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Address of the Editorial Board: *Acta Medica Academica*, Academy of Sciences and Arts of Bosnia and Herzegovina, Bistrik 7, 71000 Sarajevo, Bosnia and Herzegovina, Tel.: 00 387 33 560 718, Fax.: 00 387 33 560 703. Contact person: Nerma Tanović, E-mail: amabih@anubih.ba

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Jovan Bijelić (1884-1964), "Towers with lodgings", 1932, oil on canvas, 700x500 mm. Courtesy of the Center for culture and education Bosanski Petrovac, BA.

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Surgical coronary revascularization on-pump versus off-pump in patients with stenosis of the main tree of the left coronary artery and carotid stenosis

Edin Kabil[1], Bedrudin Banjanović[1], Emir Mujanović[1]

[1]Health Institution Special Hospital "BH Heart Centre", Tuzla, Bosnia and Herzegovina

Correspondence:
edin.kabil@bhsrce.ba
Tel.: + 387 35 309 225

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Key words: Coronary artery bypass grafting ▪ Off-pump ▪ Left main stenosis ▪ Carotid stenosis ▪ Cerebrovascular insult.

Objective. The aim of this study was to show perioperative complications of CABG procedure with and without cardiopulmonary bypass (CPB) in patients with combined coronary and carotid disease. **Patients and methods.** This retrospective survey included patients with left main stenosis greater than 50% and carotid stenosis over 50%, who had undergone CABG without carotid endarterectomy at the BH Heart Centre, from May 2009 to May 2014. The patients were divided into two groups according to the surgical method used. Group A consisted of 50 patients who underwent surgery without CPB and the second group of 50 patients with CPB, conformed according to gender, ejection fraction values, EuroSCORE and the number of bypass grafts performed. **Results.** Analysis of the basic results indicates significant differences between the groups in the time spent on a respirator or time in the ICU, the amount of postoperative bleeding or compensated blood, as well as subsequent complications. The overall incidence of neurological complications showed a difference in the observed groups. **Conclusion.** With ever easier technical performance, complete planned revascularization and the quality of performed grafts, the conditions have been created for a comparative analysis. According to the results we can say that CABG without CPB has a number of advantages over the other method, in patients with the combined disease.

Introduction

Atherosclerosis is a process that simultaneously affects the carotid and coronary arteries. It is estimated that these two conditions can coexist within a range of 2-20%, with an average incidence of 8% (1-8). Numerous statements confirm this fact. Hedelbad and associates (8) proved that more than 50% of patients, suffering from carotid arteries stenosis, simultaneously show symptoms of ischemic or coronary heart disease (9, 10). Also, it was found that in 19% of patients, with the need for coronary revascularization (coronary artery bypass grafting – CABG), carotid arteries stenosis simultaneously exists, which is of hemodynamic significance, 60% of patients undergoing carotid endarterectomy (CEA) show signs of angina pectoris and in 18% of cases coronary angiography confirmed a significant degree of coronary disease (11, 12). Patients with a significant coronary stenosis requiring CABG, with a significant simultaneous stenosis of the carotid arteries, are a major therapeu-

tic challenge. The reason for this is primarily the incidence of stroke or cerebrovascular insult (ICV) as one of the most serious CABG complications (13). Numerous studies have shown that the risk of occurrence of ICV during CABG is in direct correlation with the degree of carotid stenosis (14). As a consequence a common strategy for the treatment of such patients was „carotid before coronary" that is, first, an operation on the carotid arteries (CEA) and only then CABG in two parts, or as a simultaneous operation (15). However, in patients with significant stenosis of the main tree of the left coronary artery, „left main stenosis" (LMS) and carotid stenosis, priority is given to CABG, primarily because of the seriousness of the location of the stenosis, the symptoms, the possibility of hemodynamic instability, which is rather frequent in those patients, as well as because of the operation itself.

The main objective of this study was to show the perioperative complications of CABG procedure in surgically treated populations, with and without cardiopulmonary bypass.

Patients and methods

The survey included patients with LMS greater than 50% and carotid artery stenosis greater than 50%, who had undergone CABG at the BH Heart Center in the town of Tuzla, Bosnia and Herzegovina, within the period from May 2009 to May 2014.

Regarding the surgical method, the patients were divided into two groups. Group A consisted of 50 patients who underwent surgery without cardiopulmonary bypass (CPB), and group B 50 patients who had CPB, conformed according to gender, ejection fraction values (group on-pump: 51.5±11.4 and group off-pump 55.2±10.1), EuroSCORE (group on-pump: 3.7±1.5, group off-pump 3.5±1.1), and the number of bypass grafts performed.

These perioperative results in both groups were analyzed and compared: a) total duration of surgery (expressed in minutes); b) time spent on a respirator (expressed in hours); c) time spent in the Intensive Care Unit (ICU) (expressed in hours); d) the amount of postoperative bleeding (expressed in milliliters); e) the amount of compensated blood (expressed in milliliters) f) basic perioperative complications and their frequency (postoperative bleeding requiring revision, atrial fibrillation, myocardial infarction, deep wound infection), which was monitored within the same hospitalization; g) basic postoperative neurological complications (cognitive deficits, transient ischeamic attack (TIA), cerebrovascular insult (ICV) which were monitored within the same hospitalization; and h) length of hospitalization (expressed in days).

Statistical analysis

Distribution of the continuous variables was expressed as the mean ± standard deviation and compared by means of the unpaired two-tailed T-test or the Fisher exact test. Categorical variables were tested by $\chi 2$. Statistical significance was considered as p value less than 0.05.

Results

In both analyzed groups there were no significant differences between either the sexes or average age values, ejection fraction and EuroSCORE. Examination of preoperative risk factors showed that hypertension, hypercholesterolemia, heredity and smoking were most common in both groups. Also, a comparative analysis of the territorial distribution of bypass grafts between the groups showed an insignificant difference in the number of grafts in a particular coronary area.

An analysis of basic perioperative results is given in Table 1.

Analysis of basic perioperative results indicates significant differences between the

groups in the time spent on a respirator, the length of time spent in intensive care, the amount of postoperative bleeding and the amount of compensated blood. The duration of surgery was insignificantly shorter in the off-pump group. Also, the total number of hospitalization days was significantly lower in the off-pump group.

An analysis of the frequency of perioperative complications, such as rhythm disorder, wound infection, bleeding reoperation and myocardial infarction, for both groups is presented in Table 2.

In both groups the most frequent perioperative complication was rhythm disorder as a type of atrial fibrillation, then prolonged bleeding requiring reoperation exclusively in the off-pump group, myocardial infarction, and wound infection was noticed in the on-pump group. Analysis of death frequency in both groups leads to similar conclusions. Mortality insignificantly lower in the off-pump group.

An analysis of the frequency of perioperative complications in terms of cognitive and motility disorders or occurrence of milder cognitive deficits, then TIA and ICV in both groups is presented in Table 3.

The analysis indicates a smaller number of milder cognitive deficites in the off-pump group, also the occurrence of a stroke, noticed only in the on-pump group. In both

Table 1 Perioperative results for both groups

Monitored variables	Group A*	Group B†	p‡
Operation (min; $\bar{x} \pm SD$)	248.0±34.2	237.6±38.2	0.155
Respirator (h; $\bar{x} \pm SD$)	12.1±6.7	9.1±4.2	0.009
Stay in ICU (h; $\bar{x} \pm SD$)	59.5±88.1	31.3±14.9	0.028
Bleeding (ml; $\bar{x} \pm SD$)	584.0±232.8	456.4±168.0	0.002
Blood compensation (ml; $\bar{x} \pm SD$)	358.8±351.7	187.4±253.9	0.006
Total hospitalization (d; $\bar{x} \pm SD$)	8.4±3.5	7.1±2.1	0.033

*Surgery performed on-pump; †Surgery performed off-pump; ‡Two-tailed unpaired student's t-test; ICU=Cardiac intensive care unit.

Table 2 Perioperative complications for both groups

Complication	Group A*	Group B†	p
Rhythm disorder‡, n (%)	17 (34.0)	15 (30.0)	0.668§
Wound infection, n (%)	2 (4.0)	0 (0.0)	0.495‖
Bleeding-reoperation, n (%)	3 (6.0)	0 (0.0)	0.242‖
Myocardial infarction, n (%)	1 (2.0)	2 (4.0)	1.000‖
Mortality, n (%)	3 (6.0)	0 (0.0)	0.242‖

*Surgery performed on-pump; †Surgery performed off-pump; ‡By type of atrial fibrillation; §χ^2 test; ‖Fisher exact test.

Table 3 Neurological complications

Neurological complications	Group A*	Group B†	p‡
Milder cognitive deficit, n (%)	4 (8.0)	3 (6.0)	1.000
TIA, n (%)	4 (8.0)	1 (2.0)	0.362
ICV, n (%)	4 (8.0)	0 (0.0)	0.117
Overall incidence of neurological complications, n (%)	12 (24.0)	4 (8.0)	0.054

*Surgery performed on-pump; †Surgery performed off-pump; ‡Fisher exact test. TIA=Transient ischeamic attack; ICV=Cerebrovascular insult.

groups the most frequent perioperative complication was transitory consciousness disturbance by type of TIA, and mild cognitive deficit. The overall incidence of neurological complications showed a difference in the observed groups in terms of the more frequent occurrence of neurological deficits in the on-pump group.

Discussion

The results indicate the wide benefit of using the off-pump method in our study and bring us to the conclusion that CABG without CPB has certain advantages over the other method, in patients with a combined disease.

Numerous studies have shown that the risk of CVI during CABG is concommitant to the degree of carotid stenosis (14). In distinction from patients without a significant carotid disease in which the incidence of ICV after CABG is only 1.9%, in patients with significant stenosis it increases significantly, so that it was 3% in patients with unilateral stenosis of 50-90%, 5% in patients with bilateral 50-90% stenosis and 7-11% in patients with carotid occlusion (16). Given the well-known fact that for the occurence of ICV during CABG the most responsible are manipulation of the changed atherosclerotic aorta during cannulation, and the clamping necessary to establish the cardiopulmonary bypass (CPB) (17, 18) a logical solution in reducing the incidence of ICV appears to be operations avoiding the use of CPB. That is why CABG without CPB, so-called „beating heart surgery“ could represent an acceptable alternative to conventional CABG with CPB, in order to reduce the incidence of adverse events in patients with significant stenosis of the carotid arteries, which, as is already well-known, have an increased incidence of ICV. However, still today in the technical performance of complex cardiosurgical surgery primacy is given to the use of CPB and data from 2006 show that in the USA only 20% operations were performed on a „beating heart“.

With the increasing ease in the technical performance of surgery, a complete planned revascularization and the quality of bypass grafts performed, the conditions have been created for a comparative analysis of the results obtained with these two operating methods, primarily in high-risk patients who, theoretically, should benefit the most from surgery without CPB.

According to the results shown by Lancey and associates (19) in a sample of 76 patients, matched in terms of preoperative status and with the same surgeon, the choice of surgical technique itself determines the postoperative recovery, primarily in the prolongation of the total duration of mechanical ventilation. This fact is even more significant when the conclusions are added of the study conducted by Natarajan and associates (20) on a group of 470 patients who underwent surgery on CPB, according to which there was a significant difference in the duration of mechanical ventilation between patients with shorter or longer use or duration of CPB itself. Our results indicate similar conclusions. Namely, this study, too, shows a signifficant difference in the time spent on a respirator, that is the time spent on mechanical ventilation was shorter in patients who underwent surgery without CPB. On the other hand, the undoubted influence of CPB was shown in the study by Rosenfeld and associates (21). In 9869 patients divided into two groups, in terms of the length of stay in the ICU, a direct connection with the length of stay on CPB was shown. Patients with a prolonged stay in ICU also experienced 11 additional complications during that stay. The results of our study show that the time spent in ICU is significantly shorter in patients who did not have CPB.

The results of our study also correspond with the majority of studies pointing to the advantage of surgery without CPB related to hospitalization duration. Namely, this study too indicates a significant difference in hos-

pitalization duration in favour of patients who did not have CPB.

Our study also indicates a significantly lower amount of postoperative bleeding in patients who did not have CPB. The amount of postoperatively compensated blood was significantly lower in patients without CPB. A clear conclusion may be drawn that the off-pump method is the best choice in patients with a higher risk of operative and postoperative bleeding and an increased tendency for bleeding. Particularly significant is the group of patients, like the one included in this study, in which it was necessary to perform two surgeries, either simultaneously or with a time interval. Analysis of the overall incidence of postoperative complications in both groups indicates there were fewer in the off-pump group, but without statistical significance. In both groups the most frequent perioperative complication was rhythm disorder in terms of the type of atrial fibrillation, then prolonged bleeding requiring reoperation, solely in the on-pump group, myocardial infarction, and wound infection, noticed exclusively in the on-pump group. Analysis of the frequency of deaths in both groups leads to similar conclusions. The mortality was insignificantly lower in the off-pump group. Following the indicated differences between the groups it was obvious that the incidence of events was lower in the group of patients operated without CPB. In addition we noticed a decreased number of neurological complications in patients operated without CPB. The greatest difference was noticed in the incidence of ICV, which was also lower in patients operated without CPB.

In our study there were no registered cases of ICV in the group of patients operated without CPB, while in the other group, 4 cases of ICV were registered. The overall incidence of cerebrovascular neurological complications wasnot statistically significant. The reason why it is not statistically significant should be primarily sought in the small sample size.

Limitation of the study

This study was performed with a limited number of patients. Patients' comorbidities, such as obesity, previous insults, arterial wall thickness and many others, could possibly have influenced the results. No scale was used for detailed quantification of cerebrovascular neurological complications. Considering the cerebrovascular neurological complications, it is also important to notice the small sample size regarding significant differences.

Conclusions

According to the results of this study we can say that CABG without CPB has certain advantages over surgery using it, in patients with diseases of the coronary and carotid vessels. This is shown by the shorter operation time and the time spent on a respirator, the shorter stay in the ICU, as well as the shorter overall hospitalization time, the smaller amount of postoperative bleeding and blood compensation. The off-pump cardiac surgical method of treatment of angiopatic diseases of coronary and carotid blood vessels proved to be probably the better treatment method. The same method undoubtedly resulted in a statistically significant shorter stay on the mechanical ventilation regime in patients operated without CPB, a shorter stay in ICU, as well as in overall hospitalization duration and in the amount of bleeding and compensation of blood and blood derivatives. It turned out that other basic perioperative results were better in the patients operated without CPB, although without a statistically significant value. Also, the presumption that the frequency of neurological complications would be lower in patients operated without CPB proved true, even this is probably not statistically valid because of the sample size.

What is already known on this topic

Patients with a significant coronary disease requiring CABG, with a significant simultaneous stenosis of the carotid arteries, are a major therapeutic challenge, mainly because of the incidence of stroke or cerebrovascular insult as one of the most serious cardac surgical complications. It is well known strategy to perform surgery „carotid before coronary" usually in two phases, or as a simultaneous operation, however, in patients with significant stenosis of the main tree of the left coronary artery (left main stenosis – LMS) priority is given to CABG.

What this study adds

This study clarifies the perioperative complications of the CABG procedure with and without cardiopulmonary bypass. The results indicate significant differences between the groups in the time spent on a respirator or time in the ICU, and the amount of postoperative bleeding or compensated blood. The overall incidence of neurological complications showed a difference between the observed groups. CABG without CPB has a number of advantages over the other method.

Conflict of interest: The authors declare that they have no conflict of interest.

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Original article

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Bacterial sepsis in neonates: Single centre study in a Neonatal intensive care unit in Bosnia and Herzegovina

Izeta Softić[1], Husref Tahirović[2], Vincenzo Di Ciommo[3], Cinzia Auriti[4]

[1]Department of Paediatrics, University Clinical Centre Tuzla, Tuzla, Bosnia and Herzegovina, [2]Department of Medical Sciences, Academy of Sciences and Arts of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina, [3]Epidemiology Unit, Bambino Gesù Children's Hospital (IRCCS), Rome, Italy [4]Department of Neonatology, Bambino Gesù, Children's Hospital (IRCCS), Rome, Italy

Correspondence:
izeta.softic@bih.net.ba
Tel.: + 387 35 303 714
Fax.: + 387 35 303 740

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Objective. The aim of the study was to evaluate the incidence, mortality, risk factors, aetiology and the susceptibility to antibiotics of the bacteria responsible for sepsis. **Material and methods**. A single centre, prospective, observational study, involving 200 neonates admitted over 12 months to the NICU of the University Children's Hospital, Tuzla, Bosnia and Herzegovina. **Results**. The crude incidence of all neonatal sepsis was 68.0% (136/200) and that of late-onset sepsis (LOS) was 48.5% (97/200), yelding an incidence density of LOS of 41.6/1000 patient days. LOS represented the most frequent infection and was significantly more frequent than early-onset sepsis (EOS) (71.3% versus 28.7% p<0.001). The overall mortality was 14.0%, and 18.4% among infected neonates. Risk factors associated with LOS were: mechanical ventilation, intravascular catheter, surgical procedures, birth weight ≤1500 g, gestational age ≤ 28 weeks and Apgar score ≤ 3 at 5 minutes. Culture proven sepsis developed in 43.4% of neonates. *Klebsiella pneumoniae and Enterococcus faecalis* were the predominant bacteria. Gram-negative bacteria were susceptible to amikacin, imipenem and meropenem; gram-positive bacteria to vancomycin and amikacin. **Conclusion**. Neonatal sepsis in our NICU showed a high incidence rate, and gram-negative bacteria were predominant. Low gestational age, mechanical ventilation and an intra-vascular catheter were significantly associated with sepsis. It is necessary to develop a multidisciplinary approach for routine surveillance of nosocomial infections, to improve the asepsis of therapeutic procedures, and to implement the more appropriate use of antibiotics.

Introduction

Neonatal infections currently cause about 1.6 million deaths per year in developing countries. Sepsis and meningitis are responsible for most of these deaths (1). Late-onset sepsis (LOS) is a challenging complication that affects other morbidities, length of hospitalization, cost of care, and mortality rates (2). Improvements in outcome and successful treatment depend largely on early initiation of appropriate antibiotic therapy.

The aetiology of neonatal sepsis in developing countries differs from that in developed countries in the pattern of etiological bacteria and their antibiotic susceptibility (3). In developed countries Group B Streptococcus, (GBS) is a common aetiological agent, but the burden in the developing world is less clear (4). Ampicillin and gentamicin are used as the first-line empirical treatment and the decision is traditionally based on the pathogenic flora commonly

responsible in each neonatal unit. Antimicrobial susceptibility may vary between units and there is an increasing resistance rate of gram-negative bacteria to ampicillin, gentamicin and cefotaxime worldwide (5). Suspected bacterial infection is the main cause of neonatal admissions to hospitals in developing countries. Hospital-based studies suggest that most infections beyond the age of 72 hours are due to gram-negative pathogens, and the majority are likely to be environmentally rather than maternally-acquired. Moreover, there is a significant similarity between the causative organisms for early and late-onset sepsis in developing countries. The key pathogens are: *Klebsiella species*, *Escherichia coli, Staphylococcus aureus* and *Streptococcus pyogenes* (6).

Since in the literature there are no epidemiological data on neonatal sepsis and on the antibiotic susceptibility of the responsible bacteria in Bosnia and Herzegovina, this study was undertaken to describe the situation in a tertiary care hospital, providing neonatal intensive care services.

Methods

Clinical setting

The University Clinical Centre is a teaching hospital with 1373 beds, serving a population of 510,353. It is the referral centre for all inborn neonates (about 4500 deliveries annually) and for those born in the nearby hospitals, serving also for paediatric surgery when indicated. The NICU of the Department of Paediatrics provides intensive care for up to 18 patients. It has an area of 293 m^2, has one common and two single isolation rooms. Visitation of mothers and visitors is limited to once per day. There is a hand - washing station adjacent to the NICU, where all visitors are required to wash their hands. For all newborns, prophylactic antibiotics are routinely used (ampicillin and gentamicin) before entering the NICU or surgery, regardless of the presence of risk factors for infections, although it is questionable.

Study design and data collection

This single centre, observational study was carried out from July 1, 2012 to June 30, 2013 and included a total of 200 critically ill hospital-born neonates, admitted to the Neonatal Intensive Care Unit (NICU) at the Children's Hospital in Tuzla, Bosnia and Herzegovina. This study had a prospective design. The study was approved by the Ethical Committee of the University Clinical Centre, Tuzla. For each enrolled neonate the demographic variables collected included: sex, birth weight (BW) and gestational age (GA). Intrauterine growth restriction was defined as birth weight for GA (BW less than 10^{th} percentile for GA). Other demographic data included the clinical condition of the neonates at the time of admission to the unit [the presence of major congenital malformations (7)], 5-minute Apgar score, age at admission, rectal temperature on entry; need for intubation, mechanical ventilation or exogenous surfactant, venous catheterization, lipid-containing parenteral nutrition, peripheral venous lines, continuous enteral feeding by tube, need for bladder catheterization for surgery; maternal risk factors were: fever, prolonged rupture of membrane, meconium stained-amniotic fluid, clinical signs, laboratory tests and obstetric procedures.

Definitions of sepsis

Sepsis was defined as confirmed or clinically suspected. Confirmed sepsis was defined as the presence of at least two clinical signs and/or two laboratory findings with a positive blood culture. Sepsis was defined as suspected (or clinical sepsis) when the same clinical or laboratory signs were present

with a negative blood culture. Clinical signs of sepsis included: fever (rectal temperature >38°C), tachycardia (heart rate >180 beats per minute) or bradycardia (heart rate <100 beats per minute), apnoea lasting >20 seconds, lethargy, feeding problems, gastric billiary stasis, hemodynamic abnormalities, convulsions, and hypotonia. Laboratory signs: leucopoenia (white blood cell count <5.000 mm3) or leucocytosis (white blood cell count >20.000 mm^3), low platelet count (platelets <100.000 mm^3), blood C-reactive protein level >1.5 mg/dL, fibrinogen >150 mg/dL, white blood cell immature/total ratio ≥0.2, metabolic acidosis (base excess ≥7 mmol/L). Sepsis acquired in the hospital that became symptomatic after hospital discharge or after the first 72 hours of life is defined as late-onset sepsis (LOS) (8). Early-onset sepsis (EOS) presented within 72 hours of life with clinical and laboratory symptoms, in the presence or absence of maternal risk factors.

Microbiological analysis

Blood culture was obtained for each neonate with signs of sepsis, by the method described by Buttery (9). The blood specimen was further inoculated into a BACTEC Peds plus/F culture vial (BACTEC, Becton Dickinson, USA) and the inoculated cultures were incubated as soon as possible in the BACTEC 9120 instrument for up to five days as recommended by Becton Dickinson Microbiological Systems (10). Sensitivity of isolated bacteria to various antibiotics was assessed by the modified Kirby and Bauer method on Mueller-Hilton agar, according to the Clinical and Laboratory Standard Institute guidelines, using an automated Vitek 2 Compact system (11).

Statistical analysis

Continuous variables were described with mean ± standard deviation or as medians and interquartile ranges - IQR. Inferences about categorical data were analyzed using the chi-square or Fisher exact test, as appropriate. The main outcome measure of the analysis was LOS onset (suspected or confirmed) during hospitalization in the NICU, in relation to the other variables recorded. Rates of infection were calculated dividing the number of infections occurring during time of exposure by the total of exposure time. For each specific procedure the time of exposure was the time of administration of the procedure. The incidence density (ID) of infection was the ratio between the number of episodes of infection divided by the total sum of days at risk during the hospital stay (PD: person-days at risk), and the relative risk of infection associated with each variable was the ratio between the incidence rate in the risk-based group and the incidence rate in the reference group. Analyses were performed by IBM SPSS Statistics 22 and MedCalc statistical software for Windows Version 13 – 14.10.2; p values were considered statistically significant at a value of 0.05.

Results

Two hundred infants were admitted to the NICU. Mean BW was 2915 ± 968.74 g; of these 200 infants, 31 were very low birth weight (VLBW). The numbers of infants in each gestational age and birth weight category are shown in Table 1.

Among the 200 neonates observed in the NICU, 136 had at least one episode of neonatal sepsis. The organisms responsible for early and late onset sepsis and their antibiotic sensitivity patterns are shown in Tables 2 and 3.

The incidence of early-onset neonatal sepsis was 28.7% (39/200) and the incidence of LOS was 71.3% (97/200). There were 110 episodes of LOS, yielding an incidence density of 41.6 per 1000 patient days (110/2646). Seven of 200 neonates (3.5%) had two epi-

Table 1 Demographic characteristics of newborns

Demographic characteristic	Newborn (n=200)			
	<1500 g (n=31)		>1501 g (n=169)	
	Range	Mean±SD	Range	Mean±SD
Gestational age (Weeks)	25-36	29.71± 3.24	30-42	36.74±2.75
Birth weight (g)	580-1500	1181.32±292.09	1550-4600	2877.72±773.04

Table 2 Distribution of bacteria associated with sepsis

Pathogens	n (%)
Gram-positive bacteria	
Enterococcus faecalis	10 (16.9)
Staphylococcus epidermidis	7 (11.9)
Staphylococcus species	5 (8.5)
Streptococcus -group B	3 (5.1)
Listeria monocitogenes	2 (3.4)
Gram-negative bacteria	
Klebsiella pneumoniae	10 (16.9)
Pseudomonas aeruginosa	7 (11.9)
Escherichia coli	7 (11.9)
Acinetobacter baumanni	6 (10.2)
Enterobacter cloacae	2 (3.4)
Total	59 (100.0)

sodes of sepsis and 6/200 (3.0%) had three episodes, yielding a total number of episodes of infections of 149/200 newborns. In VLBW neonates LOS was observed in 25/31 neonates, with a crude incidence of infection of 80.6% and incidence density of 52.7 (31/588), versus an incidence density of LOS in heavier neonates (>1500 grams) of 38.4 (79/2058) (p<0.25; RR 1.37; 95% CI 0.91-2.06).

The total number of hospital days spent in the NICU was 2646, while this number for the group of 136 infected babies was 2112. The mean length of hospital stay was 13.23 ± 9.33 days for all 200 newborns. It was significantly higher in the 97 infants with LOS, than in the 64 infants that never developed sepsis, as expected (p<0.0001, mean 18.97 ± 15.49 days; median 17, IQR 5 - 27.5 days).

Mortality associated with sepsis (EOS and LOS) was 12.5% (25/200), accounting for 89.3% of overall neonatal mortality (25/28), p<0.001. The risk of death was significantly higher among infected neonates compared to the uninfected. Twenty-five of 136 infected neonates died, yielding a crude mortality rate of 18.3% versus 4.6% among uninfected neonates, (p<0.02, RR 3.92, 95% CI 2.89-10.28) and also compared to the mortality observed in the entire group of neonates enrolled (p<0.03; RR 1.31; 95% CI 0.80-2.15). The risk of death was significantly higher in the VLBW infected infants compared to the others (48% versus 12.2%, RR: 3.40; 95% CI 1.71-6.64; p<0.004).

The incidence density of sepsis in NICU by selected clinical characteristics and invasive procedures is shown in Table 4. VLBW, extreme prematurity (<28 gestational age), 5-minute Apgar score ≤3, assisted ventilation, the presence of a central venous catheter and surgical intervention were all significantly associated with a higher incidence of LOS sepsis

Table 3 Antibiotic susceptibility for of Gram-negative and Gram-positive bacteria isolated from blood cultures

Antibiotics	Gram-negative bacteria (n=32)				
	Klebsiella pneumoniae (n=10)	*Escherichia coli* (n=7)	*Enterobacter cloace* (n=2)	*Pseudomonas aeruginosa* (n=7)	*Acinetobacter baumanni* (n=6)
	n (%)	n (%)	n (%)	n (%)	n (%)
Ampicillin	R	R	R	R	R
AMC	1 (10)	R	R	R	R
Ceftriaxone	4 (40)	6 (85.7)	1 (50)	5 (71.4)	R
Cefotaxime	4 (40)	3 (42.8)	1(50)	4 (57.1)	R
Ceftazidime	4 (40)	6 (85.7)	1(50)	5 (71.4)	R
Cefepime	4 (40)	7 (100)	1 (50)	5 (71.4)	R
Imipenem	10 (100)	6 (85.7)	2 (100)	6 (85.7)	R
Meropenem	10 (100)	27 (100)	2 (100)	6 (85.7)	R
Amikacin	10 (100)	4 (57.1)	1(50.0)	7 (100)	R
Gentamicin	2 (20)	3 (42.8)	1 (50.0)	7 (100)	R
Tobramycin	10 (100)	7 (100)	2 (100)	7 (100)	6 (100)
Ciprofloxacin	1 (10)	4 (57.1)	1 (50.0)	5 (71.4)	-
Tigecycline	10 (100)	NT	1 (50.0)	7 (100)	6 (100)
TMP/SMX	5 (50)	R	1 (50.0)	R	R
Antibiotics	Gram-positive bacteria (n=27)				
	Staphylococcus epidermidis (n=7)	*Enterococcus faecalis* (n=10)	*Streptococcus Spp.* (n=5)	*Streptococcus agalactiae* (n=3)	*Listeria monocitogenes* (n=2)
	n (%)	n (%)	n (%)	n (%)	n (%)
Ampicillin	NT	2 (20)	1 (20)	3 (100)	2 (100)
Penicillin	R	R	2 (40)	1 (33.3)	2 (100)
Oxacillin	R	NT	R	NT	NT
Ciprofloxacin	3 (42.8)	NT	NT	NT	NT
Gentamicin	2 (28.5)	3 (30)*	5 (100)	3 (100)	2 (100)
Amikacin	7 (100)	10 (100)	5 (100)	NT	NT
TMP/SMX	2 (28.5)	NT	1 (20)	1 (33.3)	2 (100)
Clindamycin	6 (85.7)	1 (10)	1 (20)	3 (100)	NT
Rifampin	6 (85.7)	NT	5 (100)	3 (100)	NT
Vancomycin	7 (100)	10 (100)	5 (100)	3 (100)	NT

NT=not tested for susceptibility; R=Resistance; AMC=Amoxicillin/Clavulanic Acid; TMP/SMX=Trimethoprim/Sulfamethoxazole; *Gentamicin disk 120 mg.

Table 4: Incidence density of sepsis in NICU by selected clinical characteristics and invasive procedures

Characteristics	No. of patients	ID†	RR‡	95% CI
Birth weight (g)				
≤ 1500	31	52.7	1.37	0.91-2.06
>1500	169	38.4	1	
Gestational age§				
< 28	8	85.1	2.29	0.81-5.81
28-31	25	39.0	1	0.59-1.70
> 31-36	84	43.5	1.12	0.73-1.68
≥ 37	83	39.1	1	
Gender				
Male	115	41.8	1.02	0.70-1.47
Female	85	41.2	1	
MCM‖				
Present	14	48.2	1.18	0.64-2.16
Absent	186	40.9	1	
Apgar score¶				
≤ 3	7	98.0	2.45	1.04-5.76
4 - 6	17	44.4	1.11	0.60-2.04
7 -10	176	40.1	1	
Assisted ventilation††				
With	67	196.4	4.36	3.05-6.23
None	133	45.0	1	
CVC‡‡				
With	84	95.9	3.44	2.39-4.93
None	116	27.9	1	
Surgical intervention				
With	9	80.3	2.03	1.12-3.70
None	191	39.5	1	
Total	200	41.6	-	-

†Incidence density (the ratio between the number of episodes of infection divided by the sum of days at risk during the hospital stay); ‡Relative risk (ID1/ID0); §Weeks; ‖Major congenital malformations; ¶At 5 minutes; ††Continuous Positive Airway Pressure-Orotracheal tube; ‡‡Central venous catheter.

Discussion

To our knowledge, this is the first prospective observational study that describes the rate, aetiology and some of the risk factors of neonatal sepsis in an NICU in Bosnia and Herzegovina. The sudy by Hadzimuratovic et al. was limited to premature infants with congenital heart disease, and found a higher risk of acquiring sepsis during hospitalization in an NICU (12).

The high incidence and overall mortality rate from sepsis, especially among VLBW infants, and the high incidence density of LOS are in line with investigations from developing countries (13-15). The most common causes of death in the neonatal period in developing countries are infections (1).

Timely microbiological surveillance and assessment of antimicrobial resistance is a key component in decreasing the rate of neonatal sepsis and the associated mortality. There are a number of important gaps in our knowledge and a lack of studies looking at simple and sustainable interventions to reduce the burden of neonatal sepsis. The lack of culture driven antimicrobial therapy and limited consistent infection control practices are likely responsible for the high incidence rates of neonatal sepsis and mortality.

There are no comprehensive studies available in developing counties, because no National Nosocomial Infection Surveillance Systems (NNISS), which develop surveillance and preventive strategies to break down nosocomial infections, have been implemented. To implement such NNISS could be critical in abating this clinical and public health problem.

There is a general lack of precision in defining sepsis in developing countries. Micribiological results in many developing countries may require several days to become positive. Moreover, in neonates the incidence of positive blood cultures in the course of sepsis often does not reach 50% of samples (16). In our unit, rates of resistance for gram-negative and gram-positive bacteria were high, leading to a great deal of concern with respect to infection control and antibiotic prescribing practices. Gram-negative bacterias were more frequently isolated, which is a common finding from countries with low resources, overcrowding and poor staffing patterns in hospitals (17, 18). They were even highly resistant to ampicillin and gentamicin. In our unit there has been a questionable routine practice to start antibiotics for all new-borns regardless of clinical signs. Cephalosporins and an aminoglicoside (amikacin) have been the first line therapy because of the high resistance to recommended empirical therapy with ampicillin and gentamycin. A preponderance of enterobacteria has been noted in other similar clinical settings (19, 20). This problem might be prevented through limitation of the use of the latest generation of cephalosporin, and specific prevention measures, such as hand washing. The current standard practice of unselective use of antibiotic therapy for every new-born entering the NICU is one that needs revision.

Low birth weight and prematurity increase the risk of neonatal sepsis, but in our study it did not reach statistical significance. In other studies, 20% of VLBW preterm infants experienced a serious systemic infection. Furthermore, the mortality rate was as much as threefold higher than their counterparts without sepsis during their hospitalization (21). In this study, the overall mortality rate associated with sepsis was high for the entire group of infected babies. In the group of VLBW the incidence was twofold higher. However, there were only 31 VLBW neonates in our population.

Among the biological characteristics, extreme prematurity (<28 gestational age), and 5-minute Apgar score ≤3 were risk factors for LOS among our patients, confirming the data from major literature on this topic (22). Among the therapeutic procedures associated with LOS, assisted ventilation, the presence of a central venous catheter and surgical intervention markedly increased the risk of infection. Moreover, invasive procedures in association with prematurity are risk factors for acquiring sepsis, especially in settings with a high intensity of colonization pressure, which is characteristic for developing countries (23, 24). The current standard practice of unselective use of antibiotic therapy for every new-born entering the NICU may be a limitation and it needs to be modified.

Conclusion

A high incidence and mortality rate from sepsis especially in VLBW infants, a high incidence density of LOS, a low rate of positive blood culture and a higher frequency of isolated, very resistant gram-negative bacteria are presented in this study. The paucity of data in the medical literature on neonatal sepsis in Bosnia and Herzegovina is a matter of concern for all those who work with neonates, especially in NICUs. Another aspect that must be addressed is the rational use of antibiotics. Specific guidelines for prescribing antibiotic therapy and applying invasive procedures for neonates in NICUs are important means to limit the development of resistant pathogens. Understanding the aetiology and epidemiology of neonatal sepsis and their changes over time is a key component in the reduction of sepsis and mortality of neonates in Bosnia and Herzegovina. Only a multidisciplinary approach, with contributions from neonatologists, infection control practitioners and microbiologists, will reduce the incidence of neonatal sepsis. Limited-resource countries need to reach the quality of health care and patient safety that we find in developed countries.

What is already known on this topic

Neonatal bacterial sepsis continues to be major cause of morbidity and mortality in the developing world. Differences in epidemiology between developed and developing countries have been identified.

What this study adds

Our results from an observational study suggest the nosocomial origin of neonatal bacterial sepsis caused by predominant gram-negative bacteria. Early and focused treatment with optimal antibiotic therapy is essential for better management of neonatal bacterial sepsis in our hospital.

Authors' contributions: Conception and design: IS and HT; Acquisition, analysis and interpretation of data: IS and CA; Drafting the article: IS, CA and HT; Revising it critically for important intellectual content: HT and CA; Approved final version of the manuscript: IS, HT, CA and VC.

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Original article

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Marijuana smoking among school-aged adolescents in the Brčko District of Bosnia and Herzegovina: A cross-sectional study

Anto Domić[1], Husref Tahirović[2], Mojca Čižek Sajko[3], Borislav Đulabić[4]

[1]Government of Brčko District of BIH, Brčko, Bosnia and Herzegovina
[2]Department of Medical Sciences of the Academy of Sciences and Arts of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina, [3]Institute for Biostatistics and Medical Informatics, Faculty of Medicine, Ljubljana, Slovenia, [4]PHI Health Center Brčko, Brčko, Bosnia and Herzegovina

Correspondence:
anto.domic67@gmail.com
Tel.: + 387 65 815 000
Fax.: + 387 49 490 020

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Objective. The aim was to determine the prevalence of marijuana smoking among school-aged adolescents in the Brčko District of Bosnia and Herzegovina, with particular regard to their gender, age and residence, and the frequency of marijuana smoking in the past thirty days in relation to their peers in the rest of Bosnia and Herzegovina, the Republic of Croatia and the Republic of Serbia. **Subjects and methods.** This research, designed as a cross-sectional study and based on the ESPAD (European School Survey Project on Alcohol and Other Drugs) questionnaire, adjusted to this research, encompassed 4,188 adolescents from elementary and secondary schools. The data were collected by means of questionnaires tailored to each respondent. **Results.** A significantly lower number of adolescents smoke marijuana in comparison to those who do not smoke, but male adolescents smoke more often than female adolescents ($p<0.001$), as well as urban youth in comparison to rural youth ($p=0.04$). Every fourth adolescent, regardless of gender, who smoked marijuana, used it before the age of thirteen ($p<0.001$), male adolescents more often than females ($p=0.002$). In the previous thirty days a higher percentage of all the respondents from the Brčko District had smoked marijuana than those from the Republika Srpska and the RS ($p<0.001$), and there is no difference between them and their peers from the Federation of Bosnia and Herzegovina and the RC ($p=0.382$ and $p=0.608$). **Conclusion.** Smoking marijuana in the Brčko District is a major public health problem. Male adolescents smoke marijuana more often than female adolescents, and urban youth more in comparison to rural youth. In the previous thirty days adolescents from the Brčko District smoked more often than their peers from the Republic of Serbia and the Republika Srpska, and with the same intensity but less frequently compared to adolescents from the Republic of Croatia and the Federation of Bosnia and Herzegovina.

Introduction

Cannabis is the most commonly used illicit narcotic substance in the world (1-4). It is a generic term used to denote several psychoactive substances generated from the plant *Cannabis sativa* (5). In accordance with the Single *Convention on Narcotic Drugs*, *1961, cannabis is defined as "the* flowering or fruiting tops of the cannabis plant from which the resin has not been extracted, by whatever name they may be designated" (6). It is

the dry, greenish-gray mixture of leaves and flowers of cannabis that exists in three main forms: herbal cannabis or marijuana that originates from the dried leaves and flowering tops; cannabis resin - made by compression of the plant and it is known as hashish, and cannabis oil, mixtures that are the result of distillation or extraction of the active plant ingredients (5, 7).

Marijuana can be used in different ways such as hand-rolled cigarettes called *joints, or* many use pipes or marijuana cigars (7, 8). The main *psychoactive* chemical in marijuana, responsible for most of the psychoactive effects, is *delta-9-tetrahydrocannabinol* (THC) (9, 10). Besides THC, the plant also contains more than 400 other chemicals and more than 60 metabolic compounds chemically related to THC, with no psychoactive effects, called *cannabinoids* (10).

Smoking marijuana usually starts during adolescence. The beginning of adolescence is linked with the physiological onset of puberty, and ends when the adolescent assumes the identity and behavior of an adult (11). The World Health Organization (WHO) identifies adolescence as the period that occurs from ages 10 to19 years, and it is roughly divided into three stages: early adolescence, middle adolescence and late adolescence (12). This period is characterized by significant physical, emotional and intellectual changes, with changes in social roles, relationships and expectations (13).

Earlier studies showed that marijuana use among a general population of adolescents was a relatively common occurrence (14), and that marijuana use almost always precedes use of other illicit substances, such as cocaine, methamphetamine, hallucinogens, heroin and morphine (15). Adolescents who smoke marijuana suffer *adverse health effects* with psychosocial and physical consequences, such as poor family relationships, financial problems, education interruption, delinquent behavior, promiscuity, unplanned pregnancy, sexually transmitted diseases, poor sleep quality, *memory loss* and *attention* deficits (16). Marijuana addiction is similar to the addiction caused by other narcotics, and occurs in 14-17% of adolescents who consume it (2, 8). The influence of peers who use marijuana has been identified as one of the important risk factors associated with the onset of cannabis use (17). Several studies have shown that adolescents who use drugs often socialize with friends who also use drugs, and adolescents who do not take drugs are significantly less likely to have friends who use drugs (18-20). If they socialize with friends who use drugs and have a dominant role in society, then this could trigger their more frequent use of drugs (17-20). Perceptions about marijuana use show that it is more frequently smoked by males, who consume it longer and with more intensity, while females are less likely to use marijuana, and the consequences of its use are harmful for females health (21). Nowadays, marijuana can be found everywhere from cities to rural neighborhoods. The studies so far have generally confirmed equal access regardless of the place of residence (22, 23). According to WHO, about 147 million people or 2.5% of the world population consume marijuana, and the prevalence of cannabis use is growing much more quickly compared to other drugs (24).

In many countries the use of marijuana is continuously rising, and it is most commonly used in Western and Central Africa, Western and Central Europe as well as in North America. Europe is still one of the largest markets of marijuana, although the intensity of use varies from country to country. It is most frequently used in Western and Central Europe (25). The frequency of marijuana use is determined by public opinion, which is divided over the control and use of marijuana, and there are more and more people lobbying for liberalized marijuana access. Many countries have created a legal

basis for *decriminalization* of *marijuana* and its use for medical purposes, which affects the frequency of consumption (10, 26).

According to the European School Survey Project on Alcohol and Other Drugs (ESPAD) 2011 (25), 16% of adolescents in the Member States reported having used marijuana at least once in their life, while in the United States (USA) around 35% of them had done so (19, 25, 27, 30). In the Republic of Croatia (RC) 18% of respondents reported having used marijuana at least once in their life, while in the Republic of Serbia (RS) only 7% of respondents had done so. The studies researching the use of marijuana monitor use of marijuana in the previous 30 days, and the ESPAD research demonstrated that 7% of adolescents in the participating countries had used marijuana in the previous 30 days, while in the USA 18% of respondents had consumed cannabis in the previous 30 days; in RC 7% of respondents and 3% of the respondents in the RS had done so (25).

In Bosnia and Herzegovina (BH) there are no aggregate data for the entire country, but there are data for the Republika Srpska, where 4% of the respondents had consumed marijuana at least once in their lives, while in the Federation of BH 8% of the respondents had consumed marijuana at least once in their lifetime. One percentage of the respondents from the Republika Srpska and 3% of the respondents from the Federation of BH had consumed cannabis in the previous 30 days (25). However, this research did not include the Brčko District, a local unit of self-governance with special status, under the sovereignty of BH, located in the Northeastern part of BH, at the intersection of domestic and international routes linking East and West, and North and South.

The aim of this research was to determine the frequency of marijuana smoking among adolescents in Brčko District public schools, with particular regard to their gender, age and residence, as well as in relation to the rest of BH, RS and RC.

Subjects and methods

Area of research

The Brčko District (Figure 1) covers an area of 493.3 square kilometers. In 2011 it had 75,625 inhabitants with an average population density of 153 inhabitants per square kilometer, which represented a higher population density than some other areas in its vicinity. Of the total population, 24.2% were children and adolescents. In 2011, a total of 905 children were born in the area of the Brčko District, so the birth rate was 11.9‰, while the rate of natural increase was negative and amounted to 0.6‰ (27).

Figure 1 The area of the Brčko District of Bosnia and Herzegovina is colored green, the area of Republika Srpska is colored orange, and the Federation of Bosnia and Herzegovina is colored yellow (28).

Subjects

According to the plan, all ninth grade elementary school pupils were to be included in the research, plus all secondary school students in the Brčko District, which is 4,676 students in total. Of that number, 1,016

were ninth grade pupils from 12 elementary schools with subsidiary schools, and 3,660 students were from four high schools. The research encompassed 4,188 or 89.6% of all the students with average 15.6 years.

Methods

The research was designed as a cross-sectional study and was conducted by means of an ESPAD questionnaire (25) adapted to this research and translated into the official languages of BH. The questionnaire was created and published by a group of experts from the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and the Pompidou Group, which was founded by the Council of Europe in order to enable comparison and analysis of the results of cigarette and narcotics consumption surveys in different countries. The questionnaire contained 45 questions, divided into appropriate thematic units. The questionnaire prepared for this research was used to gather demographics for all adolescents.

After prior approval had been granted by school principals, the research was conducted during the 2011-2012 school year, from 20 October to 28 November 2011, in one school year. The students filled out the questionnaires by themselves, following an explanation given by a person specially trained for completing the questionnaire. The person supervising the test helped the students to fill out the questionnaire, explaining the questions, but did not influence the final answer. After completing the questionnaire, the students put them in envelopes, that were sealed and handed over to the person supervising the process. The adolescents' responses to the following questions were used in this article: "Have you ever smoked marijuana? When was the first time you smoked marijuana? How many times have you smoked marijuana in the past 30 days?" and the frequency of smoking in relation to the Federation of BH, the RS, the RC and the RS was calculated. Smoking marijuana for the first time was analyzed in relation to the gender, age and place of residence (rural - urban) (29) of the adolescents.

Ethics statement

The respondents voluntarily agreed to fill out the questionnaires anonymously after being informed that the results would be used for scientific purposes. The questionnaire used in the study was taken from the scientific literature and was quoted in the references.

Statistical analysis

We presented the prevalence of individual responses to the questions asked in the questionnaire as absolute and relative numbers, and we displayed them in tables or graphs. Binomial or Pearson's chi-square test were applied for analyses of the impact of the students' age, gender and place of residence on the prevalence of marijuana consumption, and to compare that prevalence in the Brčko District with the prevalence in other parts of BH and neighboring countries. Statistical significance was confirmed at $p<0.05$. We used the statistical analysis software package SPSS 20 (SPSS Inc., Chicago, Illinois, USA) for data processing.

Results

Among 4,188 respondents who accessed the research, 4,084 filled out the data about gender, of which 2,013 (49.3%) were boys and 2,071 (50.7%) girls. When asked about smoking marijuana 115 (2.8%) of respondents did not answer and the frequency of marijuana smoking by gender was calculated from a sample of 3,969 respondents, of whom 1,950 (49.1%) were boys and 2,019 (50.9%) were girls. The answer to the ques-

tion about smoking marijuana as regards the place of residence was not provided by 238 (5.7%) respondents, so the frequency of marijuana smoking among the students from rural and urban areas was calculated from a sample size of 3,950 respondents of which 54.9% (2,169) were from rural and 45.1% (1,781) from urban areas. Table 1 shows cannabis consumption among adolescents in the Brčko District by gender and place of residence.

The results showed that a statistically significant lower number of respondents had smoked marijuana in relation to those who had never smoked it [578 (14.6%):3391 (85.4%); chi-square test, p<0.001] and that male adolescents statistically significantly more often smoked marijuana in relation to female adolescents [373 (19.1%):205 (10.2%); chi-square test, p<0.001]. When comparing the marijuana smoking results in relation to the place of residence, it was established that a significantly higher number of adolescents from the city had smoked marijuana in relation to those from villages [310 (17.4%):267 (12.3%); chi-square test, p=0.04]. The results of the first marijuana smoking according to the age of students living in the *Brčko* District, by gender and place of residence are displayed in Table 2.

The correlation between age, gender and place of residence of all the respondents who reported that they had smoked marijuana and the first time of smoking mari-

Table 1 Marijuana smoking among school-aged adolescents in the Brčko District, by gender and place of residence

Gender and place of residence	Marijuana smoking among students of the Brčko District		
	No (n; %)	Yes (n; %)	Total (n; %)
Gender			
Male	1577 (80.9)	373 (19.1)	1950 (100)
Female	1814 (89.8)	205 (10.2)	2019 (100)
Total	3391 (85.4)	578 (14.6)	3969 (100)
Place of residence			
City	1471 (82.6)	310 (17.4)	1781 (100)
Village	1902 (87.7)	267 (12.3)	2169 (100)
Total	3373 (85.4)	577 (14.6)	3950 (100)

Table 2 The number of students in Brčko District according to age of first marijuana smoking by gender and place of residence

Gender and place of residence	Age of first marijuana smoking in years (n; %)				
	≤13	14	15	16	Total
Gender					
Male	42 (23.5)	27 (15,1)	47 (26.2)	63 (35.2)	179 (67.8)
Female	18 (21.2)	11 (12.9)	16 (18.8)	40 (47.1)	85 (32.2)
Total	60 (22.7)	38 (14.4)	63 (23.9)	103 (39)	264 (100)
Place of residence					
City	29 (20.4)	25 (17.7)	31 (21.8)	57 (40.1)	142 (53.8)
Village	32 (26.2)	14 (11.5)	31 (25.4)	45 (36.9)	122 (46.2)
Total	61 (23.1)	39 (14.8)	62 (23.5)	102 (38.6)	264 (100)

juana was studied. The results we obtained showed that there was a difference between male and female adolescents when they first smoked cigarettes regarding their age [179 (67.8%): 85 (32.2%); Chi-square test, p <0.001]. Nearly a quarter of adolescents, regardless of gender, who reported that they had smoked marijuana, had done so before the age of thirteen [(60/264); chi-square test, p <0.001], while males smoked marijuana at that age more often compared to their female peers [42 (23.5%):18 (21.2%): chi-square test, p=0.002]. Female adolescents more often began smoking marijuana at the age of 16 (47.1%) compared to male adolescents (35.2%). When it comes to the place of residence, the results showed that more than half of the respondents who had smoked marijuana lived in the city [142 (53.8%):122 (46.2%); Chi-square test, p<0.001]. Every fourth respondent who had tried marijuana before the age of thirteen lived in the rural area but every fifth respondent who lived in the city [(32:29); Chi-square test, p<0.001] had done so; the respondents from the city smoked marijuana more often only after the age of 14 (p <0.001).

Of all the 578 respondents who answered "yes" to the question about smoking marijuana at all in their life, 41% of them (237:578) had smoked it 1-2 times. A minimum number of adolescents (5.5%) had smoked it 20 to 39 times, and 17% of adolescents more than 40 times.Respondents living in the villages had more often smoked 1-9 times in their lifetime compared to those from the city, who had smoked it between 10 and more than 40 times.

When asked about marijuana smoking in the previous 30 days, 6.6% (256/3878) adolescents from the Brčko District answered "yes". According to the ESPAD research from 2011, 1% (31/3132) from the Republika Srpska, 6.1% (181/2966) from the Federation of BH, 7% (210/3002) from the RC and 3% (182/6084) from the RS gave the same answer. In the Brčko District, the respondents had used marijuana in a significantly higher percentage in the previous 30 days than the students from the Republika Srpska (p<0.001) and the RS (p<0.001). The differences between the Brčko District and the Federation of BH, and the RC were not statistically significant (p=0.382 and p=0.608).

Out of 256 respondents who had smoked in the previous 30 days, 115 respondents or 44.9%, had smoked one to two joints, 51 or

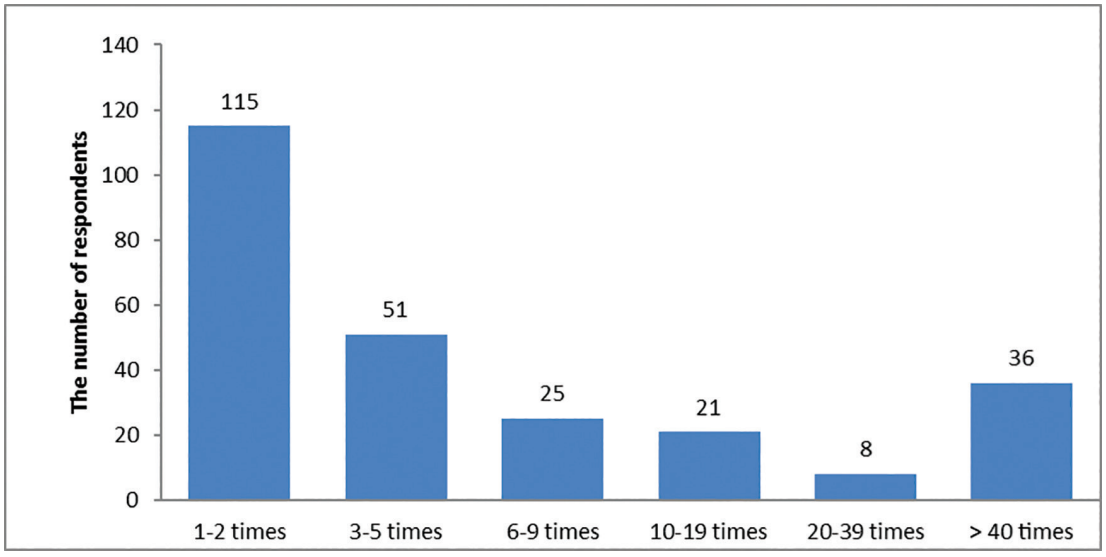

Figure 2 The number of marijuana cigarettes that respondents had smoked on average in the previous 30 days.

19.9% of the respondents had smoked 3-5 joints, 25 respondents or 9.8% 6-9 joints, 21 adolescents or 8.2% 10-19 joints, 8 respondents or 3.1% 20-39 times, and 36 or 14.1% of adolescents had smoked it more than 40 times in the previous month (Figure 2).

Discussion

This research demonstrated that almost one in seven adolescents in the Brčko District (14.6%) had smoked at least one joint of marijuana in their lifetime, which confirms the high availability and usage of marijuana. Such a high percentage of respondents who had tried to smoke marijuana at least once in their life is distressing because these are young people in early adolescence, when marijuana smoking leads to addiction, causing long term health problems (30). Comparing the data about marijuana smoking from this research with the data about their peers in other parts of BH (Federation of BH and Republika Srpska), and in the RC and the RS, we found that adolescents from the Brčko District smoked marijuana less frequently than their peers in the RC, while in relation to their peers from the RS, the Federation of BH and the Republika Srpska they did so much more frequently (25, 31). In fact, in the Republika Srpska barely every twenty-fifth (4%) adolescent had tried to smoke marijuana, while in the Federation of BH and the RS almost every twelfth (8%) adolescent had done so (25). Based on the research carried out by other authors, we learned that more than a third (35%) of adolescents in the USA had smoked marijuana at least once in their lives (19), while in Europe, on average, one in six (16%) adolescents had tried it (25). It is evident that when it comes to the prevalence of marijuana smoking, adolescents from the Brčko District are more similar to their peers from Europe and the USA than to their peers from other parts of BH, RC and the RS (19, 25, 26). It is therefore necessary to clarify the reasons why the behavior of adolescents from the Brčko District resembles more the behavior of their peers from distant countries than the behavior of their peers from the region with whom they have frequent contact, share the same geographical area and have similar habits.

When it comes to the relationship between marijuana smoking and gender, we have shown that nearly one in five male adolescents and one in ten female adolescents have tried to use marijuana at least once in their life, which is in line with the results of global research showing that male adolescents smoke marijuana much more frequently than female adolescents (17, 25, 31). In the Federation of BH, 12% of male adolescents and 4% of female adolescents had smoked marijuana, while in the Republika Srpska 6% of male and 3% of female adolescents had done so. As for the RC and the RS, the neighboring countries, research shows that male adolescents try to smoke marijuana more often compared to female adolescents. So, in the RC, 21% of male adolescents and 14% of female adolescents had smoked marijuana, while in the RS 9% of male and 4% of female adolescents had smoked marijuana (19, 25). Research carried out in the USA showed that 38% of male adolescents and 31% of female adolescents had smoked marijuana at least once in their lifetime (19, 25), while in Europe on average 19% of male and 14% of female adolescents had smoked it (25). The figures illustrate the same trends, i.e. male adolescents smoke marijuana more often than female adolescents, but with different intensity in different countries. Research carried out in the USA and in Europe in the past several years showed that the difference in drug use between men and women is decreasing and it is expected that in the near future males and females will be equally as likely to use drugs (21, 30).

This research has proven to us that boys in the Brčko District are twice as likely to try smoking marijuana than girls. One of the reasons for such behavior may be found in studies that show that women are more prone to taking sedatives and narcoanalgetics, while men are more prone to taking other narcotics, including marijuana (21, 29, 32). Based on this research, we are unable to draw a conclusion about the tendency toward marijuana smoking among male and female adolescents in the Brčko District, because this phenomenon was not the focus of research. It would be necessary to test this claim in future in order to confirm or refute research results that speak of male and female adolescents' preference for marijuana smoking. There are studies that describe the medicinal properties of marijuana that can be used for treatment of certain diseases, and that say that there is justification for the legalization of cannabis, but ultimately all studies come to one conclusion - cannabis has a harmful effect on health (26). Due to the claims that marijuana has medicinal properties, and owing to the fact that it is supposedly less addictive than other narcotics, the tolerance threshold in adolescents for engaging in experimentation with marijuana is being lowered (16). The disturbing fact is that every fifteenth adolescent in the Brčko District had smoked marijuana in the previous 30 days (25), which is almost equal to the number of adolescents from the EU, RC and Federation of BH, while twice as many respondents from the Brčko District had smoked it in the previous thirty days compared to the RS, and six times more compared to their peers in the Republika Srpska. Active drug addicts were taken to be adolescents who had smoked marijuana in the previous thirty days. Half of them had smoked one to two joints, while one in four had smoked up to five joints of marijuana. One in seven respondents, who said they had used marijuana in the past month, had smoked a minimum of one joint per day on average. This intensity of marijuana smoking suggests that it has become an integral part of the everyday activities of adolescents, and that marijuana smoking is a matter of prestige and is not perceived as a vice or something they should avoid.

From the analysis of when the respondents use marijuana, we established that more than one fifth of school-aged adolescents who had ever smoked it, had done so before the age of thirteen. This number represents 1.5% of the respondents and is significantly higher than in the rest of BH and the RS, where 1% of the respondents had tried marijuana before the age of thirteen, while in RC 3% of them had, which represents the average of the members of the ESPAD group (25). In the USA, this percentage rises to 15% (26). Bearing in mind that we are talking about early adolescence, it is difficult to understand that such a large number of respondents had had the opportunity to obtain marijuana so early in their life and decided to smoke it.

One of the elements which could be seen as a reason for the existence of these drastic differences is the location of the District, which is topographically "nested" between the Republika Srpska and the Federation of BH. Also, it is situated on the border with the EU and it is autonomous in terms of other levels of government. Consequently, migration to and from the Brčko District is extensive. This location and its autonomy make it safe for dealers to trade in marijuana and place their "goods" not only on the market in the Brčko District and BH, but also on the markets of EU countries.

This research shows that respondents from the city are prone to use marijuana more often than those from villages. It shows that one in six respondents from the city had smoked marijuana, and that one in eight respondents from villages had done so. If any difference exists in relation to the place of

residence and marijuana consumption, it has decreased and it is only a matter of time until the place of residence will be irrelevant with regard to marijuana consumption. This observation is supported by the fact that 13 year old respondents from the villages use marijuana much more frequently than their peers from the city. The claim that marijuana is finding its way to rural areas is thus affirmed. The higher intensity of marijuana smoking in rural areas could be the result of the imprecise definition of the term "village", since there are more and more rural areas which in certain circumstances are considered to be urban areas (22, 23, 33). The difference in marijuana consumption could be explained by the fact that rural people are more conservative, less educated, stricter in raising their children, and poorer in comparison to those from the city.

The influence of peers and identification with their idols who tend to use marijuana may be the trigger for adolescents to try to smoke marijuana (18). Social networking sites such as Facebook, Twitter, etc., and socializing with virtual friends and their peers who already use marijuana create more fertile ground for adolescents to engage in an "adventure" and to purchase and consume marijuana (34). Likewise, through social networks and other media, adolescents very quickly obtain information about the trends in medical usage of marijuana and the process of its legalization in some countries of the world, which can create a distorted picture of the ingenuousness of narcotic substances (26). The search for the best way to combat this scourge is aggravated by all this. Research conducted in the USA shows that unstable families have a significant impact on the behavior of adolescents, especially single-parent households (33). In such families, adolescents are much more likely to behave in a risky manner, and they consume drugs more often in comparison to those from two-parent families (35). In families where the parents have built better family relations with their children and where the parents exercise subtle control of the adolescents' actions, risky behavior and consumption of narcotics rarely appear (36). This problem was not the subject of this research, and it is necessary to design new research in order to research this phenomenon scientifically and to draw adequate conclusions.

Limitation of study

A weakness related to the research problem is the inability to check whether the respondents answered the questions truthfully. The research involved adolescents prone to turbulent emotional and social reactions that may affect their giving honest answers. One of the limiting factors was the insufficient involvement of school psychologists and teachers, who could have contributed to making respondents answer truthfully.

Conclusion

Every seventh respondent had tried smoking marijuana at least once in their lifetime, and every fifteenth respondent had smoked marijuana in the previous thirty days. Boys smoke marijuana much more frequently than girls, as do city boys compared to those from villages. Adolescents from the Brčko District had smoked marijuana in the previous thirty days more often than their peers from the RS and the Republika Srpska, and as much as their peers in the RC and the Federation of BH. On the whole, marijuana is easily available in the Brčko District, and its consumption is tolerated, which is an important public health problem.

What is already known on this topic

Marijuana is the most commonly used psychoactive substance. A high percentage of adolescents from developed, as well as from underdeveloped countries, use it. Through the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and the Pompidou Group, established by the Council of Europe,

separate research on marijuana usage has been conducted in the BH entities, the Federation of BH and Republika Srpska. However, no research was conducted before in the Brčko District, which is also part of BH. The previous research showed that marijuana is present among adolescents in BH, but the frequency of smoking varies between the two entities.

What this study adds

During this research, which was conducted in accordance with the methodology previously applied in the BH entities, data about marijuana smoking among adolescents from the Brčko District were collected and processed. The results of this research complement the perception of marijuana smoking among the BH adolescents, so that a full insight into marijuana usage in the whole of BH can be obtained, while in the Brčko District it is possible to implement marijuana smoking prevention programs and periodically repeat the research in order to monitor marijuana smoking trends.

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Original article

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The prevalence of orthodontic treatment needs of school children in northern Herzegovina

Ružica Zovko[1], Stipo Cvitanović[2], Mirela Mabić[3], Anka Ćorić[1], Katarina Vukojević[4], Kristina Goršeta[5], Domagoj Glavina[5]

[1]Health Care Center, Mostar, Bosnia and Herzegovina, [2]Health Care Center Prozor-Rama, Bosnia and Herzegovina [3]Faculty of Economics, University of Mostar, Bosnia and Herzegovina, [4]Study Program of Dental Medicine, School of Medicine, University of Mostar, Bosnia and Herzegovina, [5]Faculty of Dentistry University of Zagreb, Croatia

Correspondence:
ruzicazovko71@gmail.com
Tel.: + 387 63 321 890
Fax.: + 387 36 335 532

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Objective. The study was conducted to evaluate the Index of Orthodontic Treatment Need based on the Dental Health Component (DHC). **Materials and methods.** 300 students aged 12-15 years were included in this study. The examinations were performed in two elementary schools in the municipality of Prozor-Rama, using standard dental instruments. Children were ranked into five grades according to the DHC. **Results.** Only 12% of children were found not to have a need for orthodontic treatment. Of the others, 45.33% had a great, and 10% a very great need for orthodontic treatment. The rest of the children were found to have a need for minor or moderate orthodontic treatment. Slightly more girls than boys had a great or a very great need for treatment, although the difference by gender was not statistically significant. Analysis of the level of need by age of children showed no significant difference; children with a great need of orthodontic treatment prevailed in all age groups. About 85% of children with a great and a very great need for orthodontic treatment would agree to orthodontic treatment, while the rate of non-acceptance was about 5%. **Conclusion.** The high rate of need for orthodontic treatment in the examined students is explained by the lack of programs for this type of health care, the insufficient number of qualified orthodontic specialists, and the very low percentage of allocations from the state budget for oral health.

Introduction

Dentofacial anomalies are conditions in which there is a deviation from the normal morphology, size and function of individual parts or the whole of the craniofacial complex (1). Consequences of disorders related to dentofacial anomalies are numerous, from oral function disorders to disrupted facial aesthetics, which is a serious public health problem. Orthodontic anomalies, especially dental arch crowding, lead to the rapid formation of caries and periodontal diseases. In displaced teeth, even impeccable oral hygiene cannot eliminate food residues left in retention spaces, resulting in fermentation processes, the products of which are acids that lead to damage to the dental substance and supporting apparatus of the teeth (2, 3).

Many studies have pointed out that there is a correlation between orthodontic anomalies and periodontal conditions (4). Today, in countries with a well-developed health care system, longitudinal studies examining the prevalence and registering of dentofacial anomalies are conducted in children from

the age of three years. Timely implementation of orthodontic treatment is planned in this way (5, 6).

The objective of epidemiological studies in orthodontics is to determine the incidence and prevalence of malocclusions and identify variations in different population groups, and thus to establish priorities in implementation of orthodontic treatment in children (7). Orthodontic anomalies impair normal performance of the orofacial complex functions (mastication, deglutition and phonation), and compromise facial aesthetics (8). Distinct orthodontic anomalies, accompanied by impaired facial aesthetics and disturbed oral functions, particularly of speech, significantly affect the emotional and social development of both children and adults (9, 10).

Children with orthodontic anomalies may become introvert and avoid social contact, and often these children become the subject of ridicule and teasing by their peers (11).

Orthodontic anomalies assume epidemic proportions in the world, even in the countries with well-developed oral health care. In determining the prevalence, diagnostics based on standardized criteria are important in order to identify treatment priorities. This is successfully achieved by the Index of Orthodontic Treatment Need (IOTN) using the Dental Health Component (DHC) (12, 13).

IOTN identifies the various percentages of these needs. For example, in Italy 59.5% of elementary school children have a need for orthodontic treatment (12), in children aged 12-14 years in the United Kingdom this need ranges from 15-26% (13), in children aged 8-16 years in Sweden this need is 28.9% (14), in Germany 26.2% of the examined children need orthodontic treatment (15) and in Malaysia 47.9% (16). Similar studies on the state of orthodontic anomalies, in terms of the need for orthodontic treatment, have been carried out in a wider area of the Western Balkans (17). The last study conducted in Bosnia and Herzegovina was in 1988, before the war. The subjects were children aged 6-15 years, and orthodontic anomalies were established in 36.7% of subjects, while 30% were in need of orthodontic treatment (17). These studies did not use the IOTN according to the DHC, which is now the new standard of categorization of orthodontic treatment (3-5).

This was the motive for new research aimed at establishing the real need for orthodontic treatment and the readiness of children to agree to orthodontic treatment.

Materials and methods

The study was conducted in two elementary schools in the municipality of Prozor-Rama, Bosnia and Herzegovina. The study included a total of 300 randomly selected students from 12 to 15 years of age: 12 years of age - 78 (26.0%), 13 years - 73 (24.3%), 14 years - 76 (25.3%) and 15 years - 73 (24.3%). The genders were equally represented in the sample (150 girls and 150 boys). All school children from both surveyed schools who were younger than 12 or older than 15 years of age, as well as children who had received orthodontic treatment before these examinations, or who were undergoing orthodontic treatment during these examinations, were excluded from the study.

The first phase of the research was carried out at the Rama Community Health Center in Prozor with the approval of the Ministry of Education, Science, Culture and Sport of the Herzegovina-Neretva County.

The study was approved by the Ethics Committee of the Rama Community Health Center in accordance with the Helsinki Declaration. Informed consent was obtained from the parents. The children were informed about the study in an appropriate way and only those children who agreed to participate were included in the study. The inclusion criteria were age from 12 to 15

years and a documented medical record, as well as information from the parents, that the child had not had orthodontic treatment before this study. According to the World Health Organization's recommendations, this age group is the most appropriate for oral health examination (18). During this period, children enter adolescence, when growth significantly affects the orofacial complex.

Dental health components with dental characteristics were examined according to the checklist of the Swedish Dental Board. This method was used as a reference for determining the orthodontic treatment need based on functional and occlusal characteristics, as recommended by the WHO (10). IOTN according to the DHC is determined by the following characteristics: increased overbite depth, open bite (anterior or lateral), impeded tooth eruption, Class II and III intercuspation, missing teeth, cleft lip and/or palate (10, 11). The patients were classified into five categories accordingly.

The clinical examination was carried out using standard dental instruments, and the necessary measurements were carried out using the millimeter gauge (Dentalschiebelehre). The persons conducting the research were general dentists with several years of experience in their profession, who use IOTN assessment on a daily basis. Two investigators assessed IOTN taking into consideration inter-observer variations.

All data were entered into the research survey, which consisted of two parts. General data on each examined child were entered into the first part of the survey, and the data on function and characteristics of dentition, with an estimation of the index of orthodontic treatment need, into the second part.

Analysis of acceptance of orthodontic treatment was carried out among children found to have a great and very great need for orthodontic treatment (levels 4 and 5). Three options were offered in the following answers: "I agree to orthodontic treatment", "I have no attitude towards treatment", and "I do not agree to orthodontic treatment". The verbal response was entered into the questionnaire. The examination was carried out for both genders.

Statistical analysis

Data were analyzed in SPSS for Windows (version 17.0., SPSS Inc. Chicago, Illinois, USA) and Microsoft Excel (version Office 2007, Microsoft Corporation, Redmond, WA, USA). Results were expressed in absolute (f) and relative frequencies (%). The Chi-square (χ^2) test was used for testing the significance of differences regarding gender and age. In the absence of the expected frequencies, Fisher's exact test was used. The level of significance was p=0.05. P values that could not be expressed to three decimal places are shown as p<0.001.

Results

The subjects were divided according to the need for orthodontic treatment, by gender and by age (Table 1).

According to the results, significantly more children (almost 90%) had a need for orthodontic treatment (χ^2=173.280; df=1; p<0.001). The distribution of boys and girls by need for treatment was identical, while the distribution of children of different ages varied slightly (χ^2=0.606; df=3; p=0.895).

The distribution of children with a need for orthodontic treatment by degree, in total, by gender and by age is shown in Table 2.

There was a significant difference in the distribution of children who had a need for orthodontic treatment by degree of need (χ^2=103.394; df=3; p<0.001). Comparison of indices of orthodontic treatment need for boys and girls showed no statistically significant difference (χ^2=1.422; df=3; p=0.700), although it was shown that girls had a great

Table 1 Distribution of children by assessed need for orthodontic treatment, in total, by gender and age

Orthodontic treatment	Children (n; %)						
	Total	Gender		Age (years)			
		Boys	Girls	12	13	14	15
Not needed	36 (12.0)	18 (12.0)	18 (12.0)	9 (11.5)	8 (11.0)	11 (14.5)	8 (11.0)
Needed	264 (88.0)	132 (88.0)	132 (88.0)	69 (88.5)	65 (89.0)	65 (85.5)	65 (89.0)
Total	300	150	150	78	73	76	73

Table 2 Distribution of children with need for orthodontic treatment by index of need, in total, by gender and age

IOTN	Children (n; %)						
	Total	Gender		Age (years)			
		Boys	Girls	12	13	14	15
Minor anomaly, no treatment need	54 (20.5)	27 (20.5)	27 (20.5)	13 (18.8)	12 (18.5)	15 (23.1)	14 (21.5)
Borderline treatment need	44 (16.7)	25 (18.9)	19 (14.4)	13 (18.8)	10 (15.4)	12 (18.5)	9 (13.9)
Treatment greatly needed	136 (51.5)	64 (48.5)	72 (54.5)	34 (49.3)	37 (56.9)	32 (49.2)	33 (50.9)
Treatment very greatly needed	30 (11.4)	16 (12.1)	14 (10.6)	9 (13.0)	6 (9.2)	6 (9.2)	9 (13.9)
Total	264	132	132	69	65	65	65

IOTN=Index of Orthodontic Treatment Need.

or very great need for treatment slightly more often. Analysis of levels of need by age of children did not show any significant difference (χ^2=2.719; df=9; p=0.974), children with a great need for orthodontic treatment prevailed in all age subgroups.

Based on the examinations and data analysis, 166 children out of all those included in the study were found to have a great or very great need for orthodontic treatment. Among them, 141 (84.93%) would agree to treatment, 18 (10.84%) children did not have a personal opinion about treatment, and 7 (4.21%) would not agree to treatment. Children who did not desire treatment, despite their need, explained that they did not care about treatment, while others stated that they did not have the financial resources for the treatment. According to the survey results, 70 (81.39%) boys would agree to orthodontic treatment, 12 (13.95%) did not have an opinion on this treatment, and 4 (4.65%) would not agree to orthodontic treatment. Out of the total number of girls with a need for orthodontic treatment, 71 (88.75%) of them declared that they would agree to treatment, 6 (7.5%) did not have a personal opinion on this treatment, and 3 (3.75%) would not agree to this treatment.

Discussion

Determination of orthodontic treatment needs based on assessment of the DHC in children aged 12-15 years in two elementary schools in the municipality of Prozor-Rama, showed that a large number of children have a great and very great need for orthodontic treatment. Almost half of the examined subjects had a great need for orthodontic treatment, while 10% of the children had a very great need for orthodontic treatment.

This is in accordance with similar studies on orthodontic treatment needs in children performed in Jordan (71%), Malaysia (47.9%) and Turkey (40%) (19-21), indicating a correlation between the socio-economic status of the population and the index

of orthodontic treatment need (22). Studies have shown that external appearance, including dental appearance and speech, is essential for a person's socialization, which is why orthodontic anomalies negatively affect the social development of children (23). Introducing more reliable statistical records and monitoring outcomes of orthodontic treatments, not only currently but longitudinally over many years, is very important for improvement of care (24).

Studies indicate a significant improvement in the statistical records of orthodontic treatment at all health care levels, particularly in the private sector. This improves strategic principles for evaluation of this form of health care, which should be available to every child (25). Studies point out the importance of long-term monitoring and constant evaluation of orthodontic treatment, using the same methodology and criteria, because it is only in that way that implementation of this form of health care can be properly evaluated (26, 27). Studies conducted in England showed that the great benefit from interventions conducted was maintained after 6 years in 94% of persons who underwent orthodontic treatment (28).

Many countries have introduced mandatory testing of dental health components to the health care program for children. Studies of dental health components in children aged 10 years in New Zealand showed that one in three examined children needed orthodontic treatment (29). Although the issue of the socio-economic standard of the examined children's families was not included in our study, this issue may be one of the reasons for the great need for orthodontic treatment, due to the generally poor financial income, still felt in the region after the war.

The study by Topic et al. conducted in this area in 1990 on the need for orthodontic treatment showed that 36% of children aged 6-12 years had occlusal anomalies, of which 30% required treatment (17). Additionally, Ivankovic et al. established that after the war, in 1999, there was a significantly higher prevalence of orthodontic anomalies, in 55% of subjects, of which 49% had a great need for orthodontic treatment (30). Studies of the orthodontic treatment needs of school children aged 12-15 years in Jordan showed that 34-71% of the subjects needed orthodontic treatment (16). This is approximately equivalent to the results of our study. These countries are similar to ours in terms of economic opportunities, and this information confirms the findings of Antoff et al. that a favorable socioeconomic situation is a precondition for prevention of occlusal defects (31).

The high prevalence rates of dental caries and periodontal diseases (30) in this region have an adverse effect and cause this great need for orthodontic treatment, and are the direct negative etiological factors in occlusal anomalies (15). Unfavorable allocation of resources (staff, premises and equipment) for health care does not allow application of the principle of availability, and it is not fully respected. The high percentage of orthodontic treatment needs in the area of this study is the consequence of the concentration of health resources, which are mostly located in urban centers. Financial resources from the gross domestic product are extremely insufficient in Bosnia and Herzegovina, which also leads to the difficult situation in the field of oral health.

Limitations of the study

In order to make general conclusions, it is necessary to expand the study area, to conduct the study in several municipalities, and increase the age range of the examined children.

Conclusions

Our study showed that a high percentage of children have a great need for orthodontic

treatment. Orthodontic anomalies among the examined children have epidemic proportions, which indicates that oral health care in school children is not available to children in Prozor-Rama. Children's orthodontic care is not available in the Rama Community Health Center in the municipality of Prozor-Rama, while the study showed that orthodontic treatment would be highly acceptable by children with detected orthodontic changes. Therefore, primary health care guidelines should be developed to introduce orthodontic indices into clinical practice, while specialists for orthodontics should be involved to determine orthodontic treatment needs, as well as to provide orthodontic treatment in well-equipped orthodontic clinics.

What is already known on this topic

Children with orthodontic anomalies may become introvert and avoid social contact, and often these children become the subject of ridicule and teasing by their peers. In determining the prevalence and need for treatment, diagnostics based on standardized criteria are important, in order to identify treatment priorities. This is successfully achieved by the IOTN using the DHC.

What this study adds

Previous research in Bosnia and Hercegovina failed to use the IOTN according to the DHC, which is now a new standard for categorization of orthodontic treatment. The results will give an insight into the need for orthodontic treatment in school children in the municipality of Prozor-Rama. It is expected that the results of this research will contribute to the improvement of oral health care among school children.

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Original article

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Cystatin C, but not urinary or serum NGAL, may be associated with contrast induced nephropathy after percutaneous coronary invasive procedures: A single center experience on a limited number of patients

Emanuele Cecchi[1], Gianfranco Avveduto[2], Maria Grazia D'Alfonso[1], Alessandro Terreni[2], Emma Gelera[1], Anna Caldini[2], Cristina Giglioli[1]

[1]Department of Cardiovascular and Thoracic Medicine, Azienda Ospedaliero-Universitaria Careggi Florence, Italy, [2]Clinical Chemistry Laboratories, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy

Correspondence:
emanuelececchi@virgilio.it
Tel.: + 390 55 794 9577
Fax.: + 390 55 794 7617

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Key words: Cystatin C ▪ Neutrophil Gelatinase-Associated Lipocalin ▪ Contrast induced nephropathy ▪ Percutaneous coronary invasive procedures.

Objective. This study aimed to test the association of both the baseline values and post-procedural variations of urinary and serum Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Cystatin C (CysC) with contrast induced nephropathy (CIN) occurrence in patients undergoing percutaneous coronary invasive procedures (PCIP), and compare them to serum creatinine and the estimated glomerular filtration rate (eGFR). **Methods.** In 43 patients admitted to our Cardiac Step-Down Unit and submitted to PCIP, we measured serum creatinine and eGFR as the standard markers for CIN diagnosis, and compared them to both serum and urinary NGAL as well as serum CysC, assessed before and 4 hours after PCIP. **Results.** Patients who developed CIN (16%) were older, with significantly higher discharge creatinine values, lower eGFR values at creatinine peak, and higher baseline and post-PCIP CysC values. We did not detect any significant association between baseline serum and urinary NGAL values and their 4 hour variations after contrast medium administration and CIN occurrence. Furthermore, we observed that the baseline values of both serum and urinary NGAL were significantly higher in patients with greater neutrophil count. **Conclusion.** In our population submitted to PCIP, neither baseline serum and urinary NGAL nor their variations after PCIP were related to CIN occurrence, while CysC results were associated with CIN development, earlier than creatinine and eGFR variations.

Introduction

In recent decades, with the increasing use of intravascular iodinate contrast media for diagnostic and therapeutic purposes, contrast induced nephropathy (CIN) has become one of the more common serious complications, in particular in patients undergoing percutaneous coronary invasive procedures (PCIP) (1). In clinical practice, it is important to estimate the individual risk of developing CIN and, with regard to this, a score capable of predicting this complication has been proposed (2). Considering the classical biomarkers of renal function, serum creatinine is unfortunately poorly sensitive, being greatly influenced by many renal and non-renal factors, such as: changes in muscle mass and tubular secretion, race, age, gender, total body volume (3) and therefore it

does not help in distinguishing between the mechanisms of nephrotoxicity. Moreover, variations in creatinine values are not useful for early detection of CIN since increases in this parameter are often observed 48-72 hours after contrast medium administration.

Recent research has been addressed at finding new, more sensitive, biomarkers for the earlier diagnosis of acute kidney injury. Among them, Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Cystatin C (CysC) have been explored, but conflicting results are reported regarding their role in the early detection of CIN (4-7).

The aim of this study was to test the association of both baseline values and post-procedural variations in either urinary and serum NGAL as well as serum CysC with CIN occurrence, after PCIP compared to serum creatinine and the estimated glomerular filtration rate (eGFR).

Materials and methods

From February to August 2010 we prospectively enrolled 43 patients who were admitted to our Cardiac Step-Down Unit and submitted to PCIP, either for stable coronary artery disease such as stable angina and inducible myocardial ischemia, or for acute coronary syndromes. Exclusion criteria were the positivity of the urine culture sample and eGFR $\leq$60 ml/min/1.73 m^2. At admission and during hospitalization, the following laboratory parameters were measured in a fasting blood sample: hemoglobin (g/dL), troponin I (ng/ml), leukocyte count (N/mm^3), neutrophil count (N/mm^3), serum creatinine (mg/dL), serum CysC (ng/ml)(on admission and 4 hours after PCI), fibrinogen (mg/dl), C-reactive protein (CRP) (mg/L).

We also measured urinary and serum NGAL (ng/ml) (on admission and 4 hours after PCI); values of urinary NGAL were also adjusted for urinary creatinine values by calculating the urinary NGAL to urine creatinine ratio. For NGAL assays fresh urine and EDTA samples were collected from all patients. EDTA samples were immediately centrifuged and aliquoted. All samples were kept at -80°C until assayed in the following weeks. Serum NGAL was measured with the Triage NGAL Test (Biosite-Inverness Medical, Waltham, MA), which is a point-of-care-immunoassay for the quantitative determination of NGAL in EDTA anticoagulated whole blood or serum specimen. Urine NGAL was assessed with an automated immunoassay (Abbott Park, IL) on the Architect platform. The Architect NGAL assay is a non-competitive two-site sandwich immunoassay that utilizes two mouse antibodies, recognizing distinct NGAL epitopes. For CysC measurement, serum blood samples were kept at -20°C until assayed in the following weeks. Serum CysC was assayed by means of a nephelometric method (nephelometry Dako; IMMAGE® 800, Beckman Coulter, Inc. Fullerton, CA). All tests were performed by a well trained laboratory technician according to the manufacturers' instructions. We calculated eGFR (ml/min/1.73 m^2) with the MDRD formula. On admission, a urine culture was taken for each patient.

In agreement with previous studies, CIN was defined as an increase in serum creatinine by $\geq$25% or $\geq$0.5 mg/dl over baseline values, 48 hours after PCIP (2). The study protocol was in accordance with the Helsinki Declaration of 1975, as revised in 1983. Informed consent was obtained from all patients before enrollment. Our study had a prospective design. All data were prospectively collected in a dedicated database and analyzed.

Statistical analysis

Statistical analysis was performed with SPSS (Statistic Package for Social Sciences,

Chicago, USA) for Windows (Version 17). Categorical variables were expressed as frequencies and percentages. Continuous variables were expressed as mean values ± SD, when normally distributed, or otherwise median (range), when appropriate. Normal distribution of variables was checked by assessing the skewness and kurtosis z values, with results between -1.96 and +1.96 indicating normality, by the Shapiro-Wilk test, with a p value > 0.05, and by visual inspection of their histograms, normal Q-Q plots and box plots (data not shown). Fisher's exact test was used to compare categorical variables between CIN- and CIN+ patients. The non-parametric Mann-Whitney *U* test for unpaired data was used to compare variables between CIN- and CIN+ patients; this latter test was also used to analyze urinary and serum values of NGAL as well as the urinary NGAL/creatinine ratio either before and after PCIP in relation to the presence of inflammation, defined as neutrophil count higher than 4200/mm^3; this cutoff value for neutrophils showed association with higher leukocyte count and higher values of erythrocyte sedimentation rate or C reactive protein, confirming an inflammatory pattern. This has been previously reported in the literature to be associated with a higher prevalence of respiratory symptoms, such as bronchitis and persistent cough (8). To evaluate differences of variables analyzed between baseline and post-contrast medium administration values, the nonparametric Wilcoxon rank sum test for paired data was used. Correlations among biomarkers of renal damage and other clinical and laboratory parameters were assessed using the Pearson correlation test, when analyzing variables normally distributed, or the Spearman rank correlation test for variables not normally distributed.

To determine the best baseline CysC threshold associated with CIN occurrence, receiving operating characteristics (ROC) analysis was performed and the area under the ROC curves (AUROC) was calculated; the optimal cut off point was chosen among the coordinates of the ROC curves at the point that gave a balanced weight between sensitivity and specificity. A p value <0.05 was considered statistically significant.

Results

Our population was composed of 59 patients, of which 16 were excluded due to the positivity of their urine culture sample, or for eGFR ≤60 ml/min/1.73 m^2. In Table 1 the main clinical and angiographic characteristics of the 43 patients included in our analysis are presented. Out of them, 7 patients (16%) developed CIN. Patients who developed CIN were significantly older than those who did not.

In Table 2 laboratory data of the patients analyzed in relation to CIN occurrence are reported. Patients who developed CIN had significantly higher discharge serum creatinine values, lower eGFR values at serum peak creatinine and higher serum CysC values, either before and after PCIP. There was no significant difference in serum CysC between the values measured before and those assessed after PCIP in either of the two subgroups analyzed. Moreover, there was no significant difference between the two groups of patients in serum NGAL before and after PICP, or in urinary NGAL before and after PCIP, even after adjustment for urinary creatinine values. Patients who developed CIN underwent in a significantly higher percentage bare-metal stent implantation. In patients who developed CIN no significant variations between baseline and post-PCIP values in serum CysC, urinary and serum NGAL, or urinary NGAL/creatinine ratio were observed.

As depicted in Table 3, patients (n=14) with a positive inflammatory pattern (neutrophil count higher than 4200/mm^3)

Table 1 Clinical characteristics and angiographic data of patients enrolled

Characteristics	All patients (n=43)	CIN - (n=36)	CIN + (n=7)	p value
Age, years ($\bar{x}$ ± SD)	67.3±9.6	65.4 ±9.0	77.1±6.6	0.002
Male/Female	31/12	26/10	5/2	0.644
Overweight (BMI > 25 kg/m^2), n (%)	25 (58.1)	20 (55.6)	5 (71.4)	0.366
Diabetes mellitus, n (%)	9 (20.9)	6 (16.7)	3 (42.9)	0.147
Smoker, n (%)	24 (55.8)	22 (61.1)	2 (28.6)	0.121
Hypertension, n (%)	36 (83.7)	30 (83.3)	6 (85.7)	0.682
Previous CAD, n (%)	21 (48.8)	17 (47.2)	4 (57.1)	0.473
Previous PCI, n (%)	16 (37.2)	13 (36.1)	3 (42.9)	0.525
Previous CABG, n (%)	3 (7.0)	3 (8.3)	0 (0)	0.579
Heart Failure, n (%)	5 (11.6)	4 (11.1)	1 (14.3)	0.608
Dyslipidemia, n (%)	30 (69.8)	24 (66.7)	6 (85.7)	0.303
Family History of CAD, n (%)	15 (34.9)	13 (36.1)	2 (28.6)	0.532
Stable CAD, n (%)	17 (39.5)	14 (38.9)	3 (42.9)	0.581
NSTE-ACS, n (%)	26 (60.5)	22 (61.1)	4 (57.1)	0.581
LVEF, n (%)	55 (50-60)	55 (55-60)	55 (45-55)	0.120
Coronary artery disease extension				
No disease, n (%)	7 (16.3)	6 (16.7)	1 (14.3)	0.681
1 vessel, n (%)	6 (14.0)	5 (13.9)	1 (14.3)	0.681
2 vessels, n (%)	9 (20.9)	9 (25)	0 (0)	NA
3 vessels, n (%)	21 (48.8)	16 (44.4)	5 (71.4)	0.152
Contrast medium amount, ml ($\bar{x}$ ± SD)	264.49±136.13	266.44±140.89	254.43±117.38	0.910
PCI, n (%)	28 (65.1)	23 (63.8)	5 (71.4)	0.532
POBA, n (%)	2 (4.6)	1 (2.7)	1 (14.3)	0.302
BMS, n (%)	5 (11.6)	2 (5.5)	3 (42.8)	0.024
DES, n (%)	21 (48.8)	20 (55.5)	1 (14.3)	0.054
IABP use, *n*	0 (0)	0 (0)	0 (0)	NA
Hemodynamic instability	3 (7.0)	2 (5.6)	1 (14.3)	0.421

BMI=Body mass index; BMS=Bare metal stent; CABG=Coronary artery by-pass graft; CAD=Coronary artery disease; DES=Drug eluting stent; IABP=Intra-aortic balloon pump; LVEF=Left ventricular ejection fraction; NSTE-ACS=Non-ST-elevation Acute Coronary Syndrome; PCI=Percutaneous coronary intervention; POBA=Percutaneous only balloon angioplasty.

had significantly higher values of urinary NGAL, either pre-PCIP and post-PCIP, even when adjusted for urinary creatinine values, as well as higher values of pre-PCI serum NGAL. Moreover, using the Pearson correlation test, a significant correlation was found between age and both serum CysC pre-PCIP (r=0.62; p=0.000) and serum CysC post-PCIP (r=0.58; p=0.000), between eGFR and both serum creatinine at admission (r=-0.71; p=0.000) and serum CysC pre-PCIP (r=-0.45; p=0.003), as well as between serum CysC pre-PCIP and creatinine at the peak (r=0.47; p=0.001) and at discharge (r=0.39; p=0.01).

The Spearman rank correlation test showed a significant correlation between the serum CysC pre-PCIP and urinary

Table 2 Laboratory data in the overall study population and in relation to CIN development

Laboratory data	All patients (n=43)	CIN - (n=36)	CIN + (n=7)	p value
Admission serum creatinine, mg/dl	0.85±0.17	0.85±0.17	0.87± 0.20	0.910
Peak serum creatinine, mg/dl	0.95±0.20	0.91±0.16	1.19±0.21	0.002
Discharge serum creatinine, mg/dl	0.88±0.21	0.83±0.17	1.12±0.25	0.004
Admission eGFR, ml/min/1.73m^2	86.7±20.4	87.5± 20.4	82.7±21.9	0.356
eGFR at serum creatinine peak, ml/min/1.73m^2	77.2±19.5	81.2±17.8	56.7±15.5	<0.001
Peak TnI, ng/ml	0.21 (0.07-2.60)	0.21 (0.07-2.60)	1.80 (0.02-2.60)	0.89
Cystatin C pre-PCIP, ng/ml	1.10±0.26	1.05±0.24	1.34±0.16	0.001
Cystatin C post-PCIP, ng/ml	1.07±0.23	1.02±0.22	1.32±0.14	0.001
Urinary NGAL pre-PCIP, ng/ml	8.30 (5.50-18.60)	8.25 (5.53-17.18)	9.30 (4.60-27.70)	0.784
Urinary NGAL post-PCIP, ng/ml	8.60 (4.50-15.90)	8.25 (4.58-15.88)	9.20 (3.80-18.50)	0.735
Adjusted Urinary NGAL pre-PCIP*, ng/ml	10.92 (6.81-20.30)	10.78 (6.42-19.96)	15.87 (7.44-27.06)	0.664
Adjusted Urinary NGAL post-PCIP*, ng/ml	13.22 (8.19-30.99)	13.10 (8.28-26.63)	15.63 (7.93-38.21)	0.784
Serum NGAL pre-PCIP, ng/ml	76 (60-184)	76 (60-172)	94 (60-272)	0.640
Serum NGAL post-PCIP, ng/ml	104 (60-181)	107 (60-172)	97 (60-209)	0.936
Admission Hb, g/dl	13.5 (12.6-14.5)	13.5 (12.7-14.5)	13.5 (10.8-15.0)	0.987
Nadir Hb, g/dl	12.05±1.69	12.09±1.62	11.81±2.12	0.488
Admission Leucocytes, N/mm^3	7290 (5850-8920)	7090 (5730-8770)	7560 (6780-10400)	0.356
Peak Leucocytes, N/mm^3	8100 (6360-9710)	8040 (6295-9700)	9190 (7920-10600)	0.176
Neutrophils, N/mm^3	3360 (2350-4890)	3180 (2300-4390)	4510 (3100-7520)	0.147
Fibrinogen peak, mg/dl	483.19±98.15	468.33±91.92	559.57±100.11	0.024
ESR, mm/h	13 (7-23)	13 (7-21)	13 (6-30)	0.984

eGFR=Estimated glomerular filtration rate; ESR Erythrocyte sedimentation rate; Hb=Hemoglobin; NGAL=Neutrophil Gelatinase-Associated Lipocalin; PCIP=Percutaneous coronary invasive procedures; TnI=Troponin I. *Adjusted for urinary creatinine values. Values are presented as mean ± standard deviation when normally distributed or, otherwise, as median (interquartile range).

Table 3 Comparison between inflammatory and non-inflammatory states in relation to the timing of samples collection with respect to PCIP

Parameters	Inflammation* (n=14)	No Inflammation (n=29)	p value
Urinary NGAL pre-PCIP, ng/ml	16.60 (9.73-28.80)	7.80 (5.10-14.15)	0.005
Urinary NGAL post-PCIP, ng/ml	16.05 (9.05-20.00)	6.10 (3.80-10.65)	0.002
Urinary NGAL pre-PCIP/creatinine, ng/ml	18.11 (15.03-27.93)	8.49 (4.97-14.20)	0.002
Urinary NGAL post-PCIP/creatinine, ng/ml	20.30 (11.60-39.71)	12.43 (7.76-22.42)	0.046
Serum NGAL pre-PCIP, ng/ml	126 (86-216)	68 (60-121)	0.034
Serum NGAL post-PCIP, ng/ml	125 (102-188)	68 (60-170)	0.10

*Defined as neutrophil count higher than 4200/mm^3; NGAL=Neutrophil Gelatinase-Associated Lipocalin; PCIP=Percutaneous coronary invasive procedures. All values are reported as median (interquartile range).

NGAL post-PCIP/creatinine ratio (r=0.32; p=0.035), and urinary NGAL post-PCIP (r=0.43; p=0.004) in the overall population of patients, and a mild but significant correlation between serum creatinine at discharge and NGAL post-PCIP/creatinine ratio (r=-0.30; p=0.048).

The results from the ROC curve showed that the optimal cut-off value of serum CysC for CIN diagnosis was 1.18 ng/ml. For this value, sensitivity was 0.857 and specificity 0.778 (AUROC=0.863; p=0.003). Finally, by univariate analysis using this latter cut-off, CysC values higher than 1.18 ng/mL resulted in a significant association with CIN occurrence (p=0.003).

Discussion

The main finding of our investigation was that in patients undergoing PCIP, high values of serum CysC, even at baseline, may be considered a useful marker associated with CIN occurrence, earlier than creatinine and eGFR variations. Furthermore, in our series, neither serum nor urinary NGAL showed any significant association with the development of CIN. Data about these novel biomarkers as predictors of acute kidney injury after PCIP are inconsistent and contradictory.

It is well known that CysC, a protease inhibitor, is freely filtered in normal circumstances by the glomeruli and completely reabsorbed in the proximal tubule. In the absence of tubular dysfunction, its serum levels reflect glomerular filtration; therefore it could be used as a convenient measure of glomerular filtration rate (9). CysC is not affected by storage conditions or interfering substances: some limitations of the serum creatinine level, including the effects of gender, body muscle mass and diet, do not significantly influence serum CysC levels. Elevated urine CysC levels may also indicate tubular epithelial damage, and have been proposed as an additional urine biomarker for acute kidney injury (10). In particular, in intensive care settings, serum CysC has been able to detect acute kidney injury earlier and has been more sensitive than serum creatinine in detecting minor glomerular filtration rate reductions (11), even in patients with sepsis (12).

This early increase in serum CysC following a kidney injury is also reported in studies that investigated renal toxicity after contrast medium administration (4-6, 13-18). In all these investigations the peak of serum CysC was significantly associated with the development of CIN occurring 24-48 hours after PCIP. Moreover, in agreement with previous studies (12, 19, 20), our investigation demonstrated that high baseline values of CysC are associated with CIN occurrence.

On the other hand, in the study by Rubichini and colleagues (7), variations in serum creatinine from the baseline offered better diagnostic accuracy for predicting CIN at an earlier stage than similar changes in CysC in patients with known mild or moderate chronic renal disease, undergoing PCI. However, in our investigation we evaluated patients with eGFR $\geq$60 ml/min/1.73 m^2.

Although our study strengthens the role of baseline CysC as a biomarker early associated with CIN occurrence in the setting of PCIP, as already reported (20), no significant variation in CysC in the subgroup of patients developing CIN was observed after contrast medium administration. Interestingly, patients who developed CIN were significantly older and a significant correlation was found between CysC and patient age. Moreover, CysC pre-PCIP results were significantly correlated with values of urinary NGAL assessed after PCIP, even after adjustment for urinary creatinine values, as well as with creatinine at the peak and at discharge, confirming that even values of CysC at baseline may be associated with CIN since they correlate with later changes in creatinine, as

well as in other markers of acute kidney injury, assessed after contrast medium administration.

Furthermore, in our series we also evaluated both urinary and serum NGAL values as possible helpful markers for CIN development. NGAL, a small protein of the lipocalin superfamily, expressed by a wide variety of tissues, is primarily synthesized in the ascending loop of Henle and in the collecting ducts. It acts as a growth and differentiating factor in renal epithelium, by limiting apoptosis and maintaining the tubular structure. Raised NGAL concentrations might represent a physiological or adaptative response to injury (21), since it is has a nephroprotective effect in the proximal tubule. NGAL is rapidly upregulated in kidneys after an ischemic or nephrotoxic insult, and limits parenchymal damage (22). For these reasons NGAL has been explored as a biomarker of acute and chronic kidney injury. Both serum and urinary NGAL concentrations have been shown to be good predictors of CIN development in previous several studies (4, 23). Bachorzewska-Gajewska and colleagues (4) demonstrated in patients with stable angina that NGAL levels were significantly higher in patients with CIN from 2 hours after PCI (serum, $p<0.005$) and 4 hours (urinary, $p<0.005$), respectively. In another study (23), a significant increase in both serum and urinary NGAL occurred in patients developing CIN two hours after contrast medium administration. However, although NGAL is considered a promising biomarker for early diagnosis of acute tubular necrosis (24), serum NGAL is already elevated in patients with chronic renal disease, and it rises 6-times less than urinary NGAL in cases of renal injury (7).

Furthermore, urinary NGAL remains low in patients with pre-renal azotemia or normal renal function (25). Urinary NGAL is of interest after cardiac surgery; in this setting its values resulted in a significant association for early diagnosis of acute kidney injury (26, 27). Haase et al. (28) analyzed data from 191 patients in 3 studies and found that when urinary NGAL is measured within 6 hours after contrast medium administration, it has better sensitivity than serum creatinine alone for acute kidney injury detection. Moreover, recent studies and a meta- analysis have demonstrated the role of serum and urinary NGAL in the prediction of CIN (15, 16, 29-36) and, although this biomarker has also been reported to have a low sensitivity in predicting CIN in specific settings, such as ACS (37), a previous study also suggested that it can also predict the severity of CIN (38).

Despite the positive results of these biomarkers in predicting CIN in the above mentioned studies, in our investigation we did not detect any significant association between baseline serum and urinary NGAL values and their 4 hour variations after contrast medium administration and CIN occurrence. Our results may have several explanations: first of all, our analysis included patients with acute coronary syndromes, a condition in which inflammation is present, so that in these patients raised serum NGAL levels may reflect the degree of inflammatory status (39) due to the acute cardiac disease more than that of acute kidney injury. Accordingly, in our series, we observed that baseline values of either serum and urinary NGAL were significantly higher in patients with a higher neutrophil count, making the post-PCIP variations less significant. On the contrary, we believe that this biomarker could be useful for CIN detection in patients with stable coronary artery disease, as previously reported (4, 23), without other causes of acute inflammation, such as urinary tract infections or acute exacerbations of obstructive pulmonary disease, which is not the typical population of Cardiac Step Down or Intensive Care Units.

Though strong evidence has been provided for the use of NGAL in a variety of settings, its route into clinical management protocols is still under evaluation (40). Moreover, our results are in agreement with those of a recent review reporting the "rise and fall" of NGAL in acute kidney injury (41). In fact, authors conclude that since a rise in NGAL was first suggested more than a decade ago as a potential biomarker of early acute injury, the idea of this marker as a troponin for the kidneys has been relinquished because it also increases unpredictably during other chronic and acute inflammatory conditions frequently encountered in intensive care settings (41). The availability of assays measuring kidney-specific NGAL will improve the chance of detecting patients at risk of acute kidney injury.

Limitations of study

Our study has also several limitations: first of all, a limited number of patients investigated; and second, the heterogeneity of coronary artery disease patients, for whom PCIP were performed.

Conclusion

In conclusion, our investigation demonstrates that in a population of patients admitted to a Cardiac Step-Down Unit and submitted to PCIP, neither baseline serum and urinary NGAL nor their variations after PCIP are useful for an early diagnosis of CIN. In particular, the use of this biomarker to detect CIN is limited in ACS patients, due to its inability at discriminating between inflammatory injury associated with CIN development or with the acute cardiac disease *per se*. On the contrary, in our observation, CysC resulted in an association with CIN occurrence earlier than creatinine and eGFR variations, even though further research in this area is needed, given the limitations of this study.

What is already known on this topic

Contrast induced nephropathy (CIN) is a common and serious complication in patients undergoing percutaneous coronary invasive procedures (PCIP). Serum creatinine is poorly sensitive for CIN detection and its variations are not useful for an early diagnosis of this complication. Recent research has been addressed at finding novel, more sensitive, biomarkers for an earlier diagnosis of CIN; among them, Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Cystatin C (CysC) have been explored but with conflicting results.

What this study adds

In this study, neither baseline serum and urinary NGAL nor their variations after PCIP were related to CIN occurrence, while CysC results were associated with CIN development, earlier than creatinine and estimated glomerular filtration rate variations. Moreover, NGAL values results were significantly associated with markers of acute inflammation, suggesting that this biomarker could be useful for CIN detection in patients with stable coronary artery disease, or when no other causes of acute inflammation are present.

Authors' contributions: Conception and design: EC and CG; Acquisition, analysis and interpretation of data: GA and AT; Drafting the article: MGD; Revising it critically for important intellectual content: AC and EG; Approved final version of the manuscript: EC, CG and AC.

Conflict of interest: The authors declare that they have no conflict of interest.

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Original article

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The position of the mental foramen in the north and south Indian populations

Naveen Srinivas[1], Praveenkumar Ramdurg[1], Surekha R Puranik[1], Ketki Sali[2], Pramod Ingaleshwar[3]

[1]Department of Oral Medicine and Radiology, PMNM Dental College and Hospital, Bagalkot, [2]Private practitioner Shivleela Dental Clinic Bagalkot
[3]Department of Oral Pathology, PMNM Dental College and Hospital, Bagalkot

Correspondence:
nvns03@gmail.com
Tel.: + 919 964 140 521
Fax.: + 083 54 2 219 60

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Objective. The aim of the study was to determine and compare the position of the mental foramen MF in the north and south Indian populations using Panoramic radiographs. **Materials and methods.** A total of 100 Panoramic radiographs were selected from the archives of PMNM Dental College and the Department of Oral Medicine and Radiology, of which 50 radiographs belonged to a north Indian population and 50 radiographs belonged to a local population of Bagalkot Karnataka. The position of the MF was classified into 6 positions depending on the relationship to the mandibular teeth. The distance from the superior border of the MF to the lower border of the mandible was also measured. **Results.** The most common position of the MF was along the long axis of the second premolar in the north Indian population and in south Indian populations it was found to be between the first and second premolar. Descriptive analysis was used to compare the distance between the superior border of the mental foramen and the lower border of the mandible bilaterally. **Conclusion.** There was a significant difference in the position of the MF between the north and south Indian populations.

Introduction

The mental foramen (MF) is a funnel-like opening in the lateral surface of the mandible at the terminus of the mental canal (1). The mandibular nerve exits the mental foramen along with the mental vessels, providing both innervation and blood supply to the lower lip, the gingiva on the ipsilateral side anterior to the first molar, and the buccal vestibule (2). The MF is a significant and vital landmark in the mandible during routine clinical and diagnostic procedures, such as mental nerve block and other osteotomy procedures. It is also a very important structure for evaluation of the maturity of the lower jaw, placement of implants, remodelling activity of the bone and, more importantly, the paleoanthropological features of the lower jaw in a range of ethnic groups (3).

Due to its position, it is difficult to evaluate the position of the MF clinically. Radiographically the MF is appreciated as a round or oval radiolucent area in the inferior part of the body of the mandible on both sides (4). Due to the oblique path of the mental canal, it is difficult to identify the MF consistently on intra oral radiography. Panoramic radiographs are the most commonly used extraoral radiographic aids in dentistry since they provide a rapid and wide picture

of both the mandible and the maxilla. This technique allows the more accurate localization of the MF (5). The location of the MF may change with age. In childhood it is located between the primary canine and the 1st molar, whereas in the edentulous jaw, it is nearer to the upper border of the mandible due to bone resorption. (6). The position of the MF varies not only with age, sex and ethnicity, but even within the same race in different topographic regions, and within the same population in the same geographic location (1, 5, 7-9).

The purpose of the present study was to determine and compare the position of the MF in north and south Indian sub populations, using an orthopantomogram (OPG).

Materials and methods

The study was conducted at the Department of Oral Medicine and Radiology, Parvatha Gowda Mallanagouda Nadagouda Memorial Dental College and Hospital, from the demographic data and radiographs of patients who had visited the college for various dental treatments. A total of 2648 OPGs were examined between 2013 and 2015, and 100 radiographs were selected, of which 50 radiographs belonged to the north Indian population and 50 radiographs belonged to the local population of Bagalkot Karnataka. All the radiographs were taken using a Kodak 8000C (tube voltage 60-90 kV, tube current 2-15 mA, tube focal spot 0.5 mm, total filtration >2.5 mm eq. Al with exposure time of 4-14 seconds). Two oral radiologists were asked to analyse the radiographs, who were blinded about the study. Institutional ethical clearance was obtained. Inclusion criteria: 1. Bilateral presence of a mental foramen which could be clearly identified on the radiograph; 2. Presence of all the teeth in the mandible between the right second and left second molars; 3. Patients aged 16-45 years; 4. Absence of crowding of the mandibular premolar and canine area. Exclusion criteria: 1. Patients with periodontal problems such as mobility of the posterior teeth, supra-eruption and bone loss; 2. Periapical lesions in the mandible; 3. Patient who are taking/ had undergone orthodontic treatment; 4. Patients who have undergone maxillofacial surgery of the mandible; 5. Radiographs with impacted or mixed dentition; 6. Poor quality of radiograph. 7. Supra-eruption or pathological migration of mandibular teeth.

The position of the MF on the OPG was classified as per the criteria given by Al Jasser NM, Nwoku AL (10-12). Position 1: MF

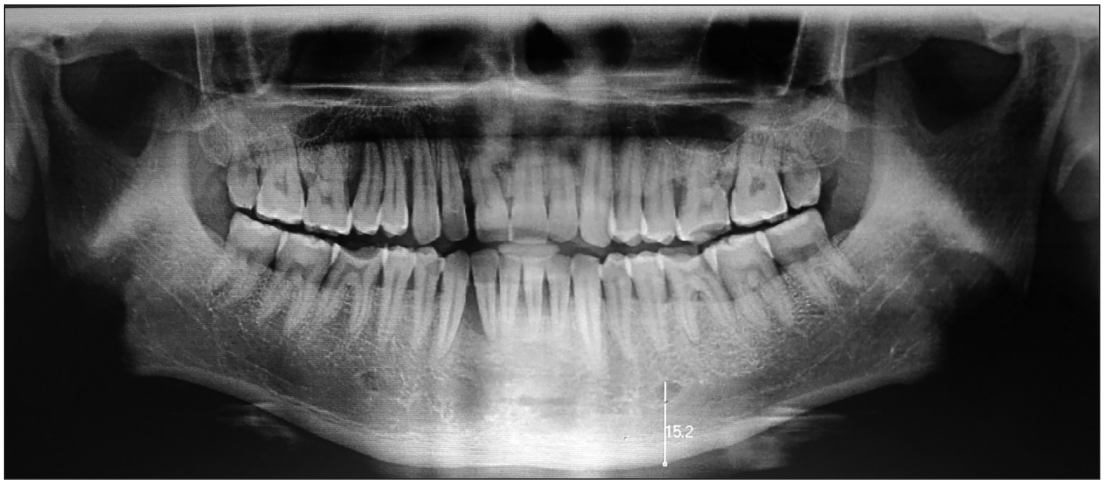

Figure 1 Orthopantomogram showing the distance between the superior border of the mental foramen to the inferior border of the mandible.

situated anterior to the first premolar tooth; Position 2: MF situated in line with the long axis of the first premolar tooth; Position 3: MF situated between the apices of the first and second premolar teeth; Position 4: MF situated in line with the long axis of the second premolar tooth; Position 5: MF situated between the apices of the second premolar and first molar teeth.; Position 6: MF situated in line with the long what the first molar tooth.

The vertical position of the MF was measured in millimetres with reference to the lower border of the mandible, using Kodak dental imaging software 6.12.11.0, from the superior border of the mental foramen on either side, to the lower border of the mandible (Figure 1).

Statistical analysis

Descriptive statistics was used to measure the percentages with respect to the position of MF in both north and south Indian populations. An independent t test was done to compare the mean value of the superior border of the mental foramen to the lower border of the mandible in both north and south Indian populations on both the left and right sides of the mandible.

Results

Table 1 shows the percentages with respect to the position of the MF in both north and south Indian populations. Position 4 was the most common, followed by position 3, with 58% and 18% respectively in the north Indian population. In the south Indian population position 3 was most common, with 62%, followed by position 4, with 24%.

Table 2 shows the comparison of the mean value of the superior border of the MF to the lower border of the mandible on the left side, giving a mean value of 1.32 and a standard deviation of 2.00 w.r.t with respect to the south Indian population, and a mean value of 1.07 and a standard deviation of 2.64 w.r.t with respect to the north Indian population, which is a highly significant difference, with a p value of <0.001. Similarly, Table 2 gives a mean value of 1.27 with a standard deviation of 2.51 w.r.t with respect to the south Indian population, and a mean value of 1.02 and a standard deviation of 2.55 w.r.t with respect to the north Indian population, which is a highly significant difference with a p value of <0.001 on the right side.

Table 3 shows statistical analysis using an independent t test for comparison of mean

Table 1 Position of the mental foramen on Orthopantomograms

Position of mental foramen	Cases out of 50 in north Indian population (n)	% of north Indian population	Cases out of 50 in south Indian population (n)	% of south Indian population
MF situated anterior to the first premolar tooth	3	6	1	2
MF situated in line with the long axis of the first premolar tooth	8	16	1	2
MF situated between the apices of the first and second premolar teeth	9	18	31	62
MF situated in line with the long axis of the second premolar tooth	29	58	12	24
MF situated between the apices of the second premolar and first molar teeth	0	0	5	10
MF situated in line with the long axis of the first molar tooth	1	2	0	0

MF=Mental foramen.

Table 2 The distance from the superior border of the MF to the lower border of the mandible in north and south Indian populations, on the left and right sides

Area	Radiograph (n)	Distance (mm)*	
		Left side (Mean±SD)	Right side (Mean±SD)
South Indian	50	1.32±2.00†	1.27÷2.51†
North Indian	50	1.07±2.64	1.02±2.55

*Between the superior border of the mental foramen and the lower border of the mandible.

value of the superior border of the mental foramen to the lower border of the mandible in north and south Indian population on the left side, with Levenes's test for Equality of Variances. A significant difference was seen, with a mean difference of 2.49 and *p* value of 0.01.

Table 4 shows analysis using an independent t test for comparison of the mean value of the superior border of the mental foramen to the lower border of the mandible in north and south Indian populations on the right side with Levenes's test for Equality of Variances. A significant difference was seen, with a mean difference of 2.51 and p value of 0.36.

Discussion

The present study is in accordance with similar studies conducted in Rajasthan, in the north-eastern states of India (13), Gujurath (14), and Kashmir (15) which are states in the northern part of India. The most common position in the south Indian population was position 3, situated between the apices of the first and second premolar teeth,

Table 3 Comparison of the mean value of the superior border of the mental foramen to the lower border of the mandible in north and south Indian populations on the left side

	Levene's test for equality of variances		t-test for equality of means						
	F	Sig.*	t	Df	Sig. (2-tailed)*	Mean difference	Std. error difference	95% CI† of the difference	
								Lower	Upper
LTSL‡	6.38	0.01	5.3	98	0.000	2.49	0.46	1.55	3.42
LTSL§	-	-	5.3	91.3	0.000	2.49	0.46	1.55	3.42

*Independent Samples Test (p value); †Confidence Interval; LTSL=Distance from the left side superior border of the mental foramen to the lower border of the mandible (‡Equal variances assumed;n§Equal variances not assumed).

Table 4 Comparison of the mean value of the superior border of the mental foramen to the lower border of the mandible in north and south Indian populations on the right side.

	Levene's test for equality of variances		t-test for equality of means						
	F	Sig.*	t	Df	Sig (2-tailed)*	Mean difference	Std. error difference	95% CI† of the difference	
								Lower	Upper
RTSL‡	0.84	0.84	0.36	4.96	98	0.000	2.51	0.50	1.51
RTSL§	-	-	4.96	97.97	0.000	2.51	0.5	1.51	3.52

*Independent Samples Test (*p* value); †Confidence Interval; RTSL=Distance from the right side superior border of mental foramen to the lower border of mandible (‡Equal variances assumed; §Equal variances not assumed).

seen in 62% of cases. The results obtained are analogous to the studies performed in the southern states of India (11, 16, 17). The present study also added another parameter, i.e. the distance between the superior border of the MF and the lower border of the mandible bilaterally. The results showed that there was a significant difference between the measurements, giving a mean value of on 1.27 and 1.02 and a SD of 2.51 and 2.55 for the south and north Indian populations respectively, on the right side. Similarly a mean value of 1.32 and 1.07 and a SD of 2.00 and 2.64 for the south and north Indian populations, respectively, was found on the left side. This indicated that the mental foramen was closer to the lower border of the mandible in the north Indian population.

The reason for the difference in the morphological alterations in the position of the MF may be attributed to the different ethnic origins. Aryans who migrated to India from central Asia constitute the north Indian population while Dravidians constitute the local south Indian population (18). Another reason may be the differences in diet and climate, which influence body height and craniofacial variability (19). It was noted from the results of our study that there is a significant difference in the position of the MF in the two study cohorts. The results may be used as a unique identification landmark in cases of mass disaster, forensics, and anthropological studies. Furthermore, they may be helpful in various dental procedures, such as nerve block, implant placement, and orthognathic surgeries.

Limitations of the study

The sample size was small considering the duration of the study which was 2 years, due to the smaller inflow of the north Indian population, as our institution is situated in the southern part of India. The other limitation of the study is that the magnification of the OPG was not taken into consideration. Hence a definite conclusion cannot be drawn.

Conclusion

The most common position of the MF in the south Indian population was between the two mandibular premolars, and in the north Indian population it was below the long axis of the second mandibular premolar, so there was a significant difference in the distance between the superior border of the MF and the lower border of the mandible bilaterally between the two populations.

What is already known on this topic

The mental foramen is a prominent radiographic landmark in the lower jaw, where the mandibular nerve exits the mandible and supplies the anterior teeth and the soft tissues. There have been various studies conducted by different authors regarding the position of the mental foramen with respect to mandibular pre-molars and molars, along with its location in different populations. However, no comparison of the position of the mental foramen between two different ethnic groups has been attempted before.

What this study adds

In the present study we attempted to find the relationship between the position of the mental foramen between the north and south Indian populations. We speculated that in the south Indian population the most common position of the mental foramen was between the two mandibular premolars, and in the north Indian population it was below the long axis of the second mandibular premolar. We also added a new parameter, which is the distance between the superior border of the mental foramen and lower border of the mandible, whereby it was calculated that the mental foramen was closer to the lower border in the north Indian population.

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Case report

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Targeted temperature management after out-of-hospital cardiac arrest in three young patients

Amina Godinjak[1], Amer Iglica[2], Adis Kukuljac[3], Ira Tančica[4], Selma Jusufović[5], Anes Ajanović[6], Šejla Rožajac[7]

[1]Medical Intensive Care Unit, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina; [2]Clinic of Heart Vascular Disease and Rheumatology Clinical Center University of Sarajevo Sarajevo, Bosnia and Herzegovina [3]Medical High School Sarajevo, Bosnia and Herzegovina; [4]Clinic of Gastroenterology Clinical Center University of Sarajevo Sarajevo, Bosnia and Herzegovina; [5]Clinic of Nuclear Medicine and Endocrinology Diabetes and Metabolic Disorders, Clinical Center University of Sarajevo, Sarajevo Bosnia and Herzegovina, [6]Clinic of anesthesiology and reanimation, Clinical Center University of Sarajevo, Sarajevo Bosnia and Herzegovina [7]Primary Health care Center Fojnica, Fojnica, Bosnia and Herzegovina

Correspondence:
aminagodinjak@gmail.com
Tel.: + 387 33 29 70 00
Fax.: + 387 33 26 59 10

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Objective. We present the use of targeted temperature management in a tertiary-level intensive care unit, in three patients who experienced an out-of-hospital cardiac arrest. **Case report**. Three young patients experienced an out-of-hospital non-coronary cardiac arrest. The causes of the cardiac arrest were: Wolf-Parkinson-White syndrome, drug overdose and long-QT syndrome. All patients were resuscitated according to the advanced cardiac life support guidelines, and treated with targeted temperature management, with a target temperature of 33°C for 24 hours. After completion of targeted temperature management, all the patients regained full consciousness and were discharged from hospital without any neurological sequelae. **Conclusion.** Targeted temperature management may improve survival and neurological outcome in patients after out-of-hospital cardiac arrest.

Introduction

The introduction of targeted temperature management (TTM) in patients after out-of-hospital cardiac arrest (OHCA) is one of the most important clinical advancements in the field of resuscitation. The American Heart Association issued a Guidelines update in 2015 for cardiopulmonary resuscitation and emergency cardiovascular care, recommending that all comatose adult patients with return of spontaneous circulation (ROSC) after cardiac arrest should be treated with TTM, and the target temperature of 32°-36°C maintained constantly for at least 24 hours (1). Although this practice is now widely used in the developed world, the developing countries have lagged behind in use of this practice, including Bosnia and Herzegovina.

We present three young patients who experienced OHCA and who were managed with TTM with a target temperature of 33°C in a tertiary-level intensive care unit.

Case report I

A 24-year-old man collapsed during a university lecture and his friends immediately

started bystander resuscitation. He was unconscious, pulseless, and ventricular fibrillation (VF) was established by the emergency medical services, which arrived 6 minutes later. The advanced cardiac life support guidelines were followed, with cardiopulmonary resuscitation (CPR) continuously performed and two defibrillations, followed by IV adrenaline and amiodarone, which resulted in ROSC after 25 minutes. The patient was hospitalized at the Medical Intensive Care Unit (MICU). Physical examination showed coma with a Glasgow coma scale (GCS) score of 6/15 (e1, v1, m3), and blood pressure (BP) 113/54 mmHg. Electrocardiogram (ECG) showed a sinus rhythm with a ventricular rate of 92 bpm, short PR interval and delta wave, without signs of acute ischemia or myocardial lesion. He was sedated and placed on mechanical ventilation. He was treated with cold IV saline, proton pump inhibitor and anticoagulant. Two hours after the OHCA, TTM was started using the Arctic Sun® Temperature Management System (Model 2000, Medivance Inc., USA) with a target temperature of 33oC achieved 4 hours after ROSC. Analgosedation was achieved by IV midazolam and fentanyl infusion, and vecuronium was used for neuromuscular blockade. Laboratory data revealed elevated creatinine kinase 675 U/L, aspartate aminotransferase 66 U/L, alanine aminotransferase 84 U/L, troponin 0.243 ng/mL and D- dimer 4.0 mg/L. Twelve hours after admission, the serum troponin level increased to 1.590 ng/ml. On the second day following admission, after completion of TTM and cessation of sedatives, he regained consciousness and was successfully extubated. Heart ultrasound showed a morphologically and functionally normal heart. He was transferred to the Clinic of Heart Disease for further evaluation of suspected Wolff–Parkinson–White syndrome. He was released from the hospital fully recovered. At a recent follow-up, he was completing his Master's degree in History.

Case report II

A 26-year-old man was found unconscious in his bedroom on the morning after a party. He was taken by car to the nearest medical practice. He was unconscious and pulseless, without spontaneous respiration. ECG showed VF. Cardiopulmonary resuscitation was immediately commenced with chest-compressions, intubation, IV adrenaline, amiodarone, naloxone and four defibrillations, which resulted in ROSC after 35 minutes. He was hospitalized at the MICU. Physical examination showed deep coma with GCS 3/15 (e1, v1, m1). ECG showed sinus rhythm with a ventricular rate of 80 bpm, and no signs of acute myocardial ischemia. Thoracic CT scan showed signs of aspiration pneumonia. Toxicology results were positive for amphetamines and cannabinoids, making a drug overdose the most probable cause of cardiac arrest. After admission he was sedated and placed on mechanical ventilation. He was treated with cold IV saline, antibiotics, proton pump inhibitor and anticoagulant. Three hours after cardiac arrest, TTM was started using the Arctic Sun® Temperature Management System (Model 2000, Medivance Inc., USA) with a target temperature of 33°C which was achieved 6 hours after ROSC. On the second day, after completion of TTM and cessation of sedatives, he regained full consciousness. Aspiration pneumonia was treated with antibiotics. After conservative treatment, he recovered fully and was released from hospital. At a recent follow-up, his sister reported that he was in good health, but she suspected that he was still abusing drugs, and he was therefore referred to the Drug and Alcoholism Rehabilitation Center.

Case report III

A 23-year-old woman collapsed during a dinner with friends. She was unconscious, with no pulse or respiration. Her friends immediately started resuscitation and called the EMS, which arrived 10 minutes later. ECG showed VF. The advanced cardiac life support recommendations were followed, with continuous CPR and one defibrillation, adrenaline and amiodarone, which resulted in ROSC after 15 minutes. She was admitted to the MICU. Physical examination showed coma with GCS 4/15 (e2, v1, m1). ECG showed sinus rhythm with HR 93 bpm, prolonged QT interval and ventricular extra-systoles (bigeminy). Immediately after admission, she was sedated and placed on mechanical ventilation. She was treated with cold IV saline, antibiotics, continuous antiarrhythmic and anticoagulant. Two hours after ROSC, TTM was started using the Arctic Sun® Temperature Management System and the target temperature of 33°C was achieved 6 hours after ROSC. Analgo-sedation was achieved by continuous IV midazolam and fentanyl infusion. Laboratory data revealed elevated lactate, 7.4 mmol/L, leucocyte count 18.5 x 10^9/L, CK 220 U/L, LDH 387 U/L, AST 51 U/L, ALT 51 U/L, and decreased potassium, 2.8 mmol/L On the second day she regained consciousness. She had no neurological deficit and was transferred to the Clinic for Heart Disease, where the diagnosis of long-QT syndrome was established. She was referred for implantation of an internal cardioverter defibrillator. At a recent follow up, her brother reported that she was on a trip overseas, fully recovered.

Discussion

Sudden cardiac arrest occurs in 250,000-300,000 people in Europe every year (2). Survival in patients with out-of-hospital cardiac arrest is less than 15%, while survival with in-hospital cardiac arrest is approximately 22% (3, 4). Only 5-35% of them leave the hospital alive, with some degree of neurological impairment (5). Seventy percent of all OHCA are due to coronary heart disease. A further 10% are caused by structural heart disease (e.g. hypertrophic cardiomyopathy) (6), and 5% to 10% are due to arrhythmic causes, in the absence of structural heart disease. In the absence of structural or electrophysiological abnormality, these entities are termed as primary electrical disease (7). Fifteen to 25% of OHCA are non-cardiac in origin (8).

The benefits of TTM have been summarized in a systematic review and meta-analysis of six randomized trials (9). According to this review, patients treated with TTM were more likely to survive than patients whose temperature was not managed with TTM.

However, TTM is is associated with a number of adverse effects and complications. The main adverse effects reported are: shivering, seizures, bradycardia, tachyarrhythmia, pneumonia, sepsis, coagulopathy, electrolyte and metabolic disturbances (10). All three of our patients experienced bradycardia with heart rate as low as 40 bpm. Recently, bradycardia during the cooling phase of TTM has been suggested to be a predictor of favorable outcome (11). Also, two patients in our case study experienced hyperglycemia during the cooling phase. Hypothermia leads to metabolic rate decline, reduces endogenous insulin secretion, induces insulin resistance and alters blood glucose homeostasis (12). Hyperglycemia is a part of the natural response to hypothermia and is regarded as being safer than the occurrence of hypoglycemia. Our patients experienced transient hypokalemia, which was appropriately treated. Hypokalemia during TTM was not associated with poor outcome, as reported in previous studies (13). Shivering was observed in one patient and was managed by the use of neuromus-

cular blockade. The lack of major adverse effects of TTM may be explained by the fact that our patients were young individuals without comorbidities, and this may be a limitation of our study.

Only a decade ago, a minority of resuscitated patients were treated with TTM in both American and European intensive care units (14). Ten years ago, TTM was utilized in only 38% of departments treating patients after OHCA in Germany (15). Although in recent years there have been major improvements in the use of TTM, this practice is still not widely used in developing countries due to limited financial resources and a lack of awareness of this treatment method. A previous study showed that 15% of health care professionals in developing countries have used TTM in comatose survivors after cardiac arrrest (16).

Before our MICU was equipped with the proper cooling system, alternative cooling methods (cold IV fluids and surface cooling with ice packs, wet blankets and a cooling fan) were utilized, which should encourage other health care professionals in intensive care units with limited resources in Bosnia and Herzegovina and further afield to start using this treatment option.

Conclusion

Sudden cardiac arrest is one of the most unexpected, dramatic, and life-threatening events in medicine. In this paper, we have presented three young patients experiencing non-coronary OHCA. The causes of cardiac arrest were: Wolf-Parkinson-White syndrome, drug overdose and long-QT syndrome. All patients were resuscitated according to the advanced cardiac life support guidelines. They were admitted to the medical intensive care unit, and treated with TTM, with a target temperature between 32°C and 36°C, which was maintained constantly for 24 hours. After completion of TTM all patients regained full consciousness and were discharged from hospital without any neurological sequelae. This report further demonstrates the feasibility of TTM in limited resource settings, and should encourage other intensive care units in Bosnia and Herzegovina and further afield to use TTM in adult patients after OHCA, because it is technically feasible in developing countries.

What is already known on this topic

Targeted temperature management is one of the most important clinical advancements in the field of resuscitation. The incidence of a neurologically good outcome is remarkably improved with this method in selected patients, after out-of-hospital cardiac arrest.

What this study adds

Although targeted temperature management is widely used in medical and coronary intensive care units in developed countries, this practice is still not widely used in developing countries. This is the first case report describing the use of targeted temperature management in patients after cardiac arrest in Bosnia and Herzegovina. When OHCA occurs in younger patients, we must diligently search for less frequent causes of cardiac arrest in the absence of structural heart disease.

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Case report

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Tissue necrosis following extravasation of acyclovir in an adolescent: A case report

Charalambos Neocleous, Eleni Andonopoulou, Alkistis Adramerina, Antigoni Pegkou, Olga Savelieva, Petroula Georgiadou, Ioannis Drikos

Department of Medical Pediatrics
Thriasio General Hospital, Athens
Greece

Correspondence:
xneoc@yahoo.gr
Tel.: + 30 695 509 99 35
Fax.: + 30 210 554 60 39

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Objective. Extravasation of intravenously infused vesicant solutions is a common problem in medical practice, which can lead to severe and progressive tissue dysfunction, ranging from persistent tissue oedema and fibrosis to delayed tissue necrosis. Acyclovir is a known vesicant medication administrated in paediatric patients, which appears to irritate venous and soft tissue if extravasated. **Case report.** We present the first case involving the extravasation of intravenously infused acyclovir in a female adolescent patient, which caused tissue necrosis and left behind a residual scar lesion. Nursing and medical staff should be aware of the potential dermatological side effects of intravenously infused acyclovir and other medications, even a long time after infusion, and the possible lack of initial local symptoms and signs. **Conclusion.** Early recognition of extravasation and prompt management are critical in preventing further morbidity, and optimizing outcomes.

Introduction

Extravasation of intravenously infused vesicant solutions is a common problem in medical practice, which can lead to severe and progressive tissue dysfunction, ranging from persistent tissue oedema and fibrosis to delayed tissue necrosis (1, 2). Acyclovir is a known vesicant medication administrated in paediatric patients, which appears to irritate venous and soft tissue if extravasated (3, 4). We report here the first case involving extravasation of intravenous (IV) acyclovir in a female adolescent patient, which caused tissue necrosis and left behind a residual scar lesion.

Case presentation

A 14-year-old female patient was admitted to our hospital for a two-day-long fever and headache. On admission, her general condition was affected and the patient appeared drowsy. Her temperature reached 37.9°C. A neurological examination revealed neck rigidity with negative Kerning and Brudzinski signs, while a further physical examination revealed no remarkable findings. A lumbar puncture revealed clear cerebrospinal fluid with 15 leucocytes/mm^3 (40% polymorphs and 60% lymphocytes). The levels of glucose and protein in the cerebral vascular fluid were within normal limits. A cerebrospinal fluid (CSF) specimen was cultured for common bacterial pathogens of the central nervous system (CNS), and it was also examined by PCR for common bacterial and viral pathogens (including Herpes Simplex Virus (HSV1 and HSV2) of CNS.

Haemoglobin, total leukocyte count, C-reactive protein, ESR, serum electrolytes,

and renal and liver function tests were within normal limits. Due to the initial altered mental status, fever and increased CSF leucocyte count, HSV meningoencephalitis was considered as possible; as such, empirical therapy with IV acyclovir was started. Acyclovir (30 mg/kg/24 h in three divided doses) was administered in 100 mL of 0.9% sodium chloride as hourly IV infusions.

Venous access was placed on the right forearm. Before infusion, the area around the catheter was routinely checked for inflammation or signs of infection. On the fifth day, 10 min after acyclovir administration, the patient complained of mild burning at the catheter insertion site. The IV line was checked and, due to the lack of patency, the infusion was interrupted. It was unknown how long the IV line had not been patent. However, the patient showed no complaints or local symptoms, which suggested inflammation until the moment that a mild sensation of burning was indicated. A painless 0.5×0.5 cm large bullous lesion was observed, with regular boundaries in the area around the catheter and along the tract of the vein. The bulla was not surrounded by erythema or induration.

The bullous lesion was opened and serous fluid was drained by plastic surgeons. An examination of the underlying tissue revealed skin breakdown and necrosis (dark black colour) (Figure 1). We opted for non-surgical treatment. The patient underwent 72 h of close observation with compartment checks, topical antiseptic and antibiotic treatment. Despite the fact that both the PCR results in CFS and the results of the cultures were negative, the therapy with acyclovir was continued because the HSV PCR test can result in false negatives, particularly among children, and early on in the course of disease (5). For the remaining nine days of treatment, IV acyclovir was infused through venous access on the other hand. After discharge from the hospital, the patient returned for a follow-up one month later. Initial evaluation revealed a residual scar at the area of the initial skin lesion, although the patient was completely asymptomatic. At the patient's request, a scar revision for aesthetic reasons was scheduled.

Figure 1 Tissue necrosis following extravasation of acyclovir.

Discussion

The reported dermatological adverse effects of acyclovir, including erythema, inflammation and phlebitis at the injection site, occur in ≤16% of patients (6). The solution is highly alkaline (pH of 11, osmolality of 278 mOsm/kg), which means that extravasation may cause significant tissue damage (4).

In the literature, only three cases of extravasation of IV acyclovir have been reported so far (1, 3, 4). De Souza and Shibu reported a 51-year-old insulin-dependent diabetic man, who presented with cellulitis and lymphatic oedema over the prior site of extravasation three months after infusion. Meanwhile, Sarica reported the case of a 14-year-old girl with acute lymphoblastic leukaemia who was given IV acyclovir for chicken pox. On the 33rd day of remission induction, the infusion extravasated and a local bullous cutaneous eruption occurred approximately 10 cm distal to the site of venepuncture. The bullous eruption subsided in eight hours and completely resolved, with a residual scar, in 24 hours. Lau and Lee reported on a 55-year-old male with suspected herpes simplex encephalitis and known HIV who was treated with IV acyclovir. A diffuse swelling, which was firm but compressible, of the right hand was noticed on the second day in hospital following extravasation of 150 mL of IV acyclovir (1). In all case reports, treatment was non-operative with compression garments and physical therapy (4), cold compresses and arm elevation in a skyhook (1), or observation (3). Additionally, in previous cases, no apparent skin breakdown or necrosis was noted.

Our report presents the first case of IV acyclovir extravasation in an immunocompetent female adolescent, which caused tissue necrosis and left a residual scar. Similar to the patients discussed above, we believe that tissue necrosis occurred in our case because of the alkalinity of the extravasated solution, resulting in local chemical inflammation and tissue damage. However, extravasation of IV medications usually presents as local swelling, erythema and pain. Only in severe cases or in patients presenting or referring late in time has serious injury including tissue necrosis and compartment syndrome been observed (4).

In contrast to previous patients, our patient was diagnosed with tissue necrosis without presenting any other symptom (pain or burning) or findings (swelling) early on in the course of tissue damage. The patient only complained about mild burning at the time when tissue necrosis was diagnosed. We believe that the lack of such symptoms and findings early on in the course of tissue damage was the main reason for the delay in diagnosis and subsequent severe tissue damage. De Souza and Shibu suggested that diabetic neuropathy was the reason why their patient did not experience any pain connected with the extravasation injury. In our patient, there was no obvious reason to explain the lack of symptoms related to the extravasation injury.

We urge medical staff to consider the potential dermatological side effects of vesicant medications, which include blistering, sloughing of the skin and varying degrees of localized tissue damage when they inadvertently leak into the tissue. Extravasation of acyclovir may also cause late complications, such as persistent lymphatic oedema, cellulitis, tissue fibrosis and necrosis. Extravasation of vesicant medications is thus a medical emergency.

Conclusion

Extravasation of vesicant medication, a common incident in medical practice, can cause severe tissue dysfunction. Nursing and medical staff should consider the potential dermatological side effects of IV acyclovir (even a long time after infusion) and

the possible lack of initial local symptoms after the extravasation. Early recognition of extravasation and prompt management are critical in preventing further morbidity and optimizing outcomes.

What is already known on this topic

Acyclovir is a known vesicant medication administrated in paediatric patients, which appears to irritate venous and soft tissue if extravasated. However, only three patients with extravasation of IV acyclovir have been reported so far. In these case reports, no apparent skin breakdown or necrosis was noted, while the treatment was non-operative.

What this study adds

We present the first patient with extravasation of IV acyclovir, which caused tissue necrosis and left behind a residual scar. In contrast to the previously reported patients, our patient was diagnosed with tissue necrosis without any other symptoms or findings early on in the course of tissue damage. It is thus important to remain vigilant at the time of the IV administration of vesicant medications in children. Early recognition of extravasation and prompt management could prevent further morbidity and optimize outcomes.

Authors' contributions: Conception and design: CN and EA; Acquisition, analysis and interpretation of data: CN, ID, OS and AA; Drafting the article: CN, OS, AP and PG; Revising it critically for important intellectual content: CN, ID, EA and AA; Approved final version of the manuscript: CN, ID and AA.

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Case report

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Central retinal vein occlusion after coronary artery bypass surgery: A case report

Ufuk Ciloglu[1], Mustafa Aldag[2], Sebnem Albeyoglu[1], Veysel Aykut[3], Canan Karakaya[1], Burcu Gonul[1]

[1]Department of Cardiovascular Surgery, Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Hospital, Istanbul, Turkey
[2]Department of Cardiovascular Surgery Istanbul Medeniyet University Medical Faculty, Istanbul, Turkey, [3]Department of Ophthalmology, Istanbul Medeniyet University Medical Faculty, Istanbul Turkey

Correspondence:
mustafa.aldag@yahoo.com
Tel.: + 90 532 374 71 70
Fax.: + 90 216 566 66 14

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Key words: Central retinal vein occlusion ▪ Cardiopulmonary bypass ▪ Coronary artery bypass graft surgery.

Objective. The aim of this study is to report a case of central retinal vein occlusion (CRVO) after coronary artery bypass graft (CABG) surgery. In this report, we present the third case in the literature with CRVO after cardiopulmonary bypass (CPB), and the first case after CABG. **Case Report.** A 59-year-old male patient complaining of chest pain was admitted to our hospital. The patient underwent an elective coronary angiography and was diagnosed with three-vessel coronary artery disease. An uneventful coronary artery bypass graft operation was performed using CPB. On the second postoperative day, the patient described blurring and decreased vision in his left eye, whereas the right eye was normal. The anterior-posterior segment was examined by an ophthalmologist who diagnosed central retinal vein occlusion, using a visual acuity test, fundoscopy. After 5 months of treatment, there has been no improvement in the patient's visual acuity. **Conclusion.** As two previous case reports indicated CRVO can be a rare complication after CPB, this study demonstrated that CRVO can also be a complication of CABG. Therefore, CRVO should always be considered as a potential complication after cardiac surgery.

Introduction

Central retinal vein occlusion (CRVO) is a retinal vascular disorder and a cause of painless visual loss, which is the second most common cause of decreased visual acuity after diabetic retinopathy. The clinical manifestations of CRVO are related to venous congestion, which leads to optic disc swelling, widespread intra-retinal hemorrhage, macular edema, and ischemia (1). Macular edema is the most common cause of decreased vision in patients with CRVO (2). Risk factors for CRVO found in previous clinical and epidemiologic studies include: age, arterial hypertension, diabetes mellitus, glaucoma, high intraocular pressure, and acquired or congenital coagulopathies (Homocysteinemia, anticardiolipin and antiphospholipid syndromes, factor V Leiden, protein S and protein C mutations). Some studies also have reported coronary artery disease and kidney disease as risk factors (3, 4). The exact pathogenesis of the thrombotic occlusion of the central retinal vein is not known. Various local and systemic factors play a role in the pathological closure of the central retinal vein.

Cardiopulmonary bypass during cardiac surgery is an important procedure which effects coagulation. There are some complications of cardiopulmonary bypass resulting from impaired coagulation cascade, including cerebrovascular accidents, peripheral embolism, renal vein thrombosis and CRVO. The relationship between cardiac surgery and central retinal vein occlusion has been previously documented. Fosnat et al. (5) reported a patient who developed CRVO immediately following extensive cardiac surgery including aortic valve, aortic root, and proximal aortic arch replacements, as well as coronary artery bypass grafts. Also, no certain method has been found so far to treat this morbidity.

In this diagnostic case report, we present the third case of CRVO after cardiopulmonary bypass, and the first one after coronary artery bypass grafting (CABG) surgery.

Case report

A 59-year-old male patient complaining of chest pain was admitted to our hospital. He was under medication for hypertension and type II diabetes mellitus. His blood pressure and blood glucose levels were within normal ranges. A treadmill stress test was carried out. Chest pain and ST segment changes were determined by the ECG, so the test was ended. Cardiac enzyme tests were negative. The patient underwent elective coronary angiography and was diagnosed with three-vessel coronary artery disease. We performed coronary artery bypass grafting surgery, including left anterior, circumflex and right coronary arteries. Massive bleeding was observed from chest tubes (1500 cc in total) in the early postoperative hours, so we operated on the patient again to locate the source, but no surgical bleeding spot was detected. In the meantime, we transfused two units of fresh blood, erythrocyte suspension and fresh frozen plasma. After the second intervention, no significant bleeding was observed and the patient was extubated on the same day.

Figure 1 The dilated fundus examination; retinal hemorrhages throughout the four quadrants, as well as disc edema and dilated, tortuous retinal veins.

On the second postoperative day, the patient described blurring and decreased vision in his left eye. The patient was then examined by an ophthalmologist and on examination the best corrected visual acuity was logMAR 1.0 (OD) and 0.8 logMAR (OS). Anterior segment examination revealed a clear cornea with grade one nuclear sclerosis in both eyes. The intraocular pressure was measured to be 14 mmHg (OD) and 17 mmHg (OS) by Goldmann applanation tonometry. The pupils were normal in size and reaction to light and accommodation. Posterior segment examination revealed clear media with normal sized optic disc and a Cup: Disc ratio of 0.3 in both eyes. The vessels were dilated and tortuous with multiple flame-shaped and dot-blot hemorrhages in all four quadrants of left eyes (Figure 1). The patient was given anti-inflammatory agent (nepefenac 0.1%) to resolve macular edema and low-molecular-weight-heparin (enoxaparin) to provide anticoagulation (6). After 5 months of treatment, there has been no improvement in the patient's visual acuity.

Discussion

Central retinal vein occlusion takes the second place (after diabetic retinopathy) of all retinal vascular diseases causing loss of visual acuity (7). Some risk factors for CRVO have been reported, including age, smoking, systemic hypertension, diabetes mellitus, open angle glaucoma and decreased physical activity, but the exact pathology remains unknown. Various local and systemic factors play a role in the pathological closure of the central retinal vein, including compression of the vein (mechanical pressure due to structural changes in lamina cribrosa, eg, glaucomatous cupping, inflammatory swelling in optic nerve, orbital disorders); hemodynamic disturbances (associated with hyperdynamic or abnormal circulation); vasculitis; homocystinuria, anticardiolipin and antiphospholipid syndromes, factor V Leiden, protein S, protein C, and prothrombin G20210A mutations. Besides these clinical data, other pathophysiological elements that effect coagulation cascade may cause clotting, resulting in vein occlusion. These are; platelet activity, aggregation of red blood cells, fibrinogen concentration, and haematocrit levels (8, 9).

CRVO may be observed as two clinically important subtypes. The first is non-ischemic CRVO, which results from venous stasis in the retina, accounting for about 75% of all CRVO cases. The second is ischemic CRVO, which results from venous occlusion. The distinction between these two types is significant, due to their different treatment and prognosis. Ischemic CRVO was defined as severe visual loss (6/60 or less), extensive retinal hemorrhages and cotton-wool spots, and poor perfusion to the retina as observed in fleuroscein angiography, which is the more severe and potentially blinding type. The non-ischemic type may advance to the ischemic type (10). Klein et al. reported the incidence of CRVO in the normal population to be 0.5% in their 15-year follow-up study of 2119 people (11).

Although there is no certain treatment, ophthalmologists are currently using several drugs in management of CRVO, such as intravitreal injections of anti-vascular endothelial growth factor (anti-VEGF) agents or steroids (12). Also anti-inflammatory agents do not form part of the standard treatment of retinal vein occlusion. Anti-VEGF and intravitreal steroid injections are not aimed at treating the CRVO, but the macular oedema secondary to CRVO.

The patient was a 59-year-old male, a smoker (20 packs/year). He also underwent a cardiopulmonary bypass, which may have caused the low coagulation factor levels and low platelet counts that resulted in major bleeding followed by massive transfusion of blood products, postoperatively. In our opinion, CRVO occurred due to the first surgery (due to use of CPB). As well as bleeding diathesis and transfusions there were other predisposing factors for the development of CRVO. Use of agents such as protamine or tranexamic acid to prevent bleeding may provoke thrombosis and cause alterations in coagulation (13). Regarding the selection of treatment of CRVO, macular edema is one of the prominent treatable causes of decreased visual acuity in patients with CRVO. Various treatment modalities have been used for different components of macular edema pathogenesis, with significant progress in stabilizing or improving visual acuity. The suggested treatments are as follows: anti-inflammatory agents; intravitreal injection of ranibizumab, aflibercept, triamcinolone and bevacizumab. Pharmacological treatment with intravitreal anti-vascular endothelial growth factor (VEGF) agents is currently first-line therapy for macular edema. We used low-molecular-weight-heparin (enoxaparin) in the treatment of this patient, according to the ophtalmologist's suggestions. Also, a topical non-steroidal anti-inflammatory agent

(nepafenac 0.1%) was administered, to reduce macular edema until the venous occlusion resolved (6). The patient's macular edema was minimally cystoid so no other treatments were considered. Moreover, intravitreal anti-VEGF Injection increases risk of heart attack in coronary heart patients (14). There was no improvement, and the patient had not regained full vision in his left eye after 5 months of treatment.

Conclusion

CRVO following cardiopulmonary bypass is very rare. As two previous case reports indicated that CRVO can be a complication after CPB, this study demonstrated that CRVO can also be a complication after CABG (15). Therefore, CRVO should always be considered as a potential complication after cardiac surgery.

What is already known on this topic

Central retinal vein occlusion is a a common cause of vision impairment and a cause of painless visual loss, and is the second most common cause of decreased visual acuity after diabetic retinopathy. The relationship between cardiac surgery and central retinal vein occlusion has been previously documented.

What this study adds

In the literature there are only two known cases of central retinal vein occlusion after cardiopulmonary bypass. This study presents the third case presented in the literature after cardiopulmonary bypass and the first after coronary bypass grafting surgery.

Authors' contributions: Conception and design: UC, MA and CK; Acquisition, analysis and interpretation of data: SA, CK and BG; Drafing the article: MA, CK and BG; Revising it critically for important intellectual content: UC and SA; Approved final version of the manuscript: UC, MA and BG.

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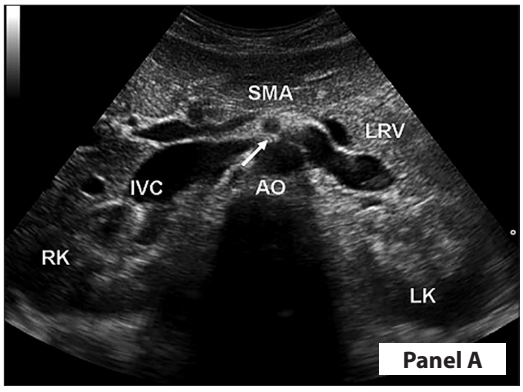
Renal nutcracker syndrome

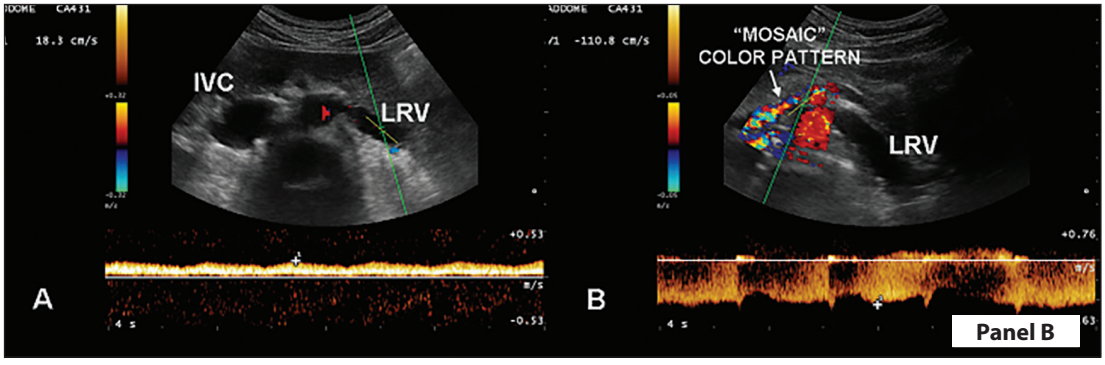
Roberto Copetti[1], Elisa Copetti[2]

[1]Emergency Department, Latisana General Hospital, Latisana (Udine), Italy, [2]Department of Anesthesia and Intensive Care, Udine, Italy

A previously healthy, 31 year old woman was admitted to the emergency room with left flank pain and macrohaematuria, which began after jogging. The physical examination and her vital signs were normal. Ultrasonography showed that both kidneys were of normal size and echostructure, with no hydronephrosis or bladder abnormalities. The left renal vein (LRV) was enlarged (15 mm) with a winding course. Examination by Doppler ultrasound showed that blood flow was low with evidence of spontaneous echo contrast. The LRV was narrow (2 mm) between the aorta and the superior mesenteric artery (SMA) (Panel A). Color Doppler ultrasound showed a "mosaic" pattern of blood flow in the LRV, beyond the origin of the SMA. The peak velocity of systolic blood flow measured in the hilar tract and in the aortomesenteric portion of the LRV was 18.3 cm/sec and 110.8 cm/sec respectively (ratio 6.05), with a pressure gradient of 5 mmHg (Panel B). Diagnosis: *Renal Nut-*



Transversal scan of the abdomen. RK=Right kidney; LK=Left kidney; IVC=Inferior vena cava; AO=aorta; SMA=superior mesenteric artery, LRV=left renal vein. The arrow indicates the point of LRV compression.



A: The peak velocity in the LRV is 18.3 cm/sec. B; The peak velocity in the LRV in aortomesenteric portion is 110.8 cm/sec. There is evidence of "mosaic" color pattern due to the turbulent flow.

cracker Syndrome. Nutcracker syndrome is a vascular compression disorder, and refers to compression of the left renal vein between the superior mesenteric artery and the aorta. Its prevalence is unknown (1). Although often asymptomatic, this condition may occur with haematuria – more frequently micro-haematuria, flank or abdominal pain and varicocele, ovarian vein syndrome, LRV hypertension and pelviureteric varices may be part of this syndrome. The haematuria is probably related to the increased left renal vein pressure, which cause small venous ruptures into the collecting system. At present there is a lack of specific diagnostic tools. Measurement of the anteroposterior diameter of the LRV, together with Doppler ultrasound peak velocities may be useful in diagnosing nutcracker syndrome (2). According to many authors, a ratio higher than 5 between the Doppler ultrasound peak velocity of the narrow tract and the distended portion, is diagnostic, as well as the ratio between the diameter of the distended and the narrowed portions. In our case these values were 6.05 and 7.5 respectively (3).

Key words: Haematuria ▪ Left renal vein ▪ Superior mesenteric artery.

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Correspondence:
robcopet@gmail.com
Tel.: + 39 340 245 4399
Fax.: + 39 040 399 4097

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Anal extrusion of the ventriculoperitoneal shunt catheter

Prastiya Indra Gunawan, IGM Reza Gunadi Ranuh, Alpha Fardah Atthiyah

Department of Pediatrics, Airlangga University College of Medicine, Dr Soetomo Hospital, Surabaya, Indonesia

An 11-month-old girl presented with a history of protrusion of a ventriculoperitoneal (VP) shunt through the anus (Panel A). The patient had a history of congenital hydrocephalus since birth and the VP shunt had already been inserted for 6 months. On examination, the child was found to be afebrile with no abdominal or meningeal symptoms. The shunt tube would protrude from the anus upon straining. The neurosurgeon decided to remove the VP shunt. The VP catheter was cut at the abdominal level and gently pulled out by the extruded anal portion. An incision at the head end was made to remove the rest of the shunt. After the procedure the patient was doing well. Abdominal ultrasound showed no evidence of free fluid collection, and there were no signs of peritonitis. VP shunting is most commonly indicated for hydrocephalus; however, there are some complications reported. Bowel perforation and anal extrusion of the distal shunt tube are rare and unusual complications of a VP shunt, and were first reported by Wilson and Bertrand in 1966. So far, to the best of our knowledge, 56 cases have been reported. The exact pathogenesis of shunt tube-related organ perforation is unclear, but various mechanisms have been suggested, including pressure necrosis, foreign body reaction, previous inflammations of the bowel wall, and poor general condition with weak bowel musculature, that may contribute to perforation. Careful attention is recommended so this complication is recognized and surgical removal of the shunt tube considered in the absence of infection, to avoid morbidity and mortality.

Key words: Anal extrusion ▪ VP shunt ▪ Hydrocephalus.

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AFA; Revising it critically for important intellectual content: RGR and AFA; Approved final version of the manuscript: PIG, RGR and AFA.

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Correspondence:
prastiya_ig@yahoo.co.id
Tel.: + 62 811 342 9476; Fax.: + 62 315 501 748

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Should the definition of the term "children born of war" and vulnerabilities of children from recent conflict and post-conflict settings be broadened?

Amra Delić[1], Philipp Kuwert[2], Heide Glaesmer[3]

[1]Department of Psychiatry and Psychotherapy University Medicine Greifswald, Greifswald Germany, [2]Department of Psychosomatic Medicine and Psychotherapy of the HELIOS Hanse Hospital Stralsund of the University of Greifswald, Stralsund Germany, [3]Department for Medical Psychology and Medical Sociology, Medical Faculty of the University of Leipzig, Leipzig, Germany

Correspondence:
a.delic@bih.net.ba
Tel.: + 387 61 666 370; Fax.: + 49 341 971 8809

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Key words: Children born of war ▪ Vulnerabilities ▪ Bosnia and Herzegovina.

Dear Editor,

The term "child born of war" (CBOW) refers to a child whose one parent is a member of military or peacekeeping forces and the other parent a local citizen (usually the mother), and it includes the following categories of children: (a) children fathered by enemy soldiers (war and civil war) and (b) occupying forces, (c) children of stationed, peacekeeping forces, and (d) children of female child soldiers (1). As Mochmann (2) suggested, the term can be applied regardless of type of conflict, historical context, geographical setting and background of procreation. It is known from the literature review that terminology used to describe this population of children varies across countries and specific periods of time (e.g., "occupation children", "war babies", "peacekeeper's babies", "children of hate", "child of rape", etc.). Despite a growing evidence that these children are exposed to stigma, discrimination, neglect, social exclusion, health risks, and other forms of violation of the rights of the child, there is no scientific consensus about recognition and involving "children born of war" into the existing classification of vulnerable children in and after conflict (3-7).

However, while conducting our research, through the process of indentifying and recruiting the potential study participants, we found that there are children that share specific living conditions and experiences with CBOW in the narrower sense, such as:

a) children whose mothers were victims of the international human trafficking being „sold" throughout Bosnia and Herzegovina (BA) (8), and of fathers who are local (BA citizens, some of which belonged to local military/police forces) or belonged to foreign stationed forces; b) children who recognized themselves as being a child of "enemy's soldiers" from fratricidal war taking place in 1993-1995 in the "Autonomous Province of Western Bosnia" (APZB), a self-proclaimed

autonomous entity, whose parents are both local citizens, but it was common that a father fought not only against members of their ethnic group but also opposing their brothers or a mother's family side. These children responded to our public invitation to participate in the study on „children born of war" in BA, reporting that although they are not guilty for their fathers' political and/or ideological views, they have been treated as children of „enemy soldier" and consequently exposed to a huge stigmatization, discrimination, and violation of the rights of child that took place in the aftermath of fratricidal war in Western Bosnia, and c) children born out of inter-ethnic („mixed") marriages, involving local parents from different ethnic backgrounds, who were considered to be „national apostates" both in war and peace (9), but a father often combated against the side of the mother's family. These children, also respondents to our public call, recognized as „children of opposing parties" reported that as such they have been affected by war since a continual ethnic politics are causing them a variety of problems associated with their mixed background (prejudices; stigmatization: „products of failed ideologies", „mixed meat", „good for soap production"; intolerance, discrimination, segregation etc.)

We think that human trafficking related to conflict and post-conflict zones, and fratricidal war – common characteristics of recent wars - are very important psycho-social issues, and that vulnerabilities of children born and raised in the above socio-political contexts (including intolerance and prejudices towards children with interethnic background) should also be addressed in the research on „children born of war". We would appreciate an input of colleagues on this topic; specifically regarding the question if understanding of the term "children born of war" should be broadened and vulnerabilities of children from various homogenous and heterogeneous non-integrated post-conflict communities be linked to this group? We see a first step towards a broadened definition by collecting empirical evidence that supports our hypothesis that these groups of children, as described above, are comparable to CBOW with respect to the core psychosocial issues of "children born of war" in general (10).

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by Nerma Tanović

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Units of measurement

Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples. Temperatures should be in degrees Celsius. Blood pressures should be in millimeters of mercury, unless other units are specifically required by the journal.

Abbreviations, acronyms and symbols

If possible for metric units use standard abbreviations. Non-standard abbreviations should be defined when first used in the text.

Sample references

Articles in journals

Standard journal article (*List the first six authors followed by et al.*):

Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002;347(4):284-7.

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Rose ME, Huerbin MB, Melick J, Marion DW, Palmer AM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res. 2002;935(1-2):40-6.

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