of respiratory dysfunctional chest injuries indicating the thoracic drainage.

Table 3 **Frequency of thoracostomies.**

All observed patients			
	Thoracostomy (N)	Total (N)	%
All patients	74	146	58.82
Age groups			
	Thoracostomy (N)	Total (N)	%
≤ 25	13	28	46.43
26-45	22	31	70.97
46-65	30	51	58.82
>65	9	36	25.00
N : number % : percentage			

DISCUSSION

Immediate actions in cases of respiratory dysfunction can be life-saving. There are different techniques for chest decompression like needle thoracocentesis and thoracostomy with tube insertion and drainage (5). Endotracheal intubation has various indications caused not only by chest trauma, but also with trauma of other systems which brings the patient into different conditions or positions (6). Given that all observed patients had multiple injuries of different body regions and that endotracheal intubation did not have to be indicated only due to chest injury, this medical procedure could not be considered as indicator of chest injury symptoms severity in this study. Also none of the observed patients underwent thoracocententesis so number of indications for tube thoracostomy was taken as a measure of chest injury severity.

Acute respiratory dysfunction is directly life threatening condition. It endangers the patients and not matter the severity of other injuries presents the main cause of death in first post injury period (4). About a half (50.68%) of the observed patients underwent emergency tube thoracostomy which is first step in chest injury management (7) and life-saving intervention (8). That means that almost half of the patients were in situation of possible fast dying because of chest injury despite other also life threatening injuries of other body systems.

Analysis between age groups showed significant difference in number of thoracostomies but also insignificant difference in severity of injuries expressed by ISS score values. Patients of age between 26-49 years had the highest incidence (70.97%) and those of age older than 65 years had the lowest incidence (25.00%) of thoracostomies.

ISS-score is created as a measure of injury severity and mortality predictor. Injuries with ISS 16-24 are considered severe and those with greater than 24 very severe or critical. Mortality rates increase proportionally with severity (9). Mean ISS value of observed patients was 35,73% what implicated that great majority of them had injuries qualified as critical with high expected mortality rate.

Presented results implicate that patients of age between 26-49 years were mostly endangered of dying in early period because of respiratory dysfunction caused by chest trauma. Despite of that, predicted mortality based on ISS value was not significantly higher than in other groups. That shows the importance of chest trauma in polytraumatized patients and gives us possibility of making conclusions of insufficiency of ISS categorization for describing the real injury severity of this body region.

Mortality rates of polytraumatized patients increase with age for same ISS values (10). The increase starts at age 40 and has even more than proportional raise (9,11). The elderly have highest mortality rate and are the most vulnerable. Majority of literature consider the age ≥65 as elderly and refer reasons for increased vulnerability in this age which causes higher trauma mortality rate in general, without specifying the time of death (12,13). Trauma mortality may be immediate, early (during the first few hours) and late (during days and weeks after injury). Immediate and early mortality takes about 80% of all mortality rates (14). Between observed patients there were no significant differences in ISS values according to age groups, so age group of ≥65 had highest expected mortality. But also, this was a group of significantly lowest incidence of thoracostomies (25.00%) what is one more confirmation of chest injury specificity. Acute respiratory dysfunction caused by chest trauma is one of the causes of early deaths. The results of this study categorize this group as the one with the lowest risk of dying for this reason and at the same time with the highest mortality rate. Taking this into account it can be concluded that ISS scoring system is not completely appropriate for expressing the severity of chest trauma.

Results of research showed that 25-46 years patients most frequently had injury caused respiratory dysfunction which required thoracostomy. Men were predominant among the observed patients. Accordingly it may be concluded that men aged 25-46 are endangered group for severe chest injuries and those characteristics are risk factors for thoracostomy indication.

CONCLUSION

Results of this study lead to conclusion that chest injuries have to be specially observed during the emergency treatment of polytraumatized patients even before the final diagnostic procedures and ISS scoring, as ISS-categorization is not appropriate for expressing the real severity and potential consequences of chest injuries. Male sex between the 25-46 years of age could be considered as risk factors for thoracostomy. Accordingly, males of this age are endangered category of people for getting severe respiratory compromising chest injuries. Further research is needed.

STUDY LIMITATION

This study enrolled patients admitted to Clinic of Emergency Medicine of the Clinical Center University of Sarajevo which is regional intrahospital Emergency department. Taking into account that thoracostomy is not allowed during the prehospital emergency care in Bosnia and Herzegovina, those results refer only to patients who were transported to our Department and did not include patients who died during the prehospital treatment and transport. Based on the fact that traumatic respiratory disfunction is cause of early death, it is possible that real number of patients with indication for thoracostomy was even bigger.

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Relapse of psychosis due to the COVID-19 isolation: case report

Relaps psihoze uslovljen izolacijom usljed COVID-19: prikaz slučaja

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ABSTRACT

The paper describes a case of psychotic symptoms in a patient who was in remission for 21 years, which correlates with the beginning of a coronavirus pandemic. The patient's daily routine was disrupted by the imposed restrictions of self-isolation, and by constantly exposing himself to news about the danger and mortality of the virus, he developed anxiety and fear for his health and the health and life of his family. After the realized hospitalization, the patient also recorded symptoms from the sphere of psychotic illnesses, which were originally treated with typical followed by atypical antipsychotics, which led to his stabilization. The direct and indirect effects of coronavirus on mental health have not yet been sufficiently investigated. Our research justifies further investigations of this phenomenon.

Keywords: psychosis, pandemic, COVID-19

SAŽETAK

U radu je opisan prikaz slučaja pojave psihotičnih simptoma kod pacijenta koji je 21 godinu bio u remisiji, a koji korelira sa početkom pandemije koronavirusa. Pacijentu je nametnutim restrikcijama samoizolacije narušena svakodnevna rutina, te je stalnim izlaganjem vijestima o opasnosti i smrtnosti virusa razvio anksioznost i strah za svoje zdravlje i zdravlje i život svoje obitelji. Po realiziranoj hospitalizaciji, kod pacijenta se evidentiraju i simptomi iz kruga psihotičnih oboljenja, koji se tretiraju prvo tipičnim, a zatim i atipičnim antipsihotikom, na što dolazi do stabilizacije. Direktne i indirektne posljedice koronavirusa na mentalno zdravlje još uvijek nisu dovoljno ispitane. Naše istraživanje opravdava dalja ispitivanja ovog fenomena.

Ključne riječi: psihoza, pandemija, COVID-19

INTRODUCTION

SARS-CoV-19 virus pandemic marked the year 2020, which significantly affected human health, but also completely changed the way we behave in social interactions. Social distance is characterized as the best method of preventing the spread of the virus, but it is also has consequences on human physical health, leads to reduced physical activity, less exposure to daylight, leads to changes in diet, and consequences for mental health, the appearance of fear and anxiety (1,2).

During a pandemic, there are a number of factors that can lead to an increase in psychosocial stress, which is a recognized risk factor for deteriorating mental health and exacerbation of psychotic symptoms in people already suffering from a mental illness. In the first place, the pandemic itself leads to heightened fear, general concern, anxiety for one's own life and health, but also the life and health of one's own family. One of the stressors is the suddenly imposed social isolation, which is additionally important for people suffering from mental illness, since studies have shown that these people use social networks less, and in situations of social isolation, it is harder to compensate for the lack of social contact. Also indirect effects of the pandemic are significant - job loss, unemployment, increased domestic violence rates, deteriorating physical health due to less access to health services.

Responses to fear and anxiety due to pandemic and imposed restrictive measures can reach a psychopathological level that requires psychiatric intervention (3).

AIM

The aim of this paper is to show the impact of pandemics and accompanying events on the occurrence of psychotic symptoms in patients in remission.

CASE REPORT

On 29 May 2020, a 48-year-old man reported at outpatient Psychiatric Clinic of the Clinical Center University of Sarajevo (CCUS) accompanied by his wife. The patient was highly educated and employed. He lived with his wife and two minor children in a small town where he used to go to work every day until the pandemic and was engaged in numerous activities working from home since the beginning of the pandemic. His medical history revealed that 21 years ago he was hospitalised in a psychiatric institution due to paranoid syndrome. The patient was subsequently discharged with the diagnosis of acute polymorphic psychotic

disorder. He stated that he had not had similar problems since then, and that after a year he had stopped taking the therapy. He felt well, finished college, got a job and started a family.

During the examination, the patient had his head down, his posture was contrite, and gaze directed towards the floor. The conversation was conducted with encouragement, so that the patient scantily answers the questions asked. He verbalized how his disturbances began two months ago, with the onset of coronavirus pandemic. He stated that he had been overly worried since then, could not sleep, and when he was able to establish sleep, he could not maintain it. He was concerned about his health and the health of his family. He was in fear of coronavirus infection, believing that the pandemic would lead to high mortality and that more than half of the planet's population would not survive. The patient stated that he believed the media was concealing information and that the situation with the pandemic was much worse than it was portrayed. He believed that it would be much worse and devastating for the majority of the population. He described how often he was anxious, tense, in a bad mood, and thinking of suicide. During the conversation, spontaneous psychotic productions were not registered. N denied the presence of auditory delusions. In the content of the opinion there was a burden of one's own condition and a nihilistic idea of general ruin. The basic mood was lowered, the affect was depressed. Instinctively voluntary dynamisms in the defect by the type of avolition, anhedonia, insomnia.

He stated that he had started to behave differently before the pandemic. We learn from his wife that N completely changed with the start of the pandemic. The wife stated that N was socially withdrawn from the beginning of the pandemic, he had stopped communicating with friends and refused to leave the house. He withdrew from his family and surroundings, watched the news reporting a large number of coronavirus deaths every day, believing that the same scenario would happen to him and his family. He told his wife that their family would fail, that they would have no food. Physical symptoms soon began to appear. N often complained of nausea, muscle aches, excessive sweating, lack of sleep.

The wife also stated that at the beginning of the pandemic, N had bought large stocks of food which he stored in their home.

CLINICAL COURSE

The patient was admitted at the Department of Emergency Psychiatry with a diagnosis of depressive disorder, the current episode severe with psychotic symptoms.

The patient underwent a comprehensive diagnostic assessment using both clinical interview and validated research instruments, and the anamnestic and heteroanamnestic data we received from the patient and follow-up were taken into account. CGI S score at the admission was 5/6. BDI score indicated moderate depression. BPRS indicated moderately severe symptoms of somatic concern, anxiety and moderate suspiciousness and blunted affect.

Patient is treated pharmacologically with a typical antipsychotic and benzodiazepines to relieve anxiety. Upon admission, a PCR test for SARS-CoV-2 was performed, which was negative.

Two days later, the patient showed signs of extrapyramidal symptoms, and the typical antipsychotic was excluded from the therapy, and quetiapine was included, increasing the over time.

During the stay on the ward, the patient was withdrawn, isolated from other patients, communicating poorly and lacking any contacts. He spends most of his time lying in his bed. He was not interested in establishing contact with his family.

After two weeks of hospitalization, the clinical picture of the patient was dominated by depressive ideas of guilt and ruin, which were paranoid and delusive, and in which the patient was highly affectively invested. The patients become even more anxious, stating that he could sleep at night, being afraid of somebody doing him harm. He also mentioned, for the first time, that he read a book about the Spanish flu at the beginning of the pandemic, and how he had since lived in the belief that a similar fate awaited the world during the coronavirus pandemic.

Since there was no improvement in the patient's clinical picture, and given that the previous therapy did not give the required effect, quetiapine was excluded from the therapy, and olanzapine was included, which dose gradually increased to 20 mg per day, and also escitalopram to relieve depressive symptoms.

The patient also received cognitive behavioural therapy (CBT). The CBT targeted social withdrawal, including psychoeducation, cognitive restructuring, goal setting, and coping strategies.

Two weeks after the prescribed therapy the patient entered the phase of stable remission. Prior to the patient's discharge, no present delusions or perceptual deceptions were registered. Depressive ideas related to one's own condition, in which the patient was less affectively invested, were still present in the content of the opinion. The basic mood was in improvement, instinctively willing dynamisms were regulated. The patient established and maintained sleep, was interested in leaving the hospital, planed his time after leaving the hospital. At the demission CGI S score was 1/2. BDI score indicated minimal depression. Previously recorded symptoms on BPRS were mild at the time of admission.

DISCUSSION

Literature was searched using the keywords 'psychosis' and 'pandemic' or 'COVID-19', finding a total of seven case reports with 12 cases.

The first paper (4) describes a series of cases - they describe three previously healthy women and three previously healthy men who developed symptoms of psychosis during the period of restrictions due to the pandemic in Italy. None of these individuals were infected with the coronavirus and their average age was 53 years. All patients had a pronounced paranoid - hallucinatory clinical picture and achieved initial remission on treatment with atypical antipsychotics.

The second publication (5) includes four cases of which two men and two women are in a hospital in Spain. In the case described, patients were on average 39 years old, with no history of previous psychiatric illness. All patients had delusions, one of the patients also had auditory hallucinations. The mean remission time was 9 days. Aripiprazole (20 to 30 mg daily), olanzapine (10 mg daily), and risperidone (1.5 mg daily) were used in the therapy.

In literature, we found four papers with one case report each. The first two case reports (6,7) described the onset of psychotic symptoms in people infected with coronavirus who had achieved rapid remission. These case reports gave the impression that patients infected with coronavirus were possibly more prone to developing delirium. The third case report (8) describes a serious clinical picture, involving a previously healthy 38-year-old woman who felt frightened after visiting a dentist who did not wear a mask during the examination. The patient subsequently developed a psychotic clinical picture and required a 14-day hospitalization.

The following case report (9) describes a 52-year-old man with a paranoid clinical picture associated with coronavirus, which resulted

in a suicide attempt and hospitalization in a psychiatric hospital. Also, the anxiety component was emphasized within the clinical picture of this man. The patient was discharged after 4 weeks of hospitalization on therapy with antipsychotics, benzodiazepines and antidepressants.

The latest case report (10) describes a previously healthy 70-year-old woman who developed hallucinatory paranoid syndrome after determining isolation measures, and who abruptly disrupted her daily life activities. The patient was treated on an outpatient basis with atypical antipsychotics, leading to remission.

Compared with the case reports found in the literature, our case report has similarities to those previously described in that our patient required hospitalization and achieved remission on atypical antipsychotic therapy. The similarities are that our patient is also middle-aged, he is part of the working population whose daily routine is disrupted by imposed measures of self-isolation.

Also, in our patient, similar to the previously described cases in the literature, the first changes in the psychological field were in the form of anxiety that followed exposure to a large amount of information about the pandemic and the number of infected and died as a result of coronavirus infection. In addition to this, N further researched the pandemic as a phenomenon, and read about the Spanish flu, known as the largest pandemic to hit Europe in the 20th century, which caused N additional anxiety and heightened fe ar.

Our case differs from other cases in the fact that N had a positive psychiatric history. He had Acute Polymorphic Psychotic Disorder 21 years ago, when he also achieved remission on treatment with atypical antipsychotics. Our case is unique as it described a patient who was in remission for 21 years, during which he was highly functional, completed higher education, started a family and worked until the pandemic.

CONCLUSION

Based on the presented cases, we can conclude that if adequate treatment is not provided in time, it is possible to have serious psychological problems and complications of a pre-existing psychiatric illness, such as suicide attempts or prolonged psychiatric hospitalization. Based on the presented cases, we can conclude which are the most common triggers of mental state worsening. First of all, it is the fear of infecting the patient or fear of infecting his family, followed by a sudden change in lifestyle due to imposed self-isolation measures, or the direct and indirect consequences of lockdown, which are economic in nature (loss of employment). Our case report suggests that further research is needed regarding direct and indirect consequences of the coronavirus pandemic on the mental health of the general population, but also of persons with a previous history of psychiatric illness.

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A huge cystic cellular intrathoracic schwannoma: a case report and literature review

Veliki cistični celularni intratorakalni švanom: prikaz slučaja i pregled literature

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ABSTRACT

Introduction: thoracic neurogenic tumors are relatively uncommon neoplasms that arise from glial cells, ganglia, paraganglia, or peripheral nerve. They account for 90% of all posterior mediastinal masses and 12-21% of all primary mediastinal tumors. Nerve sheath tumors usually appear as well-circumscribed, slow-growing tumors. Cellular schwannomas account for up to 5% of benign nerve sheath tumors in adults. They are asymptomatic in more than half of cases and are usually incidentally discovered through routine radiographic examinations. Large mediastinal cystic masses can cause difficulty in breathing and even superior vena cava syndrome. Aim: to demonstrate our experience with symptomatic thoracic cystic schwannoma, surgical treatment and follow-up of the patient. Case report: we present a case of a previously healthy 63-year-old female patient with symptomatic thoracic cystic schwannoma, which was successfully treated with surgery at our Clinic, with complete resolution of the patient's symptoms.

Keywords: cystic schwannoma, thoracic cavity, dyspnea, thoracotomy

SAŽETAK

Uvod: intratorakalni neurogeni tumori su relativno rijetke neoplazme, koje nastaju iz glijalnih stanica, ganglija, paraganglija ili vode porijeklo od perifernih živaca. Čine 90% svih masa stražnjeg medijastinuma i 12-21% svih primarnih medijastinalnih tumora. Tumori nervnih ovojnica obično se manifestiraju kao dobro ograničeni tumori koji sporo rastu. Celularni švanomi čine do 5% benignih tumora nervnih ovojnica kod odraslih. U više od polovice slučajeva, pacijenti su asimptomatski i tumori se obično slučajno otkriju rutinskim radiografskim pregledima. Velike medijastinalne cistične mase mogu uzrokovati poteškoće u disanju, pa čak i sindrom gornje šuplje vene. Cilj: demonstrirati naše iskustvo sa simptomatskim intratorakalnim cističnim švanomom, hirurškim liječenjem i postoperativnim praćenjem pacijenta. Prikaz slučaja: prezentujemo slučaj zdrave 63-godišnje pacijentice sa simptomatskim intratorakalnim cističnim švanomom, koja je uspješno hirurški izliječena na našoj Klinici, uz potpunu rezoluciju simptoma.

Ključne riječi: cistični švanom, torakalna šupljina, dispneja, torakotomija

INTRODUCTION

Intrathoracic neurogenic tumors account for approximately 12-21% of all primary mediastinal tumors (1). Nerve sheath tumors include neurofibroma, schwannoma, and peripheral nerve sheath tumors. Cellular schwannomas are relatively uncommon schwannoma variants, i.e. they make up to 5% of the benign peripheral nerve sheath tumors. These slow-growing tumors predominantly affect middle-aged adults and are located in the paravertebral region of the mediastinum and retroperitoneum (2). Most often, they do not cause any symptoms and are discovered as incidental findings on a routine chest X-ray (CXR). Symptoms are present in 35-45% of cases, and they occur mostly due to mass effects over the adjacent structures. Dry cough, shortness of breath, and vague chest pain can be initially present, but if left untreated, many of these tumors can compress mediastinal blood vessels and cause superior vena cava syndrome. Treatment is primarily based on surgery.

We present the case of a previously healthy 63-year-old female patient with symptomatic thoracic cystic schwannoma, which was successfully treated with surgery at our Clinic, with complete resolution of the patient's symptoms.

CASE REPORT

A 63-year-old female patient was admitted at Clinic of Thoracic Surgery of the Clinical Center University of Sarajevo, after being previously treated at Clinic for Pulmonary Diseases, General Hospital Tešanj. She noticed vague chest pain and progressive worsening shortness of breath that lasted for couple of months. Her past medical history was otherwise unremarkable. She had no history of tobacco, alcohol, or illicit drug use, and was not taking any medications. The patient was referred by her general practitioner (GP) for an urgent CXR. A CXR demonstrated a solitary pulmonary mass located in the left lower lobe (Figure 1).

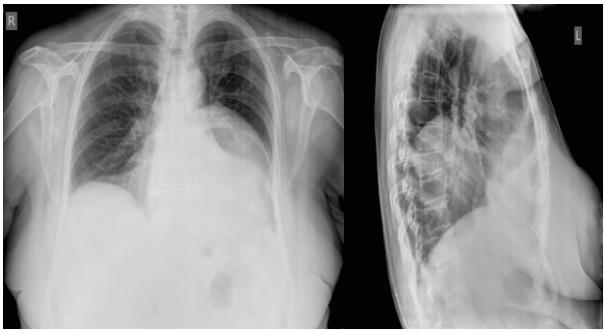

Figure 1 **Posteroanterior (PA) and lateral chest radiographs upon admission to our Clinic. A large oval, vague shadow at the level of the left lower lobe can be observed.**

The patient underwent a contrast-enhanced CT scan, which revealed a round, well-defined lesion of altered density with slightly lobulated contours, measuring 91x94x138 mm, with thickened wall and central foci of +20 Hounsfield Units (HU) - thick liquid. The tumor in the left inferior hemithorax was inseparable from the pleura, compressed the parenchyma of the left lower lobe, and pushed the diaphragm in the caudal direction. A minor pleural effusion in the left phrenicocostal sinus was observed (Figure 2).

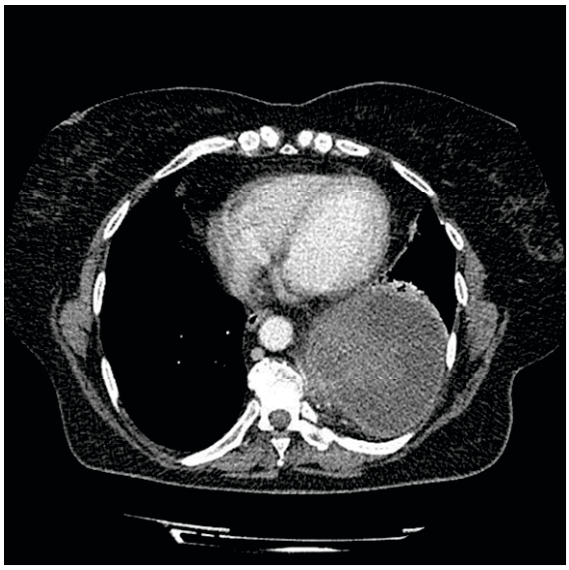

Figure 2 **Contrast-enhanced chest CT scan. A round, well-demarcated mass with thickened wall and central foci of thick liquid in the left hemithorax can be observed**.

Clinical examination was unremarkable. Flexible bronchoscopy revealed a normal finding. To rule out a pleural malignancy and to achieve a definitive diagnosis of the lesion, a histopathological assessment of tissue sample was obtained by biopsy technique through posterolateral thoracotomy. A small amount of serous fluid was found in pleural space and collected for cytological analysis and culture. A well-circumscribed cystic formation with the thickened wall measuring 90x90x140 mm in close proximity to the mediastinal pleura, chest wall, and diaphragm was detected (Figure 3).

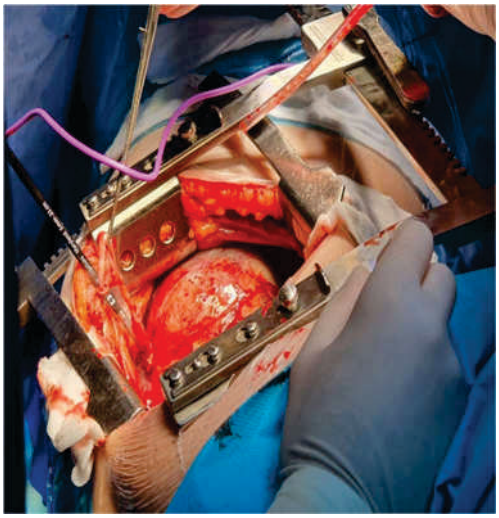

Figure 3 **A well-circumscribed cystic formation in close proximity to the mediastinal pleura, chest wall, and diaphragm was observed in the left hemithorax.**

When punctured, approximately 200 ml of thick brown content was evacuated and sent for cytological analysis. The sample of the parietal pleura was also sent for histopathological analysis. Inside the cyst, a solid tumor was identified measuring approximately 110x80x63 mm, which was removed in toto with a wall of a cyst and sent for definitive histopathological analysis (Figure 4).

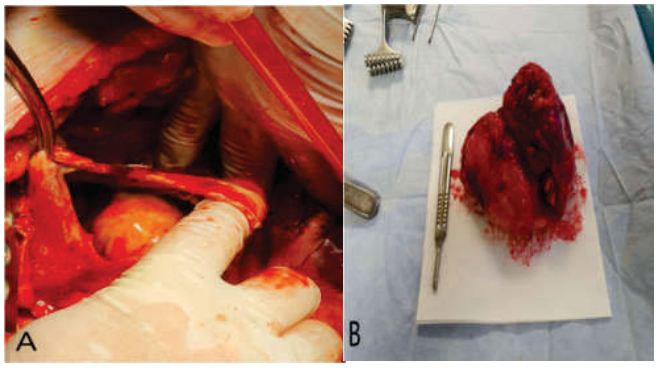

Figure 4 **Intraoperative findings. A. A solid tumor measuring 110x80x63 mm was found inside the cyst, which was removed in toto. B. Gross examination of the excised specimen showing a 91x94x138 mm encapsulated tumor**.

Histopathological examination of the tumor showed the characteristic pattern of cystic cellular schwannoma: relatively clearly demarcated fragments of tumor tissue comprised of spindle cells, with elongated nuclei, moderate pleomorphism, arranged in folds, strips, and in some places storiform. Tumor cells did not show pronounced mitotic activity, mitotic count was up to one per 10 high-power fields (hpf). In the central part of the tumor, there was a larger cavity lined with fibrin and siderophages, whereas multiplied granulation tissue with numerous small blood vessels was found at the tumor edge. The tumor was mostly hypercellular, with fewer cellular and myxoid-altered areas in which a lot of histiocytes were found. Immunohistochemical tumor cells were S-100 (+), Desmin (-), CK (-), CD34 (-), STAT6 (-), SOX10 (+), Ki67 (focal 5-7%).

The early postoperative course was uneventful, and the after 12 days the patient was discharged from the Clinic. She was symptom-free after a follow-up of four months, with unremarkable CXR (Figure 5).

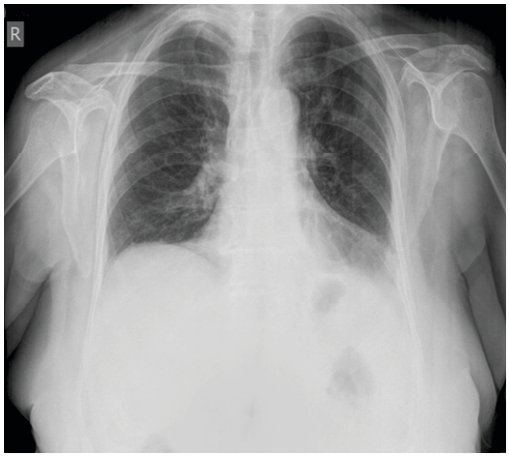

Figure 5 **A follow-up chest radiograph performed four months after surgery reveals a normal finding.**

DISCUSSION

Schwannomas and neurofibromas are the most common intrathoracic neurogenic tumors. Cellular schwannoma, which accounts for up to 5% of benign nerve sheath tumors, was first described by Woodruff et al. in 1981 (3). This uncommon variant of benign schwannoma is more commonly found in women, with a median age of 55 years, and is mostly located in the mediastinum or retroperitoneum (4). In most cases, these tumors occur in the posterior mediastinum (90% of all posterior mediastinal masses) when they originate from the intercostal nerve, but they can also originate from the phrenic nerve, vagus nerve, and brachial plexus (5). The diagnosis of cystic schwannoma is often delayed due to the paucity of symptoms, and patients usually follow an indolent clinical course related to slow tumor growth (6). According to a retrospective study by Takeda et al. (7), which analyzed the records of 146 patients with intrathoracic neurogenic tumors, most adult patients (84% of cases) and children (60% of cases) with neurogenic tumors are asymptomatic. Our patient experienced vague chest pain and progressive worsening shortness of breath that lasted for a couple of months. Conventional CXR was initially performed, followed by a contrast-enhanced chest CT scan. Brant and Osborn (8,9) defined the general imaging features of schwannomas: (a) well-demarcated masses which displace adjacent structures without direct invasion; (b) cystic and fatty degeneration is uncommon; (c) the larger a tumor, the more likely it is to show heterogeneity (cystic degeneration or hemorrhage); (d) hemorrhage from tumor occurs in up to 5% of cases; (e) tumor calcification is uncommon. A contrast-enhanced chest CT scan of our patient revealed a well-demarcated mass, with thickened wall and central area of thick liquid, with signs of cystic degeneration, and without calcification. According to the retrospective study conducted by Pekmezci, et al. (10), microscopic features of cellular schwannoma include well-defined capsules, histiocyte-rich areas, hypercellular 'Schwannian whorls' and hyalinized thick blood vessels. Moreover, most cases of cellular schwannomas have a mitotic count of fewer than four mitoses per 10 hpf. In our patient, the mitotic count was up to one per 10 hpf. Certain immunological features are inherent to cellular schwannoma to differentiate it from malignant neoplasms, such as positive S-100 stain or low Ki-67 (value of more than 20% is highly predictive of malignant peripheral nerve sheath tumor; in our patient, the value was 5-7%). Cellular schwannoma shows a favorable prognosis with total resection appearing to be the mainstay of treatment, with no metastasis and no adjuvant therapy necessary.

CONCLUSION

Cellular schwannomas represent a rare, benign variant of intrathoracic neurogenic tumors. Due to their slow-growing nature, they are usually asymptomatic and incidentally discovered. Symptoms occur due to mass effects over the adjacent structures and range from a dry cough to superior vena cava syndrome. Complete surgical resection appears to be the curative treatment, with no metastasis and no adjuvant therapy necessary.

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Article should be no longer than 10 computer pages, including figures, graphs, tables and references. The article may be submitted as a CD disk (Word Windows), or e-mail.
Spacing: 1,5: left margin: 2,5 cm; right margin: 2,5 cm; top and bottom margin: 2,5 cm.
Graphs, tables, figures and drawings should be incorporated in the text, precisely in the text, where these will be published, regardless of the program in which they are prepared. Articles are written in-extenso in English language.
The manuscript should be submitted on a good quality CD disc, or by e-mail, together with two printed copies (if possible). Sent CD disks will not be returned to the authors.

ARTICLE CONTAINS:

TITLE OF THE ARTICLE IN ENGLISH LANGUAGE
TITLE OF THE ARTICLE IN BOSNIAN/SERBIAN/CROATIAN (B/S/C) LANGUAGE
First and last name of the author/co-author(s)

Name and address of the institution in which author/co-authors is employed (same for all authors) in B/S/C and English language as well as the address of corresponding author at the end of the article.

Summary in B/S/C language with the precise translation in English. Abstract of approximately 200-250 words should concisely describe the contents of the article.
Key words (in B/S/C and in English language): up to five words should be listed under the Abstract.

ARTICLE BODY

The main body of the article should be systematically ordered under the following headings:
- **INTRODUCTION**
- **MATERIALS AND METHODS**
- **RESULTS**
- **DISCUSSION**

- **CONCLUSION**
- **REFERENCES**

INTRODUCTION

Introduction is a concise, short part of the article, and it contains purpose of the article relating to other published articles with the same topic. It is necessary to quote the main problem, aim of investigation, and/or main hypothesis which is investigated.

MATERIALS AND METHODS

This part should contain description of original or modification of known methods. If there is a method that has previously been described, it would be sufficient to include it in the reference list. In clinical and epidemiological studies the following should be described: sample, protocol and type of clinical investigation, place and period of investigation. Main characteristics of investigation should be described (randomization, double-blind test, cross test, placebo test), standard values for tests, time framework (prospective, retrospective study), selection and number of patients – criteria for inclusion and exclusion from the study.

RESULTS

Main results of investigation and level of its statistical significance should be quoted. Results should be presented in tables, graphs, figures, and directly incorporated in the text, at the exact place, with ordinal number and concise heading. Table should have at least two columns and explanation; figures clean and contrasted, graphs clear, with visible text and explanation.

DISCUSSION

Discussion is concise and refers to own results, in comparison with the other authors' results. Citation of references should follow Vancouver rules. Discussion should be concluded by the confirmation of the stated aim or hypothesis, or by its negation.

CONCLUSION

Conclusion should be concise and should contain most important facts, which were obtained during investigation and its eventual clinical application, as well as the additional studies for the completed application. Affirmative and negative conclusions should be stated.

REFERENCES – Instructions for writing references

References should follow the format of the requirements of **Vancouver rules**.

Every statement, knowledge and idea should be confirmed by reference. Each reference in the text is given its own sentence case in Arabic number in parenthesis at the end of the sentence according to the order of entering. Every further referring to the same reference, number of the first referring in the text should be stated. References are to be placed at the end of the article, and are to be numbered by ordinal numbers in the order of entering in the text (entering reference number). Journal's title is abbreviated using Index Medicus abbreviations. The names of the first six authors of each reference item should be provided, followed by "et al.".

It is very important to properly design references according to instructions that may be downloaded from addresses National Library of Medicine Citing Medicine http://www.ncbi.nlm.nih.gov/books/bv.fcg?rid=citmed.TOC&depth=2,

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UPUTSTVA AUTORIMA

Časopis "Medicinski žurnal" objavljuje originalne naučne radove, stručne, pregledne i edukativne, prikaze slučajeva, recenzije, saopćenja, stručne obavijesti i drugo iz područja svih medicinskih disciplina. Rad *in-extenso* (cjelokupan) piše se na engleskom jeziku, uz sažetak i naslov rada koji uz engleski trebaju biti napisani i na našim jezicima (bosanski, hrvatski i srpski). Autori su odgovorni za sve navode i stavove u njihovim radovima. Ukoliko je rad pisalo više autora, potrebno je navesti tačnu adresu (uz telefonski broj i e-mail adresu) onog autora s kojim će uredništvo sarađivati pri uređenju teksta za objavljivanje.
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2. da je istraživanje odobrila Etička komisija,
3. da prihvaćeni rad postaje vlasništvo "Medicinskog žurnala".

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Radovi ne smiju biti duži od deset stranica na računaru, ubrajajući slike, grafikone, tabele i literaturu. CD zapis teksta je obavezan (Word of Windows), ili e-mail.
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.
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RAD SADRŽI:

NASLOV RADA NA ENGLESKOM JEZIKU
NASLOV RADA NA NAŠEM JEZIKU

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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.".

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