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Enver Krupić (1911-1992), "Landscape" (1982), oil on canvas, 1000x1360 mm. Courtesy of the Academy of Sciences and Arts of Bosnia and Herzegovina.

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Original article

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Morphologic assessment of mandibular anterior teeth root canal using CBCT

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Key words: Mandibular anterior teeth ▪ Apical foramen ▪ Morphology ▪ Root canal ▪ Cone beam computed tomography.

Objective. The aim of this study was to evaluate the number and morphological characteristics of the roots and root canals in mandibular anterior teeth, using cone beam computed tomography. **Methods and materials.** In this cross-sectional study, 1053 anterior mandibular teeth from 200 CBCT scans were evaluated. The teeth were completely developed and should have had no fillings in the root or crown. The teeth were investigated in terms of the number of roots and root canals, the location of the apical foramen, the distance of the apical foramen to the anatomical apex, root length, crown length, dilacerations and the type of canals according to Vertucci's classification. **Results**. 87.9% of teeth had one root canal and of all of the teeth, three canines (0.3%) were found that had two roots. In 80.3% (n: 848) of cases the foramen apical location was central, then the buccal (9.3%), lingual (3.9%), distal (3.8%), and mesial (2.7%). The type of root canals, according to Vertucci's classification, with respect to prevalence, included type I (88.2%), type III (8.1%), type II (3.3%), type V (0.3%), and type VI (0.1%), respectively. In terms of the characteristics investigated, bilateral symmetry was observed. Dilaceration was not seen in any of the teeth. **Conclusion**. The root canal morphology of mandibular anterior teeth has great diversity that may differ between different races, and should be considered by all dentists in order to achieve the best dental treatment.

Introduction

Knowing the anatomy of the tooth, the morphology of the root and its morphological differences is useful in most dental procedures, especially root canal therapy. Successful root canal treatment requires appropriate and complete cavity access, full biomechanical clearing of the root canal, effective and complete chemical debridement, and filling of the canals (1-6). To achieve these goals, the dentist should have complete knowledge of the morphology of the dental root and root canal, and their variations. Insufficient knowledge of the number of roots or root canals, and root canal type and morphology will lead to treatment failure. Normally, the root canal system of mandibular central and lateral teeth is similar and oval in shape in an axial section (wider buccolingually than mesiodiastally) (7). Previous studies have shown that a high percentage of mandibular anterior teeth have one root canal (1-4, 8-19). Often a dentinal bridge within the pulp space creates two canals within the sin-

gle root.These two canals may merge together to form an apex in a single apical foramen, or may be separated from each other all the way to the end. Sometimes the inability to find the lingual root canal causes failure of endodontic treatment (3). Determining the root length also is an important factor in a successful root canal treatment because debridement and complete disinfection of the root canal is done without traumatizing the periapical tissue. Incorrect working length may lead to over instrumentation. Intraoral radiography is a common technique in determining root length during endodontic treatment. Sometimes the apical foramen opens in a position other than the apex to the lingual, buccal, mesial and distal openings. The buccal and lingual position of the apical foramen is not detected on intraoral radiography. The non-central position of the apical foramen can lead to incorrect assessment of root canal length and damage to the periapical tissue, and unsuccessful root canal treatment (20). Dilaceration is defined as a sudden change in the axial inclination of root, or between the crown and the root of a tooth. Awareness of the presence of this anomaly in the root can prevent root canal perforation and determine the best treatment plan leading to successful root canal therapy (21).

In previous studies, conventional X-ray techniques, root canal staining techniques, clearing technique, tooth sectioning and microscopic observation, etc. were used (3, 4, 7, 8, 10, 17, 22-24). Although staining the root canal and clearing techniques are the gold standard for studying the morphology of the root canal, they are only performable on extracted teeth. Recently, the CBCT (Cone Beam Computed Tomography) technique has been used to study the morphology of the root canal. The benefits include non-invasiveness, its ability to be used clinically, and the bilateral study in the patient. Given that in CBCT images are displayed in three sagittal, axial, and coronal views, it helps clinicians to observe the three-dimensional morphology features (6, 24).

The aim of this study was to evaluate the morphology of the roots and root canal, the number of roots and root canals, the type of root canal (Vertucci's classification), the distance from the apical foramen to the anatomical apex, crown length, root length, dilacerations, and the location of the apical foramen, in mandibular anterior teeth.

Methods and materials

In this cross-sectional study, 1053 mandibular anterior teeth, related to 200 CBCTs, were selected from patients who referred to a private oral and maxillofacial radiology center during 2014-2016 for treatment purposes. Of 1053 mandibular anterior teeth, 317 were central, 371 lateral, and 365 were canines. 94 (47%) of cases were male and 106 (53%) were female, and the mean ages of the women and the men were 41.5±13.7 and 47.3±13.3 years, respectively. Selected patients had to have at least one tooth in the anterior mandible; the teeth had to be completely developed and had to have no fillings in the root and crown, no cast metal post inside the canal, and no apical lesions. The CBCT images had to be of a desirable quality, and with no artifacts.

All images were prepared by Cranex3D (Soredex/Helsinki/Finland) and the scanning parameters included: 89kvp, 6mA, field of view: 6×8 cm, and voxel size: 0.2 mm. Also, Ondemand 3D Dental software was used for processing images. The teeth were investigated in axial, coronal, and sagittal sections, with a thickness of 0.1 mm. The observer used all the software features, such as zooming, and changes to contrast and brightness.

Mandibular anterior teeth were investigated in terms of the number of roots and root canals, the location of the apical foramen (Figure 1), the distance of the apical

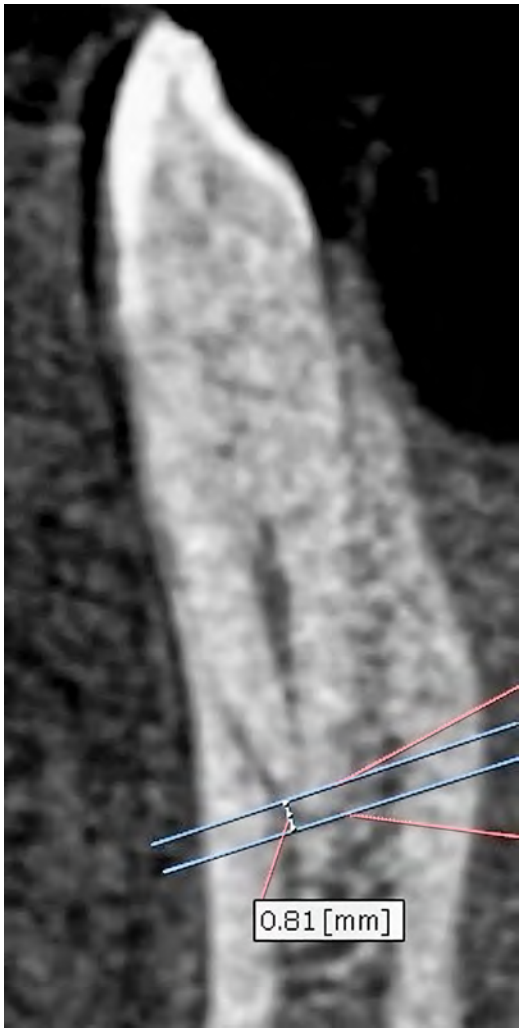

Figure 1 Illustration of foramen apical and apex and distance from foramen apical to apex.

foramen to the anatomical apex (Figure 1), root length, crown length, the type of canals according to Vertucci's classification and dilaceration. The root length was measured from the CEJ to the tooth's apex and crown length from the CEJ (Cemento Enamel Junction) to the incisal edge (Figure 2). Vertucci type I was considered single-canal and Vertucci type VIII was considered to be three canals (Figure 3).

Statistical analysis

All the data were analyzed by SPSS 17 software and statistical tests including Student's t-test,

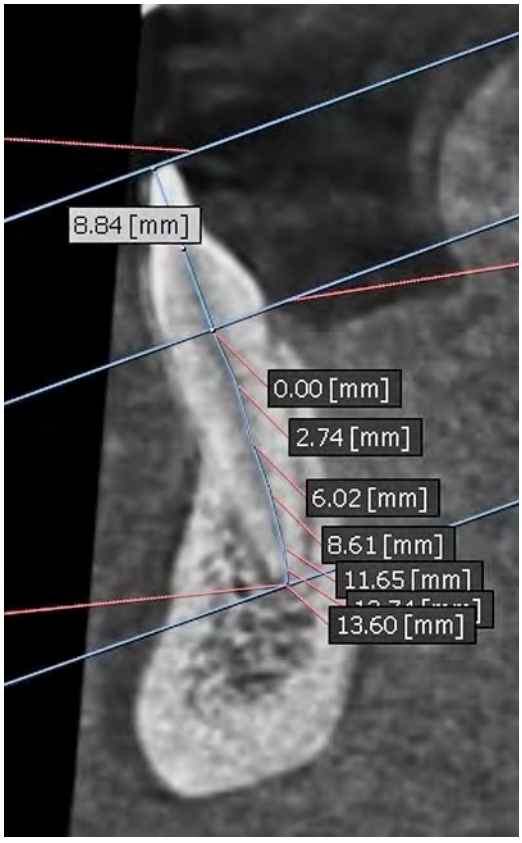

Figure 2 Measurement of crown and root length.

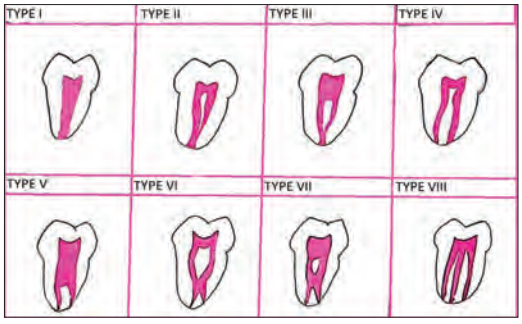

Figure 3 Vertucci classifications.

Pearson correlation, chi-square test and Fisher test. $P<0.05$ was considered significant.

Results

928 (87.9%) of the teeth had one root canal, and, accordingly, the highest percentage of single-canal teeth were canine, and the highest percentage of two root canals was related

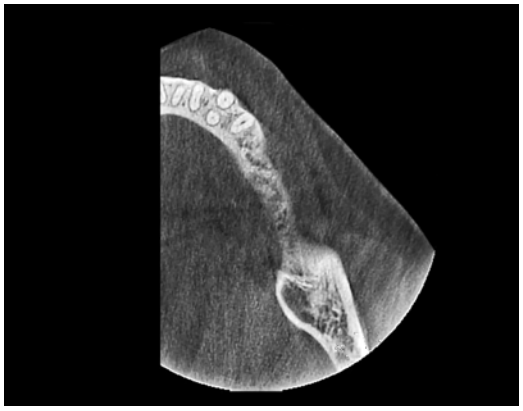

Figure 4 Mandibular canine with two roots.

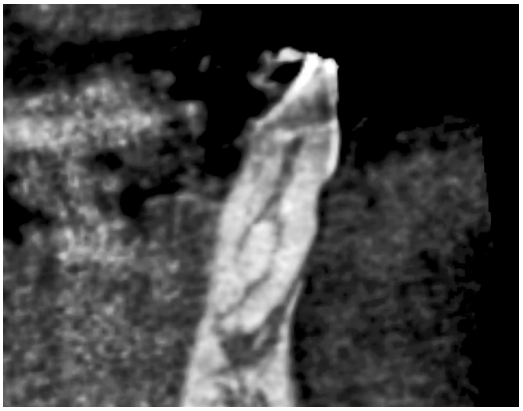

Figure 5 Type VII by Vertucci's classification.

Table 1 Distribution of the number of mandibular anterior teeth root canals in terms of sex

Tooth	Sex	Root canals		χ^2	p
		Single canal	Two canals		
		n (%)	n (%)		
Central	Men	117 (80.1)	29 (19.9)	1.92	0.166
	Women	147 (86)	24 (14)		
Lateral	Men	138 (80.2)	34 (19.8)	0.77	0.186
	Women	170 (85.4)	29 (14.6)		
Canine	Men	159 (96.4)	6 (3.6)	0.13	0.715
	Women	197 (97)	6 (3)		

Table 2 Distribution of different types of Vertucci's classification in mandibular anterior teeth on both sides of the mandible

Tooth	Side	Types of Vertucci's classification		p
		I n (%)	II, III, V, VII n (%)	
Central	Right	137 (86.2)	22 (13.8)	0.179
	Left	127 (80.4)	31 (19.6)	
Lateral	Right	156 (83.9)	30 (16.1)	0.680
	Left	152 (82.2)	33 (17.8)	
Canine	Right	180 (97.8)	4 (2.2)	0.751
	Left	179 (97.3)	5 (2.7)	
Total	Right	473 (89.4)	56 (10.6)	0.122
	Left	458 (86.9)	69 (13.1)	

p value refers to Fisher´s test.

Table 3 Apical foramen location in terms of sex

Sex	Apical foramen location				
	Central	Mesial	Distal	Buccal	Lingual
Man n (%)	388 (80.3)	21 (4.3)	24 (5)	37 (7.7)	13 (2.7)
Woman n (%)	460 (80.3)	8 (1.4)	16 (2.8)	61 (10.6)	28 (4.9)
Total n (%)	848 (80.3)	29 (2.7)	40 (3.8)	98 (9.3)	41 (3.9)

χ^2=17.36; p=0.002.

Table 4 Distribution of mean length of crown and root in terms of sex and sides of the mandible in the mandibular anterior teeth

Tooth	Sex	N	Sides	Length (mm)	Mean±SD	t	p
Central	Man	74	Right	Crown length	6.77±1.53	-1.01	0.312
		72	Left		7.03±1.58		
		74	Right	Root length	13.8±1.55	-0.39	0.696
		72	Left		13.9±1.63		
	Woman	85	Right	Crown length	7.25±1.00	-0.30	0.764
		86	Left		7.3±0.99		
		85	Right	Root length	12.7±1.88	-0.51	0.610
		86	Left		12.8±1.50		
Lateral	Man	88	Right	Crown length	7.41±1.51	-0.47	0.637
		84	Left		7.52±1.56		
		88	Right	Root length	14.5±1.66	-1.43	0.154
		84	Left		14.96±1.81		
	Woman	98	Right	Crown length	7.57±1.08	-0.15	0.883
		101	Left		7.59±0.98		
		98	Right	Root length	14.13±1.58	-1.19	0.236
		101	Left		14.4±1.63		
Canine	Man	84	Right	Crown length	8.49±1.55	0.11	0.910
		81	Left		8.46±1.56		
		84	Right	Root length	16.82±1.95	-0.69	0.492
		81	Left		17.04±2.11		
	Woman	100	Right	Crown length	8.12±1.14	-0.25	0.802
		103	Left		8.16±0.98		
		100	Right	Root length	16.04±1.85	0.75	0.452
		103	Left		15.85±1.83		

to lateral teeth (p-value >0.05). There was no significant difference between men and women or between the right and left sides of the mandible, regarding the number of canals (Table 1).

Of all the teeth, three canine (0.3%) were found that had two roots (Figure 4). The type of root canals, according to Vertucci's classification, with respect to prevalence, included type I (88.2%), type III (8.1%), type II (3.3%), type V (0.3%), and type VII (0.1%), (Figure 5) respectively. Canine teeth were less likely to have Vertucci type II and type III; and type II and type III were observed more in central and lateral teeth. In terms of Vertucci's classification, there were no differences between the right and left mandible (Table 2).

The location of the apical foramen, according to the most frequent finding, was central (80.3%), then buccal (9.3%), lingual (3.9%), distal (3.8%), and mesial (2.7%), respectively. There was a significant difference between women and men (Table 3). The mean distance of the apical foramen from the anatomical apex was 0.12±0.36 mm, the

Table 5 Length of crown, length of root and distance from apical foramen to apex in two age groups in male and female

Sex	Age (y)	Measurements	Mean±SD	t	p
Male	<50	Length of crown	8.11±1.43	-0.83	<0.001
	>=50		6.96±1.75		
	<50	Length of root	15.25±2.26	0.256	0.799
	>=50		15.20±2.12		
	<50	Distance*	0.12±0.36	-0.457	0.648
	>=50		0.13±0.40		
Female	<50	Length of crown	8.00±1.03	8.801	<0,001
	>=50		7.23±1.02		
	<50	Length of root	14.50±2.21	1.071	0.285
	>=50		14.31±2.01		
	<50	Distance*	0.14±0.37	1.130	0.259
	>=50		0.10±0.31		

*From apical foramen to apex.

mean crown length was 7.6±1.38 mm, and mean root length was 14.7±2.2 mm. The mean crown and root length of central teeth was 7.1±1.29 and 13.2±1.72 respectively and for lateral teeth 7.5±1.28 and 14.5±1.69 respectively, where canine teeth, with mean crown length of 8.2±1.38 mm, and mean root length of 16.3±1.98 mm, had the longest crowns and roots of the mandibular anterior teeth (p-value <0.05). Women had longer crowns (p-value =0.001) and men had longer roots (p-value =0.801) (Table 4). With increasing age, the crown length decreased (r= -0.313, p=0.000), but the root length(r=0.021, p=0.495) and the distance of the anatomical apex from the apical foramen(r=-0.016, p=0.594) did not change (Table 5). Crown and root lengths and the distance of the anatomical apex from the apical foramen did not differ between the two sides of the mandible, and no dilaceration was seen in any of the teeth.

Discussion

In this study, 87.9% of the mandibular anterior teeth were single-canal and the dominance of single-canal in all previous studies confirms our findings, although the proportion differs, which may be due to racial differences (1-4, 8-19). Aminsobhani et al. (1) reported that 71.7% of mandibular anterior teeth had a single root canal. Vertucci et al. (17) reported that 27.5% of the incisor teeth had two root canals. Boruah et al. studied the mandibular incisors by the clearing technique and Kamtane et al. (10, 12) studied the mandibular incisors by CBCT, and reported that 64% of teeth had one root canal. Zhao et al. (18) studied a Chinese population and reported that 6.7% of the central, 16.7% of the lateral, and 3% of canine teeth had two root canals. Zhegyan et al. (19) reported that 3.8% of the central, 10.6% of the lateral, and 2.4% of the canine teeth had more than one root canal.

In this investigation, all incisors were single-rooted and of all of the teeth, three canines (0.3%) were found that had two roots. In most studies, incisors were found to be single-rooted and two roots were more prevalent in canine teeth (1, 11-13, 15, 18, 19). In Kayaoglu's study (13), all mandibular central teeth, and 99.9% of the laterals were single-rooted, and 3.1% of the canines had two roots (13). Zhegyan et al. (19) showed

that all central teeth were single-rooted and 0.3% of mandibular lateral teeth and 0.8% of canine teeth had two roots (19). In a study by Han et al. (11) on a Chinese population, it was found that all mandibular incisors were single-rooted, and 1.32% of canines were double rooted (11). Rahimi et al. (4) reported that all incisors were single-rooted and 12.08% of the canines were double rooted.

The present study showed the prevalence of double root canals in the mandibular lateral (17%), and central (16.7%) teeth was more than in the canine teeth (9.4%) (p-value >0.05), but no significant differences were observed between men and women, or between the two sides of the mandible. In a study by Liu et al. (15) on Chinese populations, the frequency of double root canals was higher in lateral than in central teeth, and the frequency of double root canals was slightly higher in men than in women. Han et al. (11) reported that the prevalence of double root canals was higher in mandibular lateral teeth than in canine and central teeth Lin et al. (14) study showed that lateral incisor teeth tended to have more double root canals. Kayaoglu et al. (13) reported that, in a Turkish population, double root canals were observed more in lateral teeth, and double root canal teeth were observed in bilateral symmetry in the mandible, and also bilateral symmetry of the jaw was observed for all the studied characteristics. Arslan et al. (2) reported greater anatomical complexity of canals in the mandibular incisors in men than in women, while no such difference was observed between male and female participants in the characteristics studied in the present study.

The results of this study indicated that Vertucci type I (88.2%) had the highest prevalence, then type III (8.1%), type II (3.3%), type V (0.3%) and type VII (0.1%), respectively. In all studies, Vertucci type I had the highest prevalence, as in our study (1, 8, 9, 11-16, 18, 19). The study by Lin et al. (14) showed that, among double root canals, Vertucci type III was most prevalent. Sobhani et al. (1) reported that the prevalence of different types of canals, related to Vertucci classification, was types I, II, IV, III, and V, respectively. Altunsoy et al. (9) reported that Vertucci type I had the greatest prevalence among mandibular incisors, and type V had the greatest prevalence in double root canals. In the study conducted by Rahimi et al. (4) the greatest prevalence were types I, II, III, and IV, respectively. Silva et al. (25) determined the prevalence of different types of canals as types I, III, and V, respectively. Zhao et al. (18) evaluated the prevalence of different types of canals and reported types I, III, V, VII, and IV, respectively These differences may be due to the different populations studied. The mean crown length of all evaluated mandibular anterior teeth was 7.6±1.3 mm, and the mean root length was 14.7 ±2.2mm. Canine teeth with a mean crown length of 8.2±1.3 mm and mean root length of 16.3±1.9 mm had the longest crown and roots of the anterior mandibular teeth. Women had longer crowns and men had longer roots. Soleymani et al. (5) reported canine root length of 15.53 mm, and root length was greater in men than in women.

The location of the apical foramen was central (80.3%), buccal (9.3%), lingual (3.9%), distal (3.8%), and mesial (2.7%), respectively. Soleymani et al. (5), in a study on canine teeth, showed that in 68.3% of cases the apical foramen was in the central location. Boruah et al. (10) evaluated mandibular incisors and showed that the foramen apical location in 47.2% was central, 34.3% buccal, 13.3% lingual, and 5% proximal. These differences may be due to the method of studying the teeth, the population and racial differences. Boruah et al. (10) evaluated extracted teeth by the clearing method, and using a magnifying glass with 5x magnification.

No dilacerations were observed in any of the studied mandibular anterior teeth. Previ-

ous studies have also reported a very small incidence of dilaceration in mandibular anterior teeth (21, 26). Nabavizade et al.(21) determined of all teeth, dilaceration was found in the second mandible molars, the first maxillary molar, and the first mandibular molar, respectively, but not in mandibular anterior teeth. Hamasha et al. (26) examined 4655 dental x-rays; mandibular anterior teeth had the lowest prevalence of dilaceration of all teeth, with a prevalence of only 1%.

Conclusion

The findings of this study indicate that most anterior mandibular teeth have a single root and single root canal, and there is no significant morphological difference between different individuals, but the root canal morphology of the anterior mandibular teeth may be extremely diverse, and may differ between different races, and this should be considered by all dentists in order to achieve the best dental treatment. Although CBCT is very helpful in determining root canal morphological characteristics, it is not recommended for everyone.

What is already known on this topic

In mandibular anterior teeth, wide ranges of morphological features are observed, where features such as single-canal, single-root, type I Vertucci and others, have a high percentage of similarity between different individuals. However, slight differences may affect root canal therapy. So far, various ex vivo methods have been used to assess this feature, but CBCT is a new and clinical method. CBCT can help the technician to see three-dimensional images of morphological features the teeth.

What this study adds

Although most individuals have similar morphological features, the percentage of similarity between individuals differs. Bilateral symmetry of different morphological features of teeth in a jaw can be assessed using CBCT. With increasing knowledge of the morphological features of teeth and bilateral symmetry of the jaw, the best treatment can be given to prevent the failure of root canal therapy.

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Original article

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Adverse drug reactions of non-opioid and opioid analgesics reported to Croatian national authority from 2007 to 2014

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Objective. Adverse drug reactions (ADRs) are commonly observed in the health services because of system weaknesses and individual errors. Analgesics are widely used and it can be expected that with the increased use one can expect increased number of ADRs of analgesics. The aim of this study was to analyze ADRs of non-opioid and opioid analgesics reported to the Croatian Agency for Medicinal Products and Medical Devices (HALMED) from 2007 to 2014. **Methods.** HALMED provided data on generic drug name, year of the ADR report, type of report, institution, reporting person, patient's age, sex and ADR type. **Results.** In the analyzed period 796 ADRs of analgesics were reported, of which 367 (46%) were serious ADRs. Number of ADR reports was continuously increasing during the analyzed period. There were 20 analgesics that had ≥5 reports, making 597 (75%) of all ADR reports for analgesics. The most common adverse reaction reports of those 20 analgesics referred to individual drugs (n=16; 80%). Most of the ADR reports were filed by physicians (n=257; 43%), followed by pharmacists (n=252; 42%). Most side effects (n=572; 96%) were reported spontaneously through appropriate forms by patients or health professionals. ADRs were most commonly reported in women (n=352; 59%) and most of them have occurred in adults (n=354; 59%). The most common ADRs of opioid and non-opioid analgesics have been reported on the skin and mucous membranes. Most serious ADRs were result of action of opioid analgesics. **Conclusion.** Number of ADR reports in Croatia is continuously increasing and a considerable number of them refers to serious ADRs. To keep better track of medications and ADRs it is necessary to educate and encourage health professionals and patients in reporting side effects.

Introduction

Adverse drug reactions (ADRs) are commonly observed in the health services because of system weaknesses and individual errors (1). Public health burden of ADRs associated with drug use among non-hospitalized patients in community has been difficult to estimate, but the problem is being documented, it is considerable and it is expected that it will increase (2). ADRs contribute to a loss of public confidence, increase patient morbidity and mortality, as well as raise health care costs (2). Analgesics are widely used and it can be expected that with the increased use one can expect increased number of ADRs of analgesics. Large increases in the use of both opioid and non-opioid analgesics were observed worldwide (3-8).

Some medications such as aspirin, paracetamol and ibuprofen belong to the first step of the World Health Organization (WHO) pain treatment ladder, and they are used on a daily basis as an over-the-counter (OTC) medications by millions of individuals. Even though they are generally well tolerated, even apparently minor ADRs can appear significant from a public health point of view (9). These trends underscore the need for ongoing surveillance of outpatient analgesic safety. This requires obtaining timely, nationally representative surveillance data on outpatient analgesic ADRs.

This study analyzed national data on ADRs of non-opioid and opioid analgesics reported to the Croatian Agency for Medicinal Products and Medical Devices (HALMED) from 2007 to 2014. Study aims were to examine prevalence of reported ADRs of analgesics, frequency of ADRs for various analgesics, where the reports mostly come from, in which patients, types of ADRs and frequency of serious ADRs of analgesics in a national register.

Methods

Study design

This was a retrospective study.

Data collection

Information about ADRs of analgesics were obtained from the Croatian national authority for pharmacovigilance Croatian Agency for Medicinal Products and Medical Devices (HALMED). Data concerning the suspected ADRs were coded into the related Preferred Term and System Organ Class (SOC) using the Medical Dictionary for Drug Regulatory Affairs (MedDRA) adverse drug reaction terminology. ADR reports received in the eight-year period from the beginning of 2007 to the end of 2014 were analyzed.

The following data were collected: type of ADR, year of the ADR report, generic name of a drug, type of report, whether ADR was serious ADR or not), institution in which ADR was reported, the qualifications of the person who reported ADR, patient's date of birth, age group and sex. ADRs were considered serious, as defined by European Medicines Agency (EMA) if they met one of the following criteria: the ADR resulted in death, the ADR was life threatening, the ADR required inpatient hospitalization or prolongation of existing hospitalization, the ADR resulted in persistent or significant disability/incapacity, the ADR was a congenital anomaly/birth defect, or the ADR was another important medical event in accordance to important medical event (IME) list provided by EMA. For each drug we calculated percent of serious ADRs from the total number of ADRs reported for that drug. Collected data were anonymized, and any personal information removed, so patients could not be identified.

Ethics statement

The study did not include any data collection directly from patients. Instead, anonymized registry data were analyzed.

Statistical analysis

All data were inserted into the electronic spreadsheets and descriptive statistics was calculated using Microsoft Excel (Microsoft Corp., Redmond, WA, SAD).

Results

During the studied eight-year period (2007-2014) HALMED received 796 individual reports of ADRs for analgesic medications. The number of ADR reports was continuously increasing during the analyzed period, and in year 2014 this number was three times higher compared to 2007 (Figure 1).

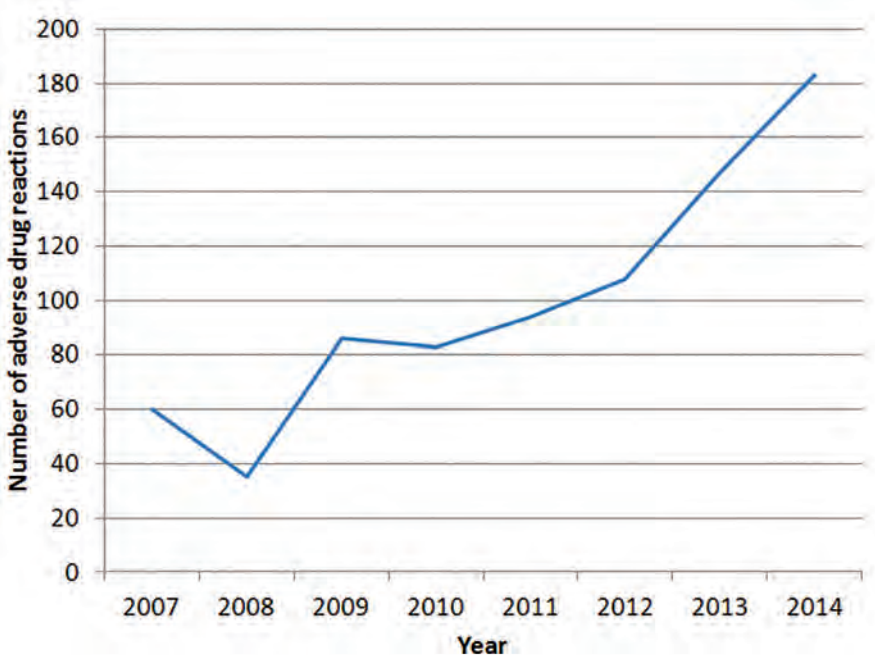

Figure 1 Number of adverse drug reactions of non-opioid and opioid analgesics reported to the Croatian Agency for Medicinal Products and Medical Devices (HALMED) from 2007 to 2014.

Most of the ADR reports were filed by physicians (n=350; 44%), followed by pharmacists (n=328; 41%), other health professionals (n=43; 5%) and patients or other non-health professionals (n=32; 4%). For 43 reports it was not recorded in HALMED database who sent the ADR report. ADRs were mostly commonly reported in women (n=352; 59%), compared to men (n=286; 36%). In 32 ADR reports sex of individuals who suffered from ADRs was not specified. Among all reported ADRs of analgesics in the analyzed period, there were 367 (46%) serious ADRs and 429 (54%) were characterized as non-serious. Some of the ADR reports referred to ADRs of individual analgesics, while other ADR reports contained various combination of multiple pharmacological interventions. There were ADR reports for 231 drugs or different combinations of various drugs. There were two drugs/combinations with 4 reports, two drugs/combinations with 3 reports, 20 drugs/combinations with 2 reports and 183 drugs/combinations with only one ADR report.

Among the reported ADRs of analgesics, there were 20 analgesics or combinations with analgesics that were reported ≥5 times, and these were analyzed in more detail. Among those 20 there were 4 drug combinations (Table 1).

ADR reports of those 20 analgesics made 597 (75%) of all ADR reports for analgesics in the analyzed period. Of the 367 ADRs that were categorized as serious, more than half were associated to only 5 drugs. The highest rate of serious ADRs was noted for drug fentanyl; of the 45 reported ADRs for fentanyl, 38 (84%) were serious. A combination of drugs acetylsalicylic acid (ASA) and clopidogrel hydrosulfate had 7/10 (78%) serious ADRs, fentanyl citrate 5/7 (71%), diclofenac potassium 8/12 (67%) and fixed combination of caffeine/paracetamol/propyphenazone/codeine phosphate sesquihydrate 8/13 (61%) serious ADRs.

Table 1 The frequency of adverse drug reactions reported for the 20 most commonly reported analgesics (2007-2014)

Generic name	n (%)*
Ibuprofen	91 (11)
Diclofenac sodium	83 (10)
Tramadol/Paracetamol	59 (7.4)
Acetylsalicylic acid	57 (7.2)
Tramadol	55 (6.9)
Ketoprofen	49 (6.2)
Fentanyl	44 (5.5)
Paracetamol	27 (3.4)
Dexketoprofen trometamol	26 (3.3)
Meloxicam	19 (2.4)
Caffeine/paracetamol/propyphenazone/ codeine phosphate sesquihydrate	13 (1.6)
Diclofenac potassium	12 (1.5)
Piroxicam	11 (1.4)
Buprenorphine	10 (1.3)
Acetylsalicylic acid; Clopidogrel hydrogen sulphate	10 (1.3)
Acetylsalicylic acid /Ascorbic acid	8 (1)
Fentanyl citrate	7 (0.9)
Etoricoxib	6 (0.7)
Metamizole natrium	5 (0.6)
Paracetamol/Pseudoephedrine HCl/Ascorbic acid	5 (0.6)

*Adverse drug reactions of medications whose reports were recorded ≥5 times in analyzed period.

Ibuprofen

Ibuprofen was drug with the most ADR reports (n=91; 11%), all of them spontaneous, and 37 (41%) were classified as serious. Number of reports for ibuprofen ADRs kept increasing from 2007 (n=3) to 2014 (n=27). Of the 196 ADRs indicated in those reports for ibuprofen, ADRs were most commonly observed on skin and mucosas (n=83; 42%), with urticaria, erythema, swollen eyelids and angioedema as the most common of those. Nausea and abdominal pain were the most common gastrointestinal ADRs. Suffocation feeling and dyspnea were the most common respiratory ADRs. Hypertension and hemorrhagic shock were the most common cardiovascular ADRs. Among other ADRs hypersensitivity reactions were the most common. All these ADRs were described only in few patients, as indicated in the Supplementary tables in the online appendix of this manuscript.

ADRs linked with erroneous use of drug were mostly due to off-label use of ibuprofen (n=4). Most of these reports were filed by healthcare workers (n=64; 70%) and pharmaceutical companies (n=13; 14%). Most of the reported ADRs were observed in adults (n=56; 62%), followed by elderly (n=16; 18%) and children (n=6; 6,6%). Patients were mostly women (n=58; 64%).

Diclofenac sodium

Diclofenac sodium was the second most common drug linked to ADR reports (n=83; 10%). Its number of ADRs kept increasing throughout the years, from 3 in 2007 to 15 in 2014. Most of the reports were spontaneous (n=79; 95%), while the others were reports from the medical literature. There were 41 (49%) of ADRs of diclofenac sodium that were classified as serious. Most of the ADRs were reported in adults, and mostly in women (n=49; 59%). In the 83 ADR reports there were a total of 206 different reported ADRs, which is the highest number of different ADRs reported for any single drug analyzed for this period. The ADRs mostly affected skin and mucosa (n=63; 31%), with edemas, erythema and rash as the most common ADRs in this group. In the gastrointestinal tract the most common ADRs were different kinds of bleeds, nausea and abdominal pain. Suffocation feeling and dyspnea were the most common respiratory ADRs, while hypertension, hypotension and tachycardia were most commonly observed ADRs in the cardiovascular system. Among other ADRs the most common were vertigo and hypersensitivity reactions. Detailed information

about ADRs of diclofenac sodium are presented in Supplementary tables in the online appendix of this manuscript.

Tramadol/paracetamol combination

There were 59 (7.4%) of ADRs reports submitted for tramadol/paracetamol combination, majority of them in the year 2014. They were mostly spontaneous and 12 (20%) were classified as serious. Majority was reported in women (n=43; 73%) and in adults (n=30; 51%). A total of 175 various ADRs were listed in those 59 reports. Most of them were ADRs in gastrointestinal system (n=58; 33%), with nausea and vomiting being most common. There were 30 (17%) of ADRs reported in the nervous system, with vertigo as the most common. A number of ADRs affected general condition of patients, with somnolence the most commonly reported ADR in this group. Confusion was the most common mental health ADR, hyperhidrosis most commonly reported skin-related ADR, while visual impairment and headache were most commonly reported other ADRs. Detailed information about ADRs of tramadol/paracetamol combination are presented in Supplementary tables in the online appendix of this manuscript.

Acetylsalicylic acid

ASA was the subject of 57 (7.2%) ADR reports, containing a total of 116 various ADRs. Number of reported ADRs for ASA kept increasing over the years, just like for other analyzed medications. Of 57 ADR reports for ASA, there were 28 (49%) serious ADRs. More ADRs were observed in men (n=30; 53%). Majority of ADRs were reported in adults (n=28; 49%) and elderly (n=20; 35%). ADRs related to skin and mucosa were most frequent (n=38; 33%), with urticaria and rash as the most common ADRs in this group. ADRs in gastrointestinal systems followed in frequency (n=27; 23%), with the most common ADR being GI bleeding. Most common ADRs in the respiratory system were suffocation feeling and dyspnea, and among other ADRs epistaxis. Detailed information about ADRs of ASA are presented in Supplementary tables in the online appendix of this manuscript.

Tramadol

For tramadol, 55 (6.9%) of ADR reports were filed; 12 (22%) were classified as serious. All the reports were spontaneous. Most of the ADR reports referred to women (n=31; 56%) and adult persons (n=31; 56%). In the 55 reports there were 169 different ADRs listed, most commonly ADRs were related to gastrointestinal system (30%), where nausea and vomiting were by far the most common. Erythema and hyperhidrosis were most common dermatological ADRs, while vertigo was the most common neurological ADR. In the cardiovascular system palpitations were most frequent ADR. Among ADRs affecting general condition of a patient, dizziness and asthenia were the most common. Detailed information about ADRs of tramadol are presented in Supplementary tables in the online appendix of this manuscript.

For the other drugs, with lower number of reported ADRs detailed description of those ADRs is provided in Supplementary tables in the online appendix of this manuscript.

Discussion

This study analyzed reports of adverse events associated with analgesics reported to national Croatian authority from 2007 to 2014. Among 896 reports there were 20 analgesics with ≥5 reports in the 8-year period and among them top five were ibuprofen, diclofenac sodium, tramadol/paracetamol combination, acetylsalicylic acid and tramadol.

ADRs are an important cause of morbidity and mortality; it is estimated that they are on the 4-6th place on the list of leading causes of death in the United States annually (10). Therefore, it is important both to encourage reporting of ADRs and to study them systematically. The main limitation in the analysis of ADRs is insufficient reporting and therefore it is important to keep encouraging all stakeholders to report the observed ADRs, in spite of a series of obstacles related to the process that have been identified in earlier studies (10).

It is especially important to closely monitor the effects of new drugs on the market because in this way, after the introduction of the drug on the market, potentially dangerous ADRs can be caught that were not identified in earlier stages of clinical trials. Spontaneous reports can provide signals that are later confirmed by other databases and complaints of patients. An example of these warning signs in recent times is myocardial infarction caused by a drug rofecoxib and heart failure caused by rosiglitazone (11).

Studies related to the promptness of reporting ADRs were not conducted in Croatia and therefore it is difficult to speculate about the accuracy of data, or to what extent the recorded adverse reactions are actually reported to the HALMED. This study was conducted specifically for the period from the year 2007 to 2014 because the HALMED established a comprehensive electronic database of ADRs in 2007. Electronic database allows for a simpler retrieval of data about ADRs and their analysis.

Historically, hospitals have relied on spontaneous reporting of harms related to drugs. This approach systematically underestimates the frequency of ADRs and recovers only a minority of ADRs. This approach is attractive because it is cheap, compared to other methods of data collection. A better approach is to use patient charts to identify ADRs, but for routine use is too expensive (12). The third approach in finding ADRs is a computer detection. This method generally uses computer data to identify signals that indicate the possible presence of ADRs. Although this approach still involves the use of the chart to confirm the event, it is much cheaper because only a small part of the scale must be reviewed and review can be focused (12).

The key benefit of electronic medical records is that it can be used to detect the frequency of ADRs and to develop methods for reducing the number of such events. Development and maintenance of the computer system of screening involves several steps. The first and biggest step is to collect information about patient in an electronic form. The second step is to apply inquiries, rules or algorithms to find objects with information in accordance with the examined adverse event. The third step is to determine the predictive value, typically, a manual review (12).

This study provides a detailed description of ADR reports of 20 analgesics or analgesic combinations that had more than 5 ADR reports, covering 75% (n=597) of all reports of ADRs of analgesics in the analyzed period. Certain ADR reports contained multiple ADRs identified in a patient. Therefore, the number of reports in some cases was not proportional to the number of different ADRs that have been listed in the reports. In this study ibuprofen was drug which had the most ADR reports in the analyzed period. However, diclofenac sodium was a drug for which the biggest number of various ADRs was listed, although it had fewer reports compared to ibuprofen. The ADR reports often record multiple different ADRs in the same patient. For the comparison, analysis of spontaneous reports of side effects of Portuguese national unit for pharmacovigilance shows that the unit received 2,408 reports containing a total of 5749 adverse reactions from 2001 to 2013 (13).

The highest number of ADRs of analgesics in this study was recorded on the skin and mucous membranes. In 11 of 20 drugs skin disorders were the most frequent. Skin disorders were followed by gastrointestinal disturbances. Problems with blood and blood coagulation were seen as the most frequent in the combination of acetylsalicylic acid and clopidogrel, and psychiatric problems during the use of opioid analgesic fentanyl as fentanyl citrate. The only drug for which death as an ADR was recorded was fentanyl; suicide was the most frequently reported ADR for this drug.

We were unable to find similar studies that covered ADRs of analgesics, and therefore we compared some of our data with similar pharmacovigilance studies on national level, which reported ADRs for all drugs. A recent study of Ozcan et al. about ADRs reported on a national level in Turkey did not present drugs in the same way that we did; they reported drugs for which ADRs were reported according to the body system in line with the ATC classification. Based on their study, drugs acting on a nervous system, which would be concordant with analgesics, were responsible for 14% of the reported drugs, ranking third among all the categories of drugs that were used (14). Bourgeouis et al. analyzed national estimates and characterizations of outpatient adverse drug events in the United States between 1995 and 2005. They showed that non-opioid analgesics were among the three most common drugs warranting a clinic visit due to adverse drug event, and in the emergency department non-opioid analgesics/antipyretics most frequently resulted in adverse drug event (15).

In this study, 46% of the ADRs were categorized as serious, and most of those serious ADRs were caused by fentanyl, combination of acetylsalicylic acid and clopidogrel hydrogen sulfate, fentanyl citrate, diclofenac potassium and fixed combination of caffeine/paracetamol/propyphenazon/codeine phosphate sesquihydrate. Portuguese data for the period 2001-2013 show that among spontaneous ADR reports 55% were categorized as serious ADRs (16). It is possible that serious ADRs make such a big percent of ADRs because of their gravity – non-serious ADRs could be perceived as less important by the healthcare workers. Different results were reported from an Australian study in 2013, which investigated medicines causing ADRs in patients older than 45 years. Studies were based on survey data collected by family physicians. ADRs were most commonly caused by opioid analgesics. Half of the patients had mild ADRs, 42% had moderate and 12% severe ADRs. Five percent of patients were hospitalized due to most recent ADR (17). These differences in the percentages of serious ADRs may be partly due to different categorizations of adverse events. In our study we analyzed data based on two pre-existing categories from the HALMED database, including serious and non-serious ADRs. However, in the Australian study there were four categories of ADRs – mild, moderate, severe and death (17).

A French study published in 2015 found that more than half of cases were serious and led to hospitalization. It was concluded that in 95% of cases it was possible to prevent those ADRs. The main factor which can be influenced to prevent adverse reactions is careless recommendations for analgesics use and wrong prescribing (17). A 2003 study found that 38% of ADRs were serious and it was estimated that 28% of all identified drug ADRs could be prevented. Most of the errors occurred in prescribing and monitoring patient therapy. Adherence errors were also frequent (18). In Croatia there is a network of pain clinics, that employ pain specialists that could be consulted for adopting interventions to prevent such ADRs (19).

In a national study which was conducted in the US in 2003, it was concluded that

many patients have adverse reactions during transition from hospital to home. According to the study, approximately one out of 5 patients has experienced an adverse event during the transition from hospital to home (20). The fact that people who are leaving hospital are more vulnerable to appearance of ADRs than other patients tells us that some populations are more vulnerable than others when it comes to ADRs. The study also came to the conclusion that one third of adverse events could be prevented. Another third was inevitable, but their weight could be reduced if there were previously implemented corrective actions. These results indicate that there are four areas of potential ADR prevention measures: assessment of patients at the time of discharge, teaching patients about medicines, ADRs and what to do if the specific problems develop, improving therapy monitoring and improving monitoring of the patient's global condition (20). Another report concluded that a larger number of ADRs could be prevented in the elderly because of complexity of their clinical presentation. The side effects and medication errors are the main target in the prevention of adverse events (21). It has been shown that a decision tree model for analysis of ADRs to discover combinations of multiple risk factors that would increase the risk; therefore, such tools could be used in clinical practice (22).

Additionally, patients are prone to self-medication, and it has been found that sharing of prescription analgesics between patients is common behavior that is mostly viewed positively by patients and even some physicians (23, 24). Therefore, educational interventions targeted to patients that will aim to reduce sharing of prescription analgesics to others may help in reducing ADRs.

In this study 96% of all ADRs reports of analgesics, reports were collected through forms that were filled out by health care workers or patients, i.e. they were spontaneous. Alshammari et al. published in 2015 an analysis of the integrity of ADR reports for all medicines in Saudi Arabia and reported for the analyzed period that out of a total of 14,873 ADR reports, 80% were spontaneous (13).

In this study 42% of the ADR reports were sent by pharmacists and 43% by physicians; 39% of reports were submitted at a pharmacy. These numbers indicate crucial importance of the pharmacist and pharmacies in recording ADRs. A study about social impacts on US pharmacists related to ADRs reporting showed that the majority of respondents intends to report serious adverse reactions, and that the most important influences for submitting ADR report are those coming from the referent regulatory body which registers ADRs, as well as from patients, pharmacy and hospital administrators. Being a woman, having less work experience and better understanding the process of reporting were associated with a greater intention for ADRs reporting (25).

US study published in 2003, with 377 pharmacists included, showed that most of the pharmacists (68%) had never reported an adverse reaction to the regulatory body. Most pharmacists (66%) thought that they do not have sufficient knowledge about the process of ADR reporting. On the assessment of knowledge they showed knowledge in the range of 56-96%. The conclusion was that pharmacists should have more education, awareness and practice related to the reporting of ADRs (26).

A study published in Croatia in 2010 analyzed pharmacists' awareness about importance of knowledge about ADRs, pharmacists' knowledge about the pharmacovigilance system in Croatia and pharmacists' personal reasons for not reporting ADRs. The study included 471 pharmacists, mostly employed in the pharmacies. The results showed that pharmacists have sufficient knowledge about ADRs and legal obligation to report ADR, but despite that, number of

ADR reports made by pharmacists was meager. After attending workshop provided by the HALMED for a target group of pharmacists, an increase in the number of ADRs reported made by pharmacists was observed, from the 0.6% before 2006 to 22% in the first 6 months of 2006. This study showed that education of pharmacists can increase the number of reports of ADRs. When they were asked for the reasons for not reporting ADRs, pharmacists indicated that they usually see ADRs which are already known, and that the patient was using more drugs at the same time and because of that it was not possible to establish an association between certain ADRs and a particular drug (27). Some of the pharmacists stated that they do not know how to report an adverse reaction. Pharmacists have a key role in pharmacovigilance (28) and therefore it is important to invest in their knowledge and skills related to the adverse reaction reports, which are legal obligation.

Regarding causality, HALMED uses the World Health Organization – Uppsala Monitoring Centre (WHO-UMC) system for causality assessment. This system has been developed together with national centers that participate in the Programme for International Drug Monitoring and is designed as a practical tool for assessing case reports. That is combined assessment that takes into account the clinical-pharmacological aspects of the case history and the quality of the documentation of the observation. The method then gives guidance to the general arguments which should be used to select one category over another. The WHO-UMC system recognizes six different causality categories: certain, probable/likely, possible, unlikely, conditional/unclassified and unassessable/unclassifiable. For the purpose of this study, we used only cases which were classified in first four categories.

Limitations

Limitations of our study include lack of causality assessment. For example, ASA is an antiinflammatory drug, but when associated with clopidogrel its therapeutic indication is as an antiplatelet drug. Therefore, ADRs related to blood and blood coagulation problems could be due to an interaction between ASA and clopidogrel, which makes causality assessment difficult. Additional such associations and cofounding variables are possible and reported ADRs may or may not be associated with the analgesics in question.

Conclusion

In conclusion, number of ADR reports for analgesics is continuously increasing and a considerable number of them refers to serious ADRs. More research in this field is necessary so that health care professionals and patients can choose the appropriate therapy that will cause less ADRs. To keep better track of medications and ADRs it is necessary to educate and encourage health professionals and patients in reporting side effects.

What is already known on this topic

Analgesics are widely used and it can be expected that with the increased use one can expect increased number of adverse drug reactions of analgesics. Large increases in the use of both opioid and non-opioid analgesics were observed worldwide.

What this study adds

This study analyzed reports of adverse drug reactions (ADRs) associated with analgesics reported to national Croatian authority from 2007 to 2014. Among 896 reports there were 20 analgesics with ≥5 reports in the 8-year period and among them top five were ibuprofen, diclofenac sodium, tramadol/paracetamol combination, acetylsalicylic acid and tramadol. We found that the number of ADR reports in Croatia is continuously increasing and a considerable number of them refers to serious ADRs. To keep better track of medications and ADRs it is necessary to educate and encourage health professionals and patients in reporting side effects.

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Original article

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Frequency and correlation of lip prints, fingerprints and ABO blood groups in population of Sriganganagar District, Rajasthan

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Objective. To investigate the frequency and uniqueness of different lip print patterns, fingerprint patterns in relation to gender and ABO Rh blood groups among a semi-urban population of Sriganganagar, Rajasthan. **Materials and methods.** The study was conducted on 1200 healthy volunteers aged 18-30 years. The cheiloscopic and dermatographic data of each subject were obtained and were analysed according to the Suzuki and Tsuchihashi and Henry systems of classification, respectively. Two forensic experts analyzed the patterns independently. The ABO Rh blood group was also recorded for each subject. The Chi square statistical analysis was done and tests were considered significant when p value <0.001 and Cohen kappa test was applied to analyze inter-observer reliability. **Results.** The B+ blood group was noted as most common in both genders while least common were A– among males and AB– in females. Type II lip pattern was most predominant while the least common was Type I' in males and Type I' and Type V in females. The UL fingerprint pattern was the most common, while RL was least noted in both genders. All the fingerprint patterns showed correlation with different lip print patterns. A correlation was found between different blood groups and lip print patterns except Type I (vertical) lip pattern. A positive correlation was observed between all the blood groups and fingerprint patterns, except for RL pattern. **Conclusion.** There is an association between lip print patterns, fingerprint patterns and ABO blood groups in both the genders. Thus, correlating the uniqueness of these physical evidences sometimes helps the forensic team members in accurate personal identification or it can at least narrow the search for an individual where there are no possible data referring to the identity of the subject.

Key words: Cheiloscopy ▪ Dermatographics ▪ Forensic ▪ Gender ▪ Personnel identification.

Introduction

The positive identification of the living or deceased using distinct traits is a cornerstone of forensic science. According to Locard's exchange principle, when two objects come into contact, there is always a transfer of material from one to the other. Traces from the scene may be carried away by a person and at the same time may be left at the scene (1). The fingerprints, lip prints and blood remains are evidence left at the crime scene which can be utilized for forensic identification purpose based on the same principle. The use of these three physical forms of evidence is of paramount importance, since undertaking personal identification

by other means such as DNA analysis is a sensitive and costly technique and therefore difficult to use for each and every case (2). Moreover, correlating this physical evidence at the crime scene altogether, rather than individual pieces of evidence alon, sometimes helps the forensic team members in accurate identification. Also, they can be used to substantiate facts in crimes where there is very little evidence, especially in regional mass disasters, accidents or crimes.

Lips surround the oral orifice and are covered by mucosa and partly by skin. When identification is concerned, the mucosal area is important, which is called Klein's zone, covered with wrinkles and grooves on the labial mucosa, forming characteristic patterns called lip prints (3). Suzuki and Tsuchihashi (4) established that the arrangement of lines on the red part of the human lip is individual and unique. The lip prints are identifiable as early as from the sixth week of intrauterine life. Mc Donell (5) reported in 1972 that two identical twins, who seem to be indistinguishable by any other means, can be distinguished by their lip prints. The lip prints on a drinking glass, clothing or cigarette butt found at crime scenes may be a link to a suspect and can be obtained for up to 30 days after being produced (6, 7). The lipstick-cellophane tape method used in lifting lip prints was first described by Bindalet al. (8). These latent and invisible lip patterns can be detected using aluminium powder, Sudan III dye, silver nitrate powder, plumb carbonate powder, fat black aniline dye, cobalt oxide, Indigo blue dye or magnet powder. The latent lip prints can be better developed using materials that are more sensitive to fatty acids. Lysochromal dyes contain an ingredient that dissolves in contact with fat and another that stains and, hence, have the ability to stain fatty acids while developing latent lip prints (9).

Moreover, in the case of a deceased person, the lip prints have to be obtained within 24 hours to prevent them from undergoing post-mortem changes. In 1967 Santos (10) classified lip grooves into four types (straight line, curved line, angled line and sine shaped line). In 1970 Suzuki and Tsuchihashi (4) devised a new classification of lip grooves as: Type I: A clear groove running vertically across the lip; Type I': Partial length groove of type I; Type II: A branched groove; Type III: An intersected groove; Type IV: A reticular pattern; Type V: Undetermined.

Dermatoglyphics is defined as the scientific study of epidermal ridges and their configuration on the volar aspect of the palmar and planter regions (11). Herschel (12) used fingerprints for personal identification in India. The palmar surfaces of hands have friction ridges, known as papillary or epidermal ridges. The establishment of the epidermal ridges takes place at from 10^{th} to 16^{th} weeks of development. As the fingerprint patterns are encoded on the interface between the dermis, the pattern cannot be destroyed by superficial skin injuries. Henry's classification is the most accepted and commonly used system, that allows for logical categorization of ten-print fingerprint records into primary groupings based on the physiological characteristics of the fingerprint pattern types. The three basic patterns of fingerprint ridges according to Henry's system (13) of classification are: a) Arch (plain and tented): An arch is a pattern where the ridges enter from one side of the finger, rise in the centre forming an arc, then exit from the other side of the finger; b) Loop (radial and ulnar, double or pocket): A loop is a pattern where the ridge enters from one side of a finger, forms a curve and tends to exit from the same side they entered c) Whorl (plain): A whorl pattern ridge forms circularly around a central point on the finger.

The blood group system was discovered in 1901 by Karl Landsteiner (14). The ABO' system is further classified as A, B, AB, O blood group types according to the corre-

sponding antigen in the plasma, while the Rhesus system is classified into Rh+ and Rh– according to the presence or absence of D antigen.

Although the studies correlating and comparing all these three variables are minimal, in a few studies (15, 16) significant correlations were found between them. Nandan et al. (17) also revealed in his study the weaker but still clear significance of lip prints and fingerprints in gender identification. Moreover, the sample size in previous studies (6, 18) was quite small (ranging from 54 to 208). Taking this shortcoming into consideration, this study was designed to be conducted on a much larger sample size that could give us a better correlation in between these three variables.

Hence, with this background, the present study was conducted to investigate the prevalence and correlation of lip print patterns, fingerprint ridges patterns and ABO blood groups among both genders in the population of Sriganganagar District, Rajasthan.

Material and methods

The present study was conducted at the Department of Oral Medicine and Radiology, Surendera Dental College and Research Institute, Sriganganagar, District Rajasthan, on 1200 randomly selected healthy volunteer subjects of both sexes, between the ages of 18 to 30 years. The study sample was considered arbitrarily. The inclusion criteria of the study consisted of: 1. Healthy males and females with no systemic, metabolic, dermatological or endocrinal disease. 2. Individuals who were non-syndromic. 3. Individuals born and brought up in Sriganganagar (North-western India) and of Indo-Aryan ethnic origin. The exclusion criteria consisted of: 1. Individuals with missing anterior teeth. 2. Individuals with permanent scars on fingers or lips caused by injuries, inflammation or surgery. 3. Individuals with worn fingerprints, extra, webbed or bandaged fingers. 4. Bacterial, viral or fungal infections affecting lips and hands.

Method of collection of lip prints

The lips of the subject were cleaned and allowed to dry for 1 minute and a red lipstick was applied uniformly to the lips. After 2 minutes, the glued portion of the cellophane tape was evenly placed and stuck to the closed lips in normal resting position, covering the entire portion of both lips, without any movement. The strip of cellophane was

Figure 1 The equipment and procedure used for recording Lip Prints: (A) Gloves; (B) Mouth mask; (C) Kidney tray); (D) Cleansing milk; (E) Red lipstick; (F) Cellophane tape; (G) Scissor; (H) Bond paper; (I) Procedure used for taking lip prints.

removed and was properly stuck to the white bond paper (A4) without any wrinkles for a permanent record. Each lip print was divided into six areas starting from the upper right area to the lower right area (A1-A6). The lip prints were analysed following Suzuki and Tsuchihashi's (4) classification using a magnifying glass, by two Forensic experts independently, and the type of pattern which was repeated the maximum number of times, was considered as described by Rajendran Sivapathasundram (19) (Figure 1).

Method of collection of fingerprints

For the fingerprints, the right hand of the subjects was considered as this is the side that most government agencies legally collect. The impressions of all five fingers were taken by asking the subjects to roll the tip of their right hand fingers across the surface of the stamp pad (Camlin of size 157×96mm) and then to transfer the fingerprint impressions onto A4-size white bond paper. The fingerprint patterns were analyzed following Henry's classification (13) using a magnifying glass by the same two Forensic experts independently (Figure 2).

Method of determining ABO blood group

The blood group of the subjects was identified by placing a drop of blood on a slide and treating it with anti-A, anti-B and then anti-Rh sera. The positive agglutination of the blood upon treatment with anti-A was considered as blood group A, a positive reaction with anti-B was considered as blood group B, if no agglutination was produced it was blood group O, and if agglutination was seen with both antisera, then blood group AB was considered. Similarly, a positive agglutination reaction with Rh antigen was considered Rh+, or otherwise Rh– (20).

Statistical analysis

The results thus obtained from lip prints, fingerprints and ABO blood group samples were tabulated and analysed using SPSS 18.0 (Microsoft Corporation Inc., Chicago, IL, USA) statistical software. The Chi-square statistical analysis was done and tests were considered significant at p value < 0.001 and the Cohen kappa test was applied to analyse inter-observer reliability.

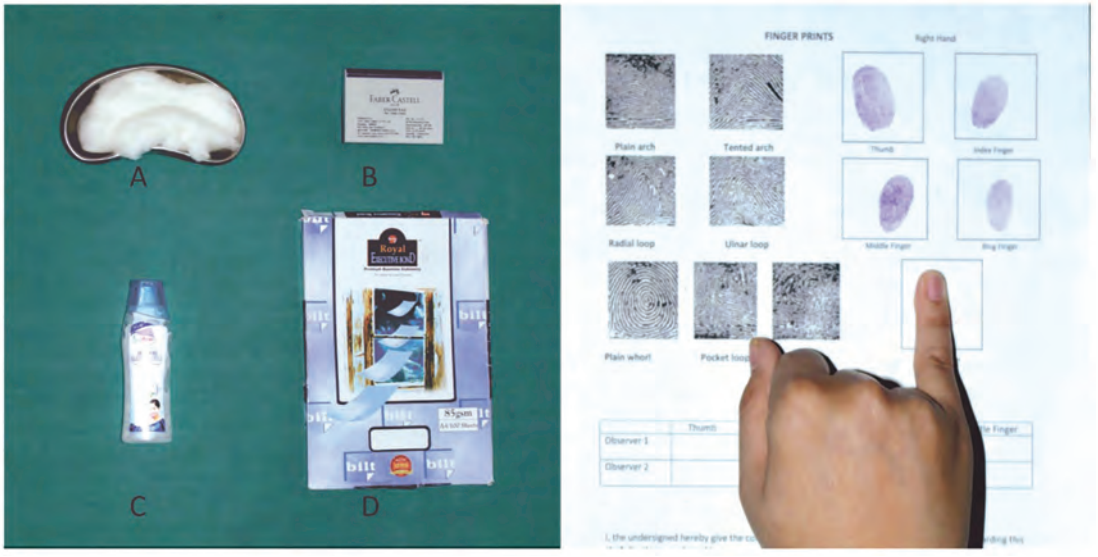

Figure 2 The equipment and procedure used for recording Finger Prints: (A) Kidney tray, cotton; (B) Blue stamp pad; (C) Cleansing milk; (D) Bond paper; (E) procedure used for taking fingerprints.

Results

Out of the total sample of 1200 randomly selected subjects aged between 18-30 years, 540 (45%) were males and 660 (55%) were females, with an overall median age of 20 years. The results of the two observers (observer 01 and observer 02) were analysed statistically for lip print and fingerprint patterns, to check inter-observer reliability, using the Cohen kappa test. Since the kappa (k) coefficient value was >0.8, which indicated good inter-observer agreement, the inter-observer bias was found to be nil (Table 1 and 2) so, for further statistical analysis the results of observer 1 were considered.

Both genders showed the Type II lip pattern to be most predominant while the least common was Type I' in males and Type I' and V in females (Table1). The ulnar loop (UL) fingerprint pattern was the most predominantly noted in both genders while the radial loop (RL) was least noted in both genders (Table 2). Among the total of 1200 individuals the B+ blood group was noted to be most common in both the genders, while the least common blood group was A- in males and AB– in females. The distribution of blood groups by gender was not statistically significant (Table 3).

Table 1 The prevalence of lip print patterns in both the genders

Types of lip print	Male		Female		Total	
	O-1 (n)	O-2 (n)	O-1 (n)	O-2 (n)	O-1 (n)	O-2 (n)
I	46	47	52	49	98	96
I'	5	4	3	3	8	7
II	278	278	317	317	595	595
III	127	128	178	182	305	310
IV	73	72	107	105	180	177
V	11	11	3	4	14	15
Total	540	540	660	660	1200	1200
Kappa value	0.81		0.82		0.84	

Type I=A clear groove running vertically across the lip; Type I'=Partial length groove of type I; Type II=A branched groove; Type III=An intersected groove; Type IV=A reticular pattern; Type V= Undetermined; O-1=Observer 01; O-2=Observer 02.

Table 2 The prevalence of fingerprint patterns in both the genders

Types of finger print	Male		Female		Total	
	O-1 (n)	O-2 (n)	O-1(n)	O-2 (n)	O-1(n)	O-2 (n)
DL	6	6	8	8	14	14
PL	28	28	24	23	52	51
PA	11	11	11	10	22	21
PW	203	204	136	134	339	338
RL	-	1	1	1	1	2
TA	13	13	6	7	19	20
UL	279	277	474	477	753	754
Total	540	540	660	660	1200	1200
Kappa value	0.64		0.83		0.80	

DL= Double loop; PL=Pocket loop; PA=Plain arch; PW=Plain whorl; RL=Radial loop; TA= Tented arch; UL=Ulnar loop. O-1= Observer 01; O-2= Observer 02.

Table 3 Frequency of types of ABO Rh blood groups among genders

Blood groups	Male n (%)	Female n (%)	Total n (%)
A+	79 (6.58)	115 (9.58)	194 (16.17)
A–	10 (0.83)	16 (1.33)	26 (2.17)
B+	253 (21.08)	283 (23.58)	536 (44.67)
B–	26 (2.17)	27 (2.25)	53 (4.42)
AB+	49 (4.08)	58 (4.83)	107 (8.92)
AB–	15 (1.25)	13 (1.08)	28 (2.33)
O+	90 (7.50)	127 (10.58)	217 (18.08)
O–	18 (1.50)	21 (1.75)	39 (3.25)
Total	540 (45)	660 (55)	1200 (100)

χ^2=4.14; p=0.764.

Table 4 Correlation of types of ABO Rh blood groups with lip print patterns (in %)

Blood groups	Lip print patterns						Total
	I	I'	II	III	IV	V	
A+	1.25	0.08	7.67	4.75	2.17	0.25	16.17
A–	0.17	0.00	0.50	0.75	0.75	0.00	2.17
B+	3.83	0.33	22.58	10.50	6.92	0.50	44.67
B–	0.50	0.08	1.92	1.33	0.58	0.00	4.42
AB+	0.50	0.00	4.50	2.33	1.25	0.33	8.92
AB–	0.17	0.00	1.33	0.50	0.33	0.00	2.33
O+	1.17	0.08	9.33	4.83	2.58	0.08	18.08
O–	0.58	0.08	1.75	0.42	0.42	0.00	3.25
Total	8.17	0.67	49.58	25.42	15.00	1.17	100.00

I=Vertically running lip pattern; I'=Partial length; II=Branched; III=Intersected; V=Reticular; V=Undetermined.

The Type II lip pattern, followed by Type III, were the most frequently observed in all blood groups, except in A- blood group, where Type III and IV lip patterns were predominantly seen. The Type I' (0.65%) followed by Type V (1.16%), Type I (8.17%), Type IV (15%), Type III (25.41%) and Type II (49.58%) lip patterns were noted in ascending percentage in any of the blood groups (Table 4).

Also the UL fingerprint pattern (62.67%) followed by the PW pattern (28.25%), PL (4.41%), PA (1.83%), TA (1.58%) DL (1.16%) and RL (0.08%) were noted most commonly in all the blood group patterns, while one subject with B^- blood group was noted with the RL fingerprint pattern. Also the primary fingerprint distribution among the Rh blood groups showed a high frequency of loops, a moderate number of Whorls, and few arches (Table 5).

The UL fingerprint pattern was most predominantly seen in all of the lip print patterns. Also the Type I' lip print pattern was noted least in all of the fingerprint patterns, followed by the Type V pattern (Table 6).

Table 5 Correlation of types of ABO Rh blood groups with fingerprint patterns (in %)

Blood groups	Fingerprint patterns							Total
	DL	PA	PL	PW	UL	TA	RL	
A+	0.08	0.25	0.75	4.25	10.67	0.17	0.00	16.17
A–	0.17	0.08	0.08	0.83	1.00	0.00	0.00	2.17
B+	0.75	0.58	2.50	12.50	27.58	0.75	0.00	44.67
B–	0.00	0.08	0.17	1.33	2.67	0.08	0.08	4.42
AB+	0.17	0.25	0.33	2.33	5.58	0.25	0.00	8.92
AB–	0.00	0.00	0.08	0.92	1.33	0.00	0.00	2.33
O+	0.00	0.50	0.42	5.25	11.58	0.33	0.00	18.08
O–	0.00	0.08	0.08	0.83	2.25	0.00	0.00	3.25
Total	1.17	1.83	4.42	28.25	62.67	1.58	0.08	100.00

DL=Double loop; PL=Pocket loop; PA=Plain arch; PW=Plain whorl; RL=Radial loop; TA=Tented arch; UL=Ulnar loop.

Table 6 Correlation of types of Lip prints with different fingerprint patterns (in %)

Types of lip print	Fingerprint pattern							Total
	DL	PL	PA	PW	RL	TA	UL	
I	0.00	0.25	0.00	2.33	0.00	0.33	5.25	8.17
I'	0.00	0.00	0.00	0.08	0.00	0.00	0.58	0.67
II	0.58	2.50	1.25	13.50	0.08	0.75	30.92	49.58
III	0.33	0.83	0.42	7.58	0.00	0.25	16.00	25.42
IV	0.25	0.83	0.17	4.33	0.00	0.25	9.17	15.00
V	0.00	0.00	0.00	0.42	0.00	0.00	0.75	1.17
Total	1.17	4.42	1.83	28.25	0.08	1.58	62.67	100.00

I=Vertically running lip pattern; I'=Partial length; II=Branched; III=Intersected; V=Reticular; V=Undetermined; Type I=A clear groove running vertically across the lip; Type I'=Partial length groove of type I; Type II=A branched groove; Type III=An intersected groove; Type IV=A reticular pattern; Type V=Undetermined; DL=Double loop; PL=Pocket loop; PA=Plain arch; PW=Plain whorl; RL=Radial loop; TA=Tented arch; UL=Ulnar loop.

Discussion

Forensic odontologists need knowledge that encompasses a number of disciplines, since dental records (21) can identify an individual or provide information needed by the authorities to establish neglect, fraud or abuse (22). Though not exclusive like an individual's DNA, fingerprints and lip prints and the ABO blood group system have the merits of being unique, permanent and are accepted evidence in a court of law (23). The correlation between variables can be used to calculate the strength between them, so the correlation of lip prints with ABO blood groups and fingerprints may be more useful in forensic science for the accurate identification of an individual than using individual parameter. Also it can narrow the search for an individual where there are no possible data referring to the identity of the subject.

Type II was the most predominant lip print pattern noticed (49.58%) in both genders, while Type I' (incomplete vertical grove pattern) was noticed the least (0.67%). The results were in accordance with previous studies (6, 15, 24-26). In another study (27) Type I and Type I' patterns were found

to be common in females while Type III and Type IV patterns were common in males, while the study by Naik et al. (18) showed that the Type IV pattern was the most prevalent in males and Type I/I' pattern was the most prevalent in females. These differences can be explained by the fact that lip print patterns are unique to different populations. Therefore, the variance can be explained by the ethnic, racial and geographical differences in the population studied (28), since in the present study only subjects from an Indo-Aryan ethnic race background were studied with their origins in the Northwestern region of India. Also, the different methods employed in the analysis of lip prints could be the reason for the disparity of the results.

The lip prints were recorded in closed relaxed position. The type of lip print was assessed in six areas and the pattern repeated the maximum number of times was considered to be the final lip print pattern, in accordance with Sivpathasundharam et al. (19), who stated that lip pattern recording depends on the way the lip muscles are relaxed to produce a particular pattern. The most widely accepted classification of lip print patterns, proposed by Suzuki and Tsuchihashi (4) in 1970, was followed. Moreover, it was found that it gave a clear description of nearly all the lip patterns commonly encountered and was easy to interpret. Its resemblance to the dental formula was also familiar to the forensic dentist (29). The 18-30 years age group was selected for the study as lips reach maturity in late adolescence (30). In the mid to late 30's, although age changes begin to occur in the upper face, the lips retain their tonicity and do not show any age-related changes (31). After 40, due to the occurrence of wrinkles on the adjacent skin and thinning of lips, lip print patterns are affected (32).

The ulnar loop (UL) fingerprint pattern was the most predominantly noted in both genders (39.50% in females and 23.25% in males) while the radial loop (RL) was least noted in both genders (0.08% in females and none in males). The results were in agreement with previous studies (33-35). Cummins (36) in 1926 postulated that this was the result of physical and topographic growth forces, tension and pressure in the skin during early embryogenesis which determined the direction of the dermal ridge.

In India, O+ is the most common blood group type (33) followed by B+, but in our study B+ was found to be most common in both genders (21.08% males and 23.58% in females) while A− was the least noted blood group in males, and AB− in females. This might be due to different regional genetic backgrounds. Moreover, according to the various antigen–antibody reactions in the bloodstream, different individuals have specific blood groups. Similar results were also noted by Srilikha et al. (2), Verma et al. (6) and Patel et al. (37). Also the majority of subjects were Rh+ (87.83%) while only 12.7% were Rh−. These results were in accordance with previous studies (14, 33).

It was noted that the Type II lip print (branched) pattern was prominent among almost all blood group subjects, while the study conducted by Patel et al. (37) showed that in blood group A+ Type II was most prominent, and in blood group B+ it was Type I, and in blood group O+ it was noted to be Type II. The study conducted by Harsha et al. (38) showed that the reticular type (Type IV) of lip pattern was more prominent among subjects with B+ blood group, and the vertical pattern (Type I) with O+. Hence, from the data obtained and the conflicting results of previous studies, it is difficult to predict the blood group of an individual from the type of lip print pattern alone (39).

A significant correlation was observed between lip print patterns and ABO blood groups in the present study. This might be due to the fact that both lip prints and

blood groups are genetically determined and developed during early foetal life (37). This contrasts with the studies conducted by Verma et al. (6), Furnari et al. (40) and Hunasgi et al. (41), due to the smaller study sample considered by past researchers, and moreover the present study reflected the most common whole upper and lower lips patterns (six quadrants considered), rather than partial lip area patterns. The UL fingerprint pattern was the most predominantly noted in both genders in all blood groups. Also, the three fingerprint patterns (Whorls, Arches and Loops) were seen more among Rh+ subjects. Similar results were obtained by previous studies (35, 42).

A highly significant correlation was found between lip prints and fingerprints in both genders in the present study. This can be explained by the fact that the epidermal ridges of the fingers and palms, as well as the facial structures such as the lips, alveolus, teeth and palate, are formed from ectoderm during the same embryonic period (6-9 weeks) with a somewhat similar genetic background (43). Loop patterns of fingerprints were most predominantly seen in all the ABO blood groups while past studies (44, 45) found no or only a weak correlation between them. This again may be due to the small study samples of previous studies.

Nowadays the digital analyses of both lip prints and fingerprints are available (Adobe Photoshop, Veri Finger SDK, Precise Biometrics Biomatch™, etc) to avoid human error in manual analysis. A few studies have been conducted using digital analysis (46, 47), while others have been done using manual methods (6, 34), but both methods have showed no significant differences in their findings. Taking into consideration the large sample size, we performed the manual method with two observers. However more extensive and detailed research studies using digital analysis among different populations, considering racial and ethnic backgrounds, are required to establish a definite correlation between these variables. Also, the fingerprints of only the right hand were considered for the study, so the results could be fuller using fingerprints from both hands.

Conclusion

Both genders showed the Type II lip pattern and the ulnar loop (UL) fingerprint pattern to be the most predominantly recorded ectodermal patterns. The B+ blood group was noted as most common in both genders. A highly significant correlation was found between lip prints, fingerprints and ABO blood groups in both genders in the Indo-Aryan (North-western India) ethnic background study population. Our regional, large sample sized study clearly reflects that the supplementary physical evidence (lip prints, fingerprints and ABO blood groups) can be assessed by simple and non-expensive techniques, and can be used as an additional tool in forensic investigations in the Sriganganagar population.

What is already known on this topic

With the ever increasing demands placed upon law enforcement to provide sufficient physical evidence linking a person to a crime, it makes sense to utilize any type of physical characteristic to identify suspects guilty of a particular offense. Personal identification plays an inevitable role in forensic investigations. Lip prints, fingerprints and ABO blood groups are geno-typically determined and remain unchanged from birth until death.

What this study adds

Although extensive scientific research into the study of lip prints, fingerprints and ABO blood groups is available the studies correlating and comparing all these three variables are minimal. However correlating these physical evidences at the crime scene may be used to substantiate facts in crimes where there is very little evidence. Moreover, regional data from the Sriganganagar district, Rajasthan (North-western India) on variables add to the existing data pool for forensic use.

Authors' contributions: Conception and design: PV and HK; Acquisition, analysis and interpretation of data: HK, SP, and SSR; Drafting the article: HK; Revising it critically for important intellectual content: PV,

HK, SSR and SP; Approved final version of the manuscript: PV, and SSR.

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Original article

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Metformin use associated with protective effects for ocular complications in patients with type 2 diabetes – observational study

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Objective. The aim was to study the association of the use of an oral antihyperglycemic agent metformin with the presence of ocular complications in patients with type 2 diabetes (T2D). **Methods.** Medical records were reviewed for 234 patients with diagnosed T2D. 81.2% (n=190) patients were using metformin and 18.8% (n=44) using other oral antihyperglycemic agents. Plasma glucose concentration, glycated haemoglobin, and the presence of ocular complications in patients treated with metformin were compared to those in patients treated with other oral antihyperglycemic agents. **Results.** Ocular complications occurred in 65 patients (27.8%). Patients treated with metformin had fewer ocular complications compared to patients treated with other oral antihyperglycemic agents (χ^2=19.985; p<0.0001). After adjustment for gender, age, duration of T2D, serum concentration of cholesterol, smoking, body mass index and presence of other diseases, treatment with metformin decreased the odds of both glaucoma (OR=0.14, 95% CI: 0.03-0.57, p=0.006) and diabetic retinopathy (OR=0.33, 95% CI: 0.14-0.82, p=0.017) compared with other oral antihyperglycemic agents. **Conclusion.** Our results suggest that metformin may have a protective effect on ocular complications, especially glaucoma, in patients with T2D. The effects of metformin either regarding prevention of ocular complications or ocular complications already developed in patients with T2D, should be further investigated.

Introduction

Type 2 diabetes (T2D), as a chronic systemic metabolic condition, causes changes to the eyes, leading to ocular complications such as glaucoma, diabetic retinopathy, cataracts and other ocular lesions (1, 2). This has been proven to be one of the major causes of visual impairment in these patients (3).

A relationship between T2D and glaucoma has been described (4). It is confirmed that diabetes mellitus (DM) is an etiological factor for neovascular glaucoma, but there are still controversial opinions about its association with open-angle glaucoma or angle-closure glaucoma (5). Factors identified to increase the risk of glaucoma in patients with T2D are corneal stiffness, corneal hysteresis and enlargement of the optic cup, recognized as an important morphological feature of the glaucomatous eye (6). However, the age of the patient is a major risk factor for the onset of glaucoma (5).

Diabetic retinopathy is the most common ocular complication of DM and, if not treated, may lead to vision loss (7). Factors that affect the occurrence of diabetic retinopathy are the duration of DM, the severity of hyperglycaemia and the age of the patient (8). Diabetic retinopathy is also related to hypertension, dyslipidaemia, pregnancy, nephropathy, and anaemia (6). Inflammation-mediated pathways and angiogenesis are thought to underlie the progression of diabetic retinopathy (9, 10). Due to its anti-inflammatory, antiangiogenic and anti-aging activity, the oral anti-hyperglycaemic agent metformin could possibly exercise a protective mechanism by inhibiting neovascularization in diabetic retinopathy, as well as in other retinopathies (11-13). Some studies have documented a less known but also important mechanism of metformin, the reduction of ischemia that may underlie the beneficial effects of metformin in ischemia-related retinopathy (10, 14). However, some recent studies have described the pro-angiogenic effects of metformin that could favour diabetic retinopathy (14, 15).

Numerous studies have documented the association between T2D and cataracts (5). Patients with T2D are more likely to have cataracts diagnosed at an earlier age, even before any diabetic retinopathy is seen (16, 17). The suggested mechanisms that may precipitate the formation of diabetic cataracts are: increased osmotic stress caused by activation of the polyol pathway, non-enzymatic glycation of lens proteins and deposition of its end-products in the lens, and increased oxidative stress (5, 18, 19). Based on the risk factors for ocular complications in patients with T2D on the one hand, and the effects of metformin on the other, the potential protective effects of metformin treatment for ocular complications could be hypothesized. However, the data regarding this are controversial and limited to a few studies (2, 20-24).

We aimed to study the association of the use of the oral anti-hyperglycaemic agent metformin with the presence of ocular complications in patients with T2D.

Patients and methods

Study design and data collection

This was an observational study. Patients with T2D treated at the Sarajevo Public Institution Health Centre in the period between 1997 and 2014 were screened for inclusion in this study. Out of 500 records reviewed, patients with confirmed diagnosis of T2D and who were treated with the same oral antihyperglycemic agents for at least one year, were included in the study (n=234, 46.8%). Patients treated with insulin and patients without complete medical records were not included in the study. Data were collected on the age of patients, gender, duration of T2D, body mass index (BMI), the medication used in the treatment of T2D, smoking habits, plasma glucose concentration, glycated haemoglobin (HbA1c), serum cholesterol concentration, the presence of ocular complications and the presence of other diseases at the last check-up.

Ethics statement

The study was approved by Local Ethics Committee organized at the School of Medicine of the University of Sarajevo. All procedures were performed in accordance with the ethical standards of the institutional and/or national research committee, the 1964 Helsinki Declaration and its later amendments, or comparable ethical standards.

Statistical analysis

Upon completion of the data collection, data were analysed using IBM SPSS Statistics software (Statistical Package for Social Sci-

ences, SPSS Inc, Chicago, Illinois, USA) version 20.0. Continuous numerical variables with normal distribution were expressed as mean±standard deviation, while those not normally distributed were expressed as median and interquartile range. Depending on the type of distribution of continuous numerical variables, a comparison between two treatment groups was made either by using the unpaired *Student's t-test* or the *Mann-Whitney test* as appropriate. Comparison between patients with different ocular complications was performed using ANOVA. The *Chi-square test* was used to determine the relationship between categorical variables. The univariate associations between the presence of ocular complications and metformin treatment were assessed with logistic regression. For multivariate analysis, models were adjusted for gender, age, duration of T2D (model II) and additionally for serum cholesterol concentrations, smoking, BMI and the presence of other diseases (model III). The Bonferroni adjustment at $\alpha=0.05/3=0.017$ was applied. To assess whether the findings were influenced by the older age of some patients, a sensitivity analysis was performed by excluding the patients in the highest tertile of age (>70 years). P-values less than 0.05 were considered statistically significant.

Results

This study included 234 patients with diagnosed T2D. For at least one year, 81.2% (n=190) of the patients were treated with only metformin, and 18.8% (n=44) of the patients were treated with only one of other oral antihyperglycemic agents, either sulfonylureas (glibenclamide, glimepiride, gliclazide) or thiazolidinediones (pioglitazone). Patients treated with metformin were more likely to be women, younger, with shorter duration of T2D and with fewer other comorbidities compared to the patients treated with other oral antihyperglycaemic agents. The subjects' characteristics are presented in Table 1.

For the plasma glucose concentration measured at the last check-up, the unpaired *t-test* showed a marginally significant difference with slightly higher plasma glucose con-

Table 1 Patients characteristics

Parameters	Patients treated with	
	Metformin (n=190)	Other *AHG agents (n=44)
Age, years	65.6±10.5	72.1±12.4
Gender (male), n (%)	86 (45.3)	25 (56.8)
Duration of †T2D (years)	5.0 (2-11)	8.0 (4-11)
‡BMI	29.9±5.07	28.6±4.31
Smoking, n (%)	87 (45.8)	20 (45.5)
Serum concentration of cholesterol (mmol/l)	5.6±1.29	5.1±1.35
Plasma glucose concentration (mmol/l)	8.2 (6.9-9.3)	7.4 (6.8-8.4)
§HbA1c (%)	7.2 (6.8-8.8)	7.0 (6.7-7.9)
Presence of other diseases, n (%)	182 (95.8)	44 (100)
Ocular complications		
Glaucoma, n (%)	6 (3.2)	5 (11.4)
Diabetic retinopathy, n (%)	11 (5.8)	6 (13.6)
Cataract, n (%)	24 (12.6)	13 (29.5)

Data are presented as mean±standard deviation or median (interquartile range) or percentages. *Antihyperglycemic; †Type 2 diabetes; ‡Body Mass Index; §Glycated hemoglobin.

centrations in patients treated with metformin, compared to patients treated with other oral antihyperglycemic agents (8.7±4.5 mmol/L vs. 7.7±1.9 mmol/L; p=0.05; 95% CI: -1.36-0.01). The median HbA1c value in the metformin group was 7.2% and in the other treatment group 7.0%. The *Mann-Whitney* U-test showed no significant difference between the two groups (p=0.179). The one-way ANOVA test showed no significant difference in plasma glucose concentrations [F(3,230)=0.236, p=0.871] and HbA1c levels [F(3,230)=0.669, p=0.572] between patients with different ocular complications. The median HbA1c levels in patients with different ocular complications are presented in Figure 1.

Out of 234 patients with diagnosed T2D, ocular complications occurred in 65 patients (27.8%). Patients treated with metformin had fewer ocular complications compared to patients treated with other oral antihyperglycemic agents (c2 test=19.985; p<0.0001) (Table 1). The association of T2D therapy with ocular complications are shown in Table 2. When the association between metformin use and the presence of ocular complications was tested, metformin use was associated with fewer ocular complications in an unadjusted model. The odds ratio of ocular complications in patients treated with metformin (OR=0.23, 95% CI: 0.12-0.46, p<0.001) remained significant after adjustment for gender, age and duration of T2D (OR=0.22, 95% CI: 0.10-0.49, p<0.001) and also after adjustment for gender, age, duration of T2D, serum cholesterol concentration, smoking, BMI and the presence of other diseases (OR=0.19, 95% CI: 0.08-0.42, p<0.0001).

Next, the association between metformin use and each ocular complication was tested. Table 2 shows that the odds ratio of glaucoma was significant in unadjusted models

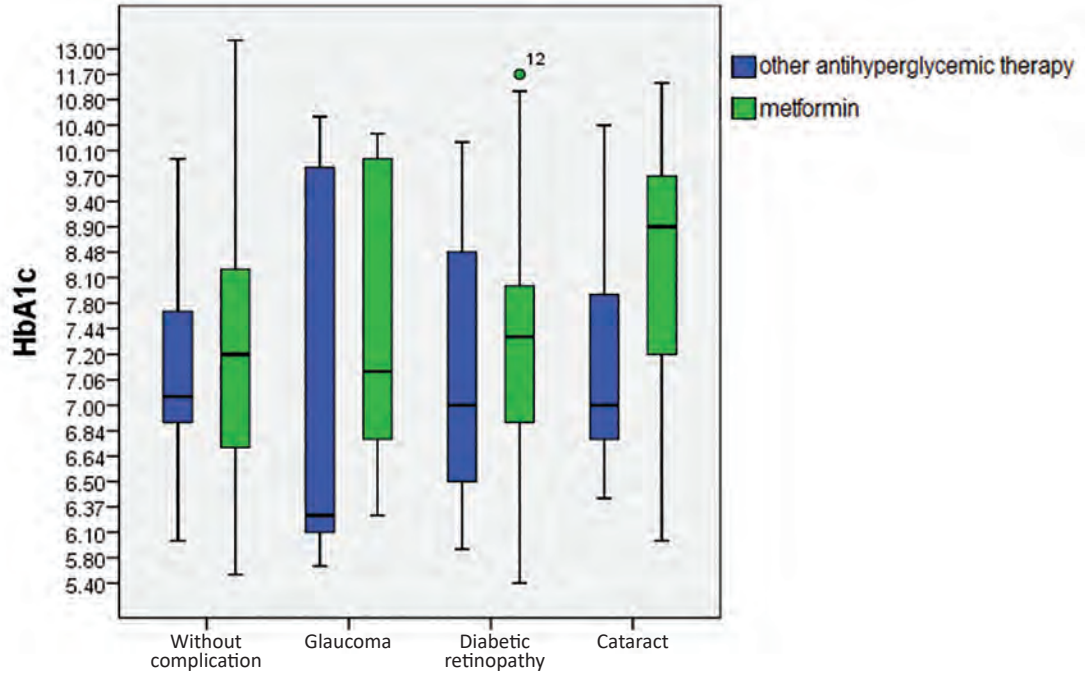

Figure 1 Glycated haemoglobin (HbA1c) levels in patients from the two treatment groups with different ocular complications. The solid horizontal line denotes the median value of HbA1c in two treatment groups with different ocular complications; the box represents the 25th and 75th interquartile range. The whisker represents the minimum and maximum value.

Table 2 Metformin therapy and ocular complications in patients with T2D

Outcome	Metformin therapy					
	Model I		Model II		Model III	
	OR (95% CI)	p	OR (95% CI)	P	OR (95% CI)	p
Ocular complications	0.23 (0.12, 0.46)	<0.001	0.22 (0.10, 0.49)	<0.001	0.19 (0.08, 0.42)	<0.0001
Glaucoma	0.25 (0.07, 0.88)	0.030	0.21 (0.06, 0.78)	0.020	0.14 (0.03, 0.57)	0.006
Diabetic retinopathy	0.35 (0.16, 0.75)	0.007	0.40 (0.17, 0.94)	0.037	0.33 (0.14, 0.82)	0.017
Cataract	0.39 (0.14, 1.12)	0.079	0.36 (0.10, 1.26)	0.11	0.40 (0.10, 1.52)	0.18

T2D= Type 2 diabetes; Model I: Univariate analysis; Model II: Analysis adjusted for gender, age and duration of T2D; Model III: Analysis adjusted for gender, age, duration of T2D, serum concentration of cholesterol, smoking, BMI and presence of other diseases; Predictor: Metformin use; other therapy was used as a reference category.

(OR=0.25, 95% CI: 0.07-0.88, p=0.030) and also in two adjusted models (OR=0.21, 95% CI: 0.06-0.78, p=0.020; OR=0.14, 95% CI: 0.03-0.57, p=0.006). It is also shown that the odds ratio of diabetic retinopathy in patients treated with metformin (OR=0.35, 95% CI: 0.16-0.75, p=0.007) remained significant after adjustment for gender, age and duration of T2D (OR=0.40, 95% CI: 0.17-0.94, p=0.037) and also after adjustment for gender, age, duration of T2D, serum cholesterol concentration, smoking, BMI and the presence of other diseases (OR=0.33, 95% CI: 0.14-0.82, p=0.017).

The association of metformin use and the presence of cataracts was also analysed. The odds ratio of cataracts was not significant for metformin use in either unadjusted or adjusted models. The results remained significant after Bonferroni adjustment at α=0.05/3=0.017. In sensitivity analysis, the highest tertile of age (>70 years) was excluded and the results remained significant for overall ocular complications (OR=0.184, 95% CI: 0.05-0.67, p=0.010) and glaucoma (OR=0.10, 95% CI: 0.01-0.91, p=0.041).

Discussion

Although with no significant differences in the control of the HbA1c levels and the duration of T2D, and with a marginally significant difference in fasting plasma glucose concentrations between the two treatment groups, patients with T2D treated with metformin presented with less glaucoma, diabetic retinopathy, and cataracts. This suggests the possible protective effect of metformin treatment against these ocular complications in patients with T2D. Besides the duration of DM, glycaemic control is recognized as one of the strongest predictors for the development of diabetic retinopathy (25, 26). However, our study showed that patients treated with metformin had a lower incidence of ocular complications, although the control of plasma glucose concentrations and HbA1c levels were not within the recommended range for most of the patients in both treatment groups.

The micro- and macro-vascular complications of T2D lead to morbidity and reduced life expectancy (2, 27, 28). Use of metformin as calorie restriction (CR) mimetic drug has been shown to be associated with reduced risk of late-onset eye diseases such as glaucoma, but also diabetic retinopathy, cataract and macular degeneration (20). In this regard, our study showed that the prevalence of glaucoma was lower among patients treated with metformin compared to patients treated with other oral anti-hyperglycaemic agents (3.2% v. 11.4%). Although the relationship between DM and glaucoma was until recently controversial, new studies have identified an association between these

two disorders (4). Glaucoma is a type of neuropathy and DM could cause optic neuropathy. In addition, it has also been found that the central cornea is thicker in patients with DM, and this could also cause higher intraocular pressure (5). To our knowledge, only one retrospective cohort study has shown that metformin use is associated with a reduced risk of developing open-angle glaucoma and the authors suggested that metformin could have an impact on glaucoma risk on multiple levels, including glycaemic control, but also as a CR mimetic drug (20, 21). As previous studies suggested that age-related tissue changes significantly contribute to glaucoma development (29), the need has already been postulated to explore whether metformin, as a CR mimetic drug with an anti-aging effect, could delay progression of tissue damage (13).

Similar to our study, Richards et al. found that glaucoma risk reduction in a population with DM was dose-dependent for metformin and independent of glycaemic control (21). They also found that other anti-hyperglycaemic agents did not result in a similar glaucoma risk reduction (21). New prospective clinical trials are necessary to evaluate whether metformin might also have protective effects on the occurrence of glaucoma in non-diabetic patients. Such findings could lead to novel treatment approaches and better glaucoma control.

Our study showed the possible beneficial protective role that metformin may have for diabetic retinopathy, compared to other antihyperglycemic agents (13.6% vs. 5.8% patients). This protective effect of metformin on diabetic retinopathy has also been shown in an animal study (10). Moreover, our results are consistent with the results from Ryu et al. (22) who found that 45.5% of patients from the non-metformin group developed diabetic retinopathy compared to 27.3% of the patients from the group treated with metformin. Additionally, the United Kingdom Prospective Diabetes Study (UKPDS) Group showed a significantly lower (p=0.044) rate of progression of retinopathy in overweight patients with intensive blood-glucose control, treated with metformin, compared to those treated with other treatments (diet or intensive treatment with chlorpropamide, glibenclamide, or insulin) (2).

Limitations of the study

The total number of patients included in the study was small, and therefore replication in a larger study is needed. Also, as data on the onset or duration of ocular complications were not available, we cannot conclude whether patients had any complications at the moment when the therapy started, with either metformin or other oral antihyperglycemic agents.

Conclusion

The results of our study suggest that metformin use might be associated with protective effects for ocular complications in patients with T2D. As this is an observational study and a causal/protective relationship cannot be established, the effects of metformin either on the prevention of ocular complication or on ocular complications already developed in patients with T2D should be further investigated through large prospective clinical trials.

What is already known on this topic

Diabetes-associated ocular complications are often associated with inflammation-mediated pathways, angiogenesis, and age-related tissue changes. Besides its effects on glycaemic control, metformin has also been shown to have anti-inflammatory, antiangiogenic and calorie restriction related anti-aging activity. There are very limited data suggesting the protective role of metformin treatment against glaucoma and retinopathy in patients with type 2 diabetes.

What this study adds

This observational study confirmed the protective effects of metformin treatment against glaucoma and diabetic retinopathy, and suggested its protective effects on cataracts in patients with type 2 diabetes.

Authors' contributions: Conception and design: SM, JK and EG; Acquisition, analysis and interpretation of data: JK and AK; Drafting the article: SM, JK and AK; Revising it critically for important intellectual content: MRT, DŠ and LBR; Approved final version of the manuscript: SM, JK and AK.

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Evaluation of the water sorption of luting cements in different solutions

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Objective. To evaluate and compare the water sorption of three luting cements in three different solutions: distilled water and artificial saliva with different pH values (7.4 and 3.0). **Materials and methods**. Resin-modified glass-ionomer cement (GC Fuji Plus) and two resin cements (Multilink Automix and Variolink II) were used. A total of 45 specimens - 15 specimens (15x1 mm) for each cement were prepared according to ISO standard 4049:2009. The water sorptions of the cements were calculated by weighing the specimens before and after immersion and desiccation. **Results.** Nonparametric statistic methods were applied. GC Fuji Plus cement showed significantly higher values of water sorption in all three solutions of both resin cements ($p<0.009$) and significantly higher values of sorption in artificial saliva pH 3.0. Multilink Automix showed significantly higher values of water sorption compared with Variolink II in artificial saliva pH 7.4, and higher values of sorption in this solution compared with pH value 3.0. **Conclusion.** Water sorption values are mainly influenced by the proportion of hydrophilic matrix, the type and composition of filler, and the pH value of solutions.

Introduction

Materials placed for long periods in the oral environment will undergo interaction with the oral fluids (1). Cements for permanent cementation must have the ability of long ageing in an environment such as the oral cavity, where, on the edge of the restoration area, cement is in contact with saliva, a fluid which contains organic and inorganic water soluble components (2).

The pH value of saliva is slightly alkaline. Food and liquids in the oral environment have various pH values and they can change the saliva`s pH value (3, 4). Water sorption is important in evaluation of dental cement clinical durability. Water sorption and cement dissolution may result in decomposition of cement, which leads to debonding of the restoration, post-operative hypersensitivity, pulpal inflammation and periodontal disease (5-7). Water sorption leads to dimensional changes, loss of retention, color change and breaks in the margin contours, and it can also affect the mechanical properties of the cement, such as flexural strength, hardness and mechanical stability (8-10).

Numerous authors have pointed out that water may affect the bond strength between

the dentin and the bonding agents. (11-14). Water sorption can lead to material discoloration and deterioration of the esthetic quality of the restoration (15). Resin-based cements constitute an important link in the adhesive luting technique in prosthodontics, but their water sorption behavior and dimensional change have not been sufficiently studied (6). In a moist environment, the polymer matrix of a resin-based cement can absorb water and swell, resulting in a decrease in elastic modulus and ultimate strength, and an increase in creep (16). Water sorption by polymers is a diffusion controlled process, and water uptake occurs mainly in the resin matrix (13). The water absorbed by the polymer matrix could cause filler-matrix debonding, or even hydrolytic degradation of the filler-resin interface (16). Hydrolytic degradation is a result of either the breaking of chemical bonds in the resin or softening, through the plasticizing action of water (17). The interaction between resin-based cements and water involves two opposing phenomena: the first is water sorption, which leads to the swelling of the material and an increase in weight, and the second is dissolution of materials (fillers or monomers) in the water, contributing to the shrinkage, weight, and reduction of the final specimens (6, 18).

As the luting agent for all ceramic restorations, resin-modified glass-ionomer cement can be used, and it is also widely used for luting metal-ceramic crowns and cast post. Resin-modified glass-ionomer cement possesses the benefits of conventional glass-ionomer cements, such as fluoride release and adhesion to the tooth structure, along with the benefits of composite cement, such as improved strength, water sorption and solubility (19).

The objective of this research was to measure the water sorption of three cements for permanent cementation, in three different solutions: distilled water and artificial saliva of two different pH values, and to examine the influence of the pH value of the artificial saliva on water sorption. The null hypothesis was that there are no differences between the water sorption of different dental cements after immersion in three different solutions, and the pH value of artificial saliva has no influence on the water sorption of dental cements.

Material and methods

The cements used in this study are shown in Table 1.

Fifteen specimens of each cement were made according to ISO specification 4049:2009 (20). The specimens were made in Teflon molds of inner diameter 15 ±0.1 mm and thickness 1±0.1 mm. Preparation of specimens of self-curing GC Fuji Plus ce-

Table 1 Name, type and main component of cement

Name of cement and manufacturer	Type of cement	Main components
GC Fuji PLUS CAPSULE (reinforced glass-ionomer cement) GC Corporation Tokyo, Japan.	Resin-modified glass-ionomer cement.	Powder: aluminofluorosilicate glass Liquid: polyacrylic acid, HEMA, metadimethacrylate, water.
Multilink Automix IvoclarVivadent AG; FL-9494 Schaan, Liechhtenstein.	Resin cement.	Monomer: dimethacrylate , HEMA; Inorganic filler (40%): barium glass, ytterbium trifluoride, spheroid mixed oxide; Additional content: catalysts, stabilizer, pigments. The mean particle size is 0.9 μm.
Variolink II IvoclarVivadent AG; FL-9494 Schaan, Liechhtenstein.	Resin cement.	Monomer: Bis-GMA, urethane dimethacrylate, triethylenglicoldimethacrylate; Inorganic filler (40%): barium glass, Ba-Al-fluorsilicate glass, ytterbium trifluoride, spheroid mixed oxide; Additional content: catalysts, stabilizers, pigments. The mean particle size is 0.7μm.

ment (GC Corporation Tokyo, Japan) was carried out as follows: a 50 μm thick polyester film was put on a metal plate and over it the mold in which the cement was slightly overfilled, being careful to minimize air inclusion. Another polyester film was put on top of the material in the mold and was covered with a second metal plate to remove the excess material. The metal plates were bound together by clamps and the specimens were immediately stored in an incubator at 37±1°C. After 60 minutes the specimens were removed from the mold. Specimens were trimmed and polished with 1000 grit silicon carbide grinding paper until the final diameter of 14.9±0.1 mm was attained. The diameter was measured with a TESA 0-25 mm micrometer for external measurements, with measurement accuracy of 0.001 mm (TESA, Renens, Switzerland).

For preparation of specimens of dual cure cements, the metal plate was replaced by a glass plate, over which polymerization of specimens was performed. The polymerization light was tested for light output by means of a digital radiometer (Bluephase Meter, Ivoclar Vivadent, Schaan, Liechtenstein). The tip of the polymerization lamp (Bluephase 20, Ivoclar Vivadent Ag, and FL-9494 Schaan, Liechtenstein) was directed over the center of the specimens for the correct time of exposure, and then eight peripheral overlapping sectors were irradiated for 20 seconds each, until the whole area had been irradiated. After that the lower side of the specimens was polymerized in the same way as the upper one. The specimens were immediately stored in an incubator at 37±1°C for 60 minutes, and then finished as the previous ones. After treatment was complete, all specimens were stored in desiccators with silicate gel, and the entire set was stored in an incubator maintained at 37±1°C. After 22 hours the specimens were moved into another desiccator maintained at 23±1°C for 2 hours, and after that weighed on an analytical balance, Sartorius LE244S 0-240 g, accuracy of measurement 0.0001 g (Sartorius Göttingen, Germany) until a constant mass of m1 was obtained or until the mass loss of each specimen was not less than 0.01mg over 24h. The diameter-r (mm) and thickness h (mm) of each specimen was measured by micrometer, with accuracy up to 0.001 mm according to the ISO specification, and the volume V (mm^3) was calculated according to the formula: $V=\pi \times r^2 \times h$.

Five specimens were immersed in distilled water, five specimens in artificial saliva pH value 7.4, and five specimens of each cement were immersed in artificial saliva pH value 3.0. All specimens were stored in a Culture Incubator (Ivoclar Vivadent, Schaan, Liechtenstein) at 37±1°C for 7 days. Tomasi's solution of artificial saliva pH value 7.4 was prepared for this research at the Department of Chemistry, Faculty of Natural Science and Mathematics of Sarajevo University (2).

In order to obtain a pH-value of 3.0, Tomasi's solution was modified with the intentional acidification of the solution. After 7 days of storage all specimens were taken out of the liquid, washed with water, air dried for 15 s and weighed one minute after removal from the water to record the mass of the second cycle-m2. After weighing in the second cycle the specimens were again stored in the desiccator and incubator in the same way as in the initial cycle, and afterwards the mass - m3, was recorded. The value of water sorption (Wsp) expressed in mg/mm^3 for each of the five specimens was calculated using the following formula (ISO 4049:2009): Wsp = (m2-m3)/V, where: m2 = mass of specimens (mg) immersed in solution after 7 days, m3 = mass of refined specimens (mg) and V– volume of specimens (mm^3).

Statistic methods

All data were analyzed by the statistical software IBM SPSS v.17. Preliminary statisti-

cal analyses were carried out to determine the distribution of dependent variables and make a decision on the application of parametric or nonparametric statistical methods. Since the dependent variables were asymmetric, the Mann-Whitney U nonparametric statistical method was applied. As the required alpha level of significance for rejection of the null hypothesis, the level 0.05 (5%) was taken. To avoid first category statistical error, in subsequent (Post hoc) sample comparisons, matching with Bonferroni was used, where the required alpha level of significance of 5% was corrected, i.e. divided by the comparison number ($p<0.05/3 = p<0.017$).

Results

The arithmetical mean and standard deviations of water sorption for each dental cement in the three different solutions are shown in Table 2. A difference in water sorption levels between the cements GC Fuji Plus and Variolink II; p=0.009 (effect size=0.826), and GC Fuji Plus and Multilink Automix; p=0.009 (effect size=0.826), in all three solutions was confirmed. Multilink Automix showed a statistically significant difference in water sorption; p=0.009 (effect size=0.826), in comparison with Variolink II in a solution of artificial saliva pH 7.4. Post hoc comparisons are shown in Table 3. Sta-

Table 2 Mean and Standard Deviation of water sorption between groups

Water sorption in different solutions	Type of cement	n	Mean	SD
Water sorption (m2-m3)/V1 - distilled water	Multilink Automix	5	19.61	2.89
	GC Fuji Plus	5	181.50	5.17
	Variolink II	5	15.74	0.84
Water sorption (m2-m3)/V1 - in artificial saliva pH 7.4	Multilink Automix	5	21.30	0.49
	GC Fuji Plus	5	173.72	4.66
	Variolink II	5	15.87	0.21
Water sorption (m2-m3)/V1 - in artificial saliva pH 3.0	Multilink Automix	5	19.31	2.09
	GC Fuji Plus	5	185.81	4.61
	Variolink II	5	15.19	2.70

Table 3 Post hoc comparison between groups

Type of cement	Multilink Automix	GC Fuji Plus	Variolink II
	Water sorption (m2-m3)/V1 - distilled water		
Multilink Automix	-	p=0.009 (0.826)	NS
GC Fuji Plus	p=0.009 (0.826)	-	p=0.009 (0.826)
Variolink II	NS	p=0.009 (0.826)	-
	Water sorption (m2-m3)/V1 - in artificial saliva pH 7.4		
Multilink Automix	-	p=0.009 (0.826)	p=0.009 (0.826)
GC Fuji Plus	p=0.009 (0.826)	-	0.009 (0.826)
Variolink II	p=0.009 (0.826)	p=0.009 (0.826)	-
	Water sorption (m2-m3)/V1 - in artificial saliva pH 3.0		
Multilink Automix	-	p=0.009 (0.826)	NS
GC Fuji Plus	p=0.009 (0.826)	-	p=0.009 (0.826)
Variolink II	NS	p=0.009 (0.826)	-

Mann Whitney U test ; p (effect size =$Z/\sqrt{n}$); NS=not statistically significant.

Table 4 Post hoc comparison between groups

Solutions	A.S. pH 7.4	A.S. pH 3.0
	Multilink Automix	
A.S. pH 7.4	-	p=0.009 (0.826)
A.S. pH 3.0	p=0.009 (0.826)	-
	GC Fuji Plus	
A.S. pH 7.4	-	p=0.016 (0.759)
A.S. pH 3.0	p=0.016 (0.759)	-

Mann Whitney U test; p (effect size =Z/√n); NS=not statistically significant.

tistical analysis of the effect of Ph value on the water sorption of dental cements confirmed a statistically significant difference p=0.009 (effect size=0.826), in water sorption in Multilink Automix cement between the solution of artificial saliva pH value 7.4 and artificial saliva pH value 3.0. Post hoc comparisons are shown in Table 4.

A statistically significant difference, p=0.016 (effect size=0.759), in the levels of water sorption for GC Fuji Plus cement was confirmed between the solutions of artificial saliva pH 7.4 and artificial saliva pH 3.0 (Table 4). Studying the effect of pH value on the water sorption of Variolink II dental cement, in this case we did not find any statistically significant difference in the levels of water sorption with cement Variolink II between the different solutions, with a probability of p=0.248.

Discussion

The resin modified glass ionomer (GC Fuji Plus) cement tested in this study exhibited statistically significant higher water sorption in relation to the composite cements in all three solutions. Resin-modified glass-ionomer had a dual setting reaction, involving mainly an acid-base reaction and free radical polymerization. The polymerized structure of resin-modified glass-ionomer cement contains a high percentage of hydrophilic functional groups in a well-networked matrix, and it may be similar to synthetic hydro gels. Synthetic hydro gels are often prepared from HEMA copolymer and are designed to hold huge amounts of water, possibly up to 80% of their mass (5). Materials which have more HEMA in their composition will have higher water sorption. As GC Fuji Plus cement contains HEMA in its composition, it is considered that this is the main reason for the statistically significantly greater water sorption in this material in relation to both the composite cements in all three solutions. The presence of hydroxyl ethyl methacrylate (HEMA) was the main reason for the hygroscopic expansion, which leads to retention stress on the tooth and restoration, and could result in postoperative sensitivity (6, 21). The clinical implication of this examination shows that these cements, because of their significant dimensional changes, are not applicable for cementing all ceramic restorations and composite posts, because their expansion may eventually result in the fracture of the restoration or tooth root (22-24). One must be careful with cementing a post in the very thin walls of the tooth root canal.

Our data agree with the data of Beriat (25), Messe (6), Gordole (26) and Mortier (15). In Gerdolea's research, GC Fuji Plus showed some higher values of water sorption than in this research, and this could be explained by the fact that mechanically mixed capsulated cement was used in this research, while Gerdole used hand mixed cement. The manual mixing process can lead to the formation of air voids that

can accelerate water sorption by increasing the surface area exposed to the water and, at the same time, sorption (18, 27-29). Although resin-modified glass-ionomer cement showed significantly higher sorption than resin cements, those values are lower compared with conventional luting cements. The best choice of cement for cementing metal-ceramic restorations is resin-modified glass- ionomer cements, due to the fact that they have other characteristics which are superior in comparison with conventional cements.

There is not a large amount of data in the literature about the effect of pH value on the water sorption of resin-modified glass-ionomer cements. Fano et al. suggest that the decrease in pH value accelerates erosion by increasing the number of micro cracks (30). Specimens with more micro cracks will absorb more water, so the water sorption of CG Fuji Plus cement was significantly higher in artificial saliva with lower pH values. Hydrolytically, degradation which resulted in water sorption is based on micro cracks appearing in the acidic environment. Czarnecka confirms that water sorption depends on the solution composition, and that increases in lactic acid resulting in formation of erosion (31).

There was a small amount of water sorption in the resin cements. Water sorption by polymers is a controlled process of diffusion which mainly takes place in the resin matrix (15, 16, 18). The sorption depends on the resin composition, which is determined by hydrophilicity, mobility kinetic parameters, and filler content (18, 32).The presence of hydroxyl, carboxylic and phosphate groups in monomers and polymers makes them more hydrophilic. The resulting polymers are not considered to be extremely hydrophilic, but nevertheless they will absorb water (33).The water sorption which occurred in Variolink II may be explained by the Bis-GMA monomer in its composition, which contains pendant hydroxyl groups within its molecular structure (34). Mese (6) and Gordole (26) obtained results for Variolink II in samples stored in water which correspond with this research. HEMA flows more easily in water than Bis-GMA because of its lower molecular weight and hydrophilic chemical structure. It contains hydroxyl groups with a high affinity for hydrogen bonds (35), which can explain the slightly higher arithmetic mean of water sorption of Multilink Automix cement in comparison with Variolink II in all three solutions, and by the Mann Whitney U-test, Multilink Automix showed a statistically significant difference in comparison with Variolink II in the solution of artificial saliva with a pH value of 7.4. The results for resin cement corresponded with the results of Mese (6), Gordole (26), Vrochari (36), and the results which Mortier (15), Malacarne (9), Ortengren (13) and Berger (37) obtained for composite restorative materials. Taking into account the fact that the pH value changes constantly in the oral environment, it seems that dental composites are designed to endure acidic conditions (38, 39). The effect of the alkaline medium on composite properties explained its interaction with OH-ions during the process of hydrolysis. Accelerated degradation is expected in a medium with an excess of hydroxyl ions. Besides the possibility of debonding, hydrolysis of the filler may occur (38). This may explain the statistically significant difference in water sorption levels in Multilink Automix cement between the solutions of artificial saliva with pH values of 7.4 and 3.0. It must be pointed out that the level of water sorption of Multilink Automix in artificial saliva 7.4 is lower than the maximum water sorption value (40 mg/mm^3 according to ISO specification 4049). As is the case with most in vitro studies, caution must be used when the results are extrapolated to the oral environment. The solution of artificial saliva used in our re-

search did not consist of enzymes, therefore higher water sorption could be expected in the mouth, because they may lead to degradation, which would be expressed in reality because of the anhydrase, amylase, peroxidase, lysozumes and other esterases, which can cause less resistance in materials based on resin (40, 41). If the material is exposed to enzymes for a long time, degradation of the surface may be the result of the acidic products of bacteria (33). Curing specimens in vitro represents an ideal processing procedure (29). In the complex oral environment, maximum polymerization cannot be expected, which can result in a reduction in the mechanical properties of the cement, increasing micro leakage, post-operative sensitivity, secondary caries and aesthetic failure (42, 43). Therefore, higher sorption than the reported values is expected because of incomplete polymerization in the oral cavity.

Aesthetic dentistry composite cements are becoming increasingly used in clinical practice for permanent cementing of porcelain veneers, all ceramic crowns, inlays, onlays and composite posts. Resin modified cement can be used for cementing all ceramic restorations and it is also widely used for luting metal-ceramic crowns and cast posts. Multilink Automix, Variolink II, GC Fuji Plus were selected because there are commonly used in our clinical practice. The composite cements showed the best mechanical properties of all luting cements. One shortcoming is that the clinical procedure is more complicated (44). New types of cements have been developed such as self-etching composite cements for reducing the multiple clinical steps required for application of the composite cement. Nevertheless, self-etching composite cements simultaneously demineralize and infiltrate the tooth substrate (44, 45). Some future studies could examine self-adhesive composite cements for luting of prostodontic restorations.

In this study, the water sorption of a material was assessed in distilled water over a one-week period, according to the ISO standard 4049. Müller et al. concluded that water sorption according to ISO 4049 provides reliable results (46). Some studies have shown that polymeric materials absorb water continuously over a long period of time (6, 47). A future study could be extended to a longer period of time, such as 30, 90, and 180 days, and one year of the specimens being immersed in water.

Conclusion

The resin modified glass-ionomer cement Fuji Plus showed the significantly highest water sorption values in all three examined solutions, and did not satisfy Standard 4049. Multilink Automix and Variolink II were found to comply with ISO requirements regarding water sorption. The values of water sorption of cements were found to depend on the matrix hydrophilicity, type and composition of the filler. The pH value of saliva affected the water sorption of dental cements.

What is already known on this topic

Water absorption is an important factor in the evaluation of the clinical durability of dental cements. Knowing the cement's material properties will enable the appropriate choice of cement for permanent cementation and the durability of fixed prosthetic restoration.

What this study adds

There are numerous studies of water absorption in dental cements while studies with artificial saliva are rare. This research contributes to the knowledge about water absorption in artificial saliva with various pH values.

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Original article

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Assessment of inpatient psychiatric readmission risk among patients discharged on an antipsychotic polypharmacy regimen: A retrospective cohort study

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Objective. Patients are frequently prescribed multiple antipsychotic medications, leading to higher healthcare costs and increased risk for side effects. The efficacy of multiple versus single antipsychotics to prevent acute relapse, measured by incidence of inpatient readmission, is investigated in Arizona, USA. **Method.** A retrospective chart review compared socio-demographic and clinical data from 1,010 patients discharged on a single and 377 discharged on multiple antipsychotic medications. Case management records were reviewed for readmission within one year of discharge. **Results.** Younger age, diagnosis of Schizophrenia or Schizoaffective Disorder, prescription of mood stabilizer, shorter length of stay, and discharge to residential treatment or crisis recovery unit were associated with multiple antipsychotics at discharge. Readmission rates of the single (13.7%) versus multiple (15.9%) antipsychotic groups were not statistically different (p=0.286). Logistic regression analysis established that only age (younger) and the prescription of a mood stabilizer at discharge were significant predictors for increased risk for readmission (p=0.010 and p=0.049, respectively). A Cox survival analysis supported these findings. **Conclusions.** Concomitant antipsychotic polypharmacy at discharge did not reduce readmission risk over a one-year period. Given the increased risk of side effects and financial costs of polypharmacy, this study did not provide evidence to support this practice. Strikingly, only two variables predicted readmission risk, younger age and prescription of mood stabilizer. Although practitioners should follow practice guidelines more closely to prevent unnecessary exposure to potentially lethal side effects of antipsychotic polypharmacy, further studies are needed to better identify patients at high risk for readmission.

Introduction

The concomitant use of multiple antipsychotic medications or antipsychotic polypharmacy (APP) is a routinely utilized treatment modality in the psychiatrist's armamentarium (1). In the Clinical Antipsychotic Trial of Intervention Effectiveness (CATIE) study, six percent of patients were taking two antipsychotics upon study entry (2). Additional studies indicate that the rates of APP in the United States may be as high as 50%, depending on the treatment setting and population being evaluated (3-7). The tendency to initiate multiple antipsychotics does not seem to be limited to country of

training or practice setting, as one Canadian study indicated that 27.5% of patients were prescribed an APP regimen at discharge from a tertiary care psychiatric service (8). Furthermore, long-term prescription surveys report increased use of APP in the same treatment setting over time, and the advent of second-generation antipsychotics is associated with an increase in the rates of APP (1, 4, 9).

While the pharmacologic factors driving the widespread use of APP are unclear, there are many postulated rationales, including combining high and low potency antipsychotics or paring more and less sedating medications (1). However, it is uncertain how combining antipsychotic medications is correlated with clinical improvement. There is some evidence to support that the efficacy of antipsychotic medications is correlated with other factors. For example, multiple studies indicate that the occupancy of the dopaminergic D_2 receptor is a determinant of antipsychotic efficacy. In these studies, a range of 65%-75% occupancy was needed to produce efficacy; at over 80% occupancy there was increased risk of extrapyramidal side effects and tardive dyskinesia (10-12). Given the high clinical efficacy of clozapine and its low affinity for D_2 receptors, it is unclear whether D_2 affinity and occupancy are correlated or if there are other potential undiscovered mechanisms of clozapine's therapeutic efficacy (13). In either case, current evidence for APP combinations grounded upon pharmacologic principles is limited.

Studies seeking to understand the clinical motivators of APP have implicated a wide number of factors: intent to minimize potential side effects induced by a specific compound (for instance clozapine-induced weight gain), short term combinations that are extended beyond original intent, such as the use of both a typical and atypical antipsychotic as a bridge to a new treatment regimen, the use of APP when it is reasonable to try different compounds as monotherapy, and failure of or patient refusal to continue monotherapy (1, 9). Given that schizophrenia and schizoaffective disorder are chronic and disabling, it is possible that the severity and persistence of symptoms of psychosis contribute to the use of multiple antipsychotics (14, 15). Additional factors associated with APP include diagnostic inaccuracy, a need to make decisions under time constraints due to short hospital stays, inadequate knowledge of receptor pharmacology, and poor understanding of the differences among antipsychotics (16).

Despite use of APP, there is ample evidence of its potential harm. APP is associated with a greater risk for side effects, adverse reactions and drug-drug interactions (17). For example, investigations of mortality in schizophrenia implicate APP as a significant predictor of reduced survival (18, 19). Antipsychotic combinations may also increase QTc intervals, especially when ziprasidone is used (20). Other potential side effects of antipsychotics, such as sedation and anticholinergic toxicity, may be potentiated when two or more antipsychotics are used concomitantly, or when anticholinergic medications are used to alleviate extrapyramidal side effects (17). Combinations of first and second generation antipsychotics may further increase the risk of tardive dyskinesia posed by the first generation agent (21). Furthermore, the high prevalence of APP is costly for patients and insurers. A one year follow up study comparing patients with treatment initiated on quetiapine, risperidone or olanzapine, reported that each dollar spent on the index antipsychotic was accompanied by an additional $1.31, $0.64 and $0.38 cost for concomitant antipsychotics for these medications, respectively (22).

These factors have led to evidence-based guidelines that recommend using more than one antipsychotic only after at least four trials of antipsychotic monotherapy have been

tried, one of which must have been clozapine (1, 23-25). The apparent discrepancy between clinical practice and evidence-based guidelines suggests that ongoing empirical research is needed to place the implied benefits and drawbacks of APP into perspective (26).

We sought to compare both socio-demographic and clinical variables of two cohorts of patients discharged on either antipsychotic monotherapy or polypharmacy, and their associated acute relapse rates, as measured by readmissions to an acute inpatient facility within one year. Logistic regression and Cox survival analyses helped to address confounders and other clinical or demographic factors associated with acute relapse, as measured by readmission.

Methods

Study setting

The study was completed at the Maricopa Integrated Health System (MIHS), which operates the county hospital and related healthcare safety net facilities within greater Phoenix and Maricopa County, Arizona. MIHS also receives all persons within Maricopa County who are petitioned to undergo an involuntary inpatient psychiatric evaluation. If these patients are then placed on court ordered treatment (COT), inpatient care is provided at MIHS inpatient psychiatric facilities and outpatient care is provided by a provider agency contracted by the state as the county's Regional Behavioral Health Authority (27). Value Options (VO) was the contracted outpatient provider at the time of the study. Within Arizona, persons may be placed on COT for up to one year across both inpatient and outpatient settings. Additionally, it should be noted only patients placed on COT were assessed in this study in order to accurately keep track of discharges and readmission from the provider agency contracted by the state. The study was approved by the MIHS Institutional Review Board (IRB 2006-016).

Study design

An inquiry of the MIHS electronic health record for patients discharged from psychiatric units over a two-year period (2003-2005) yielded 2,587 discrete inpatient stays coded with one of the following inclusion diagnoses: Schizophrenia, Schizoaffective Disorder, and other Psychotic Disorders (Psychotic Disorder and Brief Psychotic Disorder). Each admission and discharge primary diagnosis was confirmed by chart review, with repeat hospitalizations during the study period dropped and only the most recent stay retained. Outpatient treatment data from the Regional Behavioral Health Authority's case management database was queried to identify patients who were readmitted to an acute psychiatric inpatient facility within one year of discharge. Each patient had to be prescribed at least one antipsychotic medication at discharge and receive outpatient case management to be included in the study. The final sample size (n=1,387) included 377 unique records of subjects discharged on multiple antipsychotics and 1,010 discharged on a single antipsychotic medication.

Basic demographic and additional clinical information were retrieved, including additional diagnoses, discharge disposition location (i.e., home, residential placement, or sub-acute psychiatric facility), presence and severity of substance use, and medications prescribed at discharge (antipsychotic, anti-manic, anti-anxiety, and/or anti-depressants). Clinical case management records were reviewed and admission to one of the county's two emergency psychiatric facilities within one year of discharge was recorded as a psychiatric readmission. For readmitted patients, the number of days until readmission was recorded.

Statistical analysis

The sample was separated into two cohorts: those subjects prescribed a single antipsychotic and those prescribed multiple antipsychotics at discharge. Descriptive statistics for the socio-demographic and clinical characteristics between the two cohorts were assessed by two-tailed t-tests, Mann Whitney U tests, and chi-square tests where indicated. Differences between rates of readmission for the two cohorts were calculated. To identify variables associated with readmission and potential confounders, multivariate logistic regression models and a Cox regression survival analysis were conducted. All analyses were conducted with the Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS Inc, Chicago, IL) software, unless otherwise indicated, and p values less than 0.05 were considered statistically significant.

Results

Socio-demographic and clinical characteristics associated with the concomitant use of multiple antipsychotics

We sought to determine the baseline socio-demographic and clinical characteristics to determine if these variables are associated with antipsychotic mono- or polypharmacy. The study sample (n=1,387) consisted of 60% males and 36% non-white ethnicities. The average age of the subjects was 38.5 years. At discharge, 72.8% of the sample (1,010 subjects) was prescribed a single antipsychotic, and 27.2% (377 subjects) of the sample was prescribed multiple antipsychotics. The rate of APP was consistent with values reported elsewhere (1-8).

Unsurprisingly, due to the lack of randomization associated with retrospective cohort studies, differences in the treatment cohorts were seen. For socio-demographic variables, younger age was significantly associated with polypharmacy, while gender and ethnicity were not (p=0.008, p=0.080, and p=0.212, respectively, Table 1). These differences were expected, as younger age has also previously been associated with polypharmacy (15).

Substance use can be an important confounder in psychiatric symptoms and clinical assessment and might contribute to treatment decisions. We therefore examined the prevalence of substance use between the two cohorts, including abuse, dependence and those with insufficient data recorded to determine the severity of their substance use in this group. Surprisingly, there was no statistical difference amongst the study cohorts (p=0.518, Table 1). As with socio-demographic factors, significant differences were seen for clinical variables. Multiple clinical diagnoses were significantly associated with either mono or polypharmacy. Both Schizophrenia and Schizoaffective Disorder were associated with polypharmacy (p<0.0005 and p=0.008, respectively, Table 1) though the prevalence of Schizophrenia was nearly one in three of the patients in the monotherapy group (32.8% vs 44.3% of patients in the mono or polypharmacy groups, respectively, Table 1). Interestingly, the diagnosis of Psychotic Disorder was significantly associated with monotherapy (p<0.0005, Table 1). Neither the diagnosis of a personality disorder nor intellectual disability was significantly associated with one treatment modality (P = 0.265 and p=0.056, respectively, Table 1).

Additional treatment variables were associated with polypharmacy. Interestingly, both the concomitant use of a mood stabilizer and a decreased length of inpatient stay (LOS) were associated with APP (p<0.0005 and p<0005, respectively, Table 1). However, the concomitant prescription of an anxiolytic or antidepressant was not (p=0.069 and p=0.793, Table 1). Finally, each discharge disposition was significantly associated with either mono or polypharmacy. Discharge

Table 1 Socio-demographic and clinical variables by single and multiple antipsychotic discharge cohorts[a]

Characteristic	Single antipsychotic discharge group (n=1,010)[b]	Multiple antipsychotic discharge group (n=377)[b]	p[c]
Total readmission rate	1,010 (13.7%)	377 (15.9%)	0.286
Age, mean years	39.0	37.1	0.008
Gender, male	58.2 (588)	63.4 (239)	0.080
Ethnicity, non-white	35.9 (357/994)	39.6 (148/374)	0.212
Substance use	53.7 (535/996)	55.7 (206/370)	0.518
Schizophrenia	32.8 (331)	44.3 (167)	<0.0005
Schizoaffective disorder	35.9 (361)	43.8 (165)	0.008
Psychotic disorder	32.0 (323)	11.9 (45)	<0.0005
Personality disorder	16.6 (168)	19.4 (74)	0.265
Intellectual disability	4.2 (42)	6.6 (25)	0.056
Mood stabilizer	34.1 (344/1008)	47.9 (179/374)	<0.0005
Anxiolytic	26.6 (266/1000)	31.6 (118/374)	0.069
Antidepressant	29.3 (294/1005)	28.5 (107/375)	0.793
Length of stay, mean days	38.3	36.6	<0.0005
Discharge home	59.7 (603/1010)	43.8 (165/377)	<0.0005
Discharge to residential treatment	19.4 (196/1010)	26.5 (100/377)	0.004
Discharge to crisis recovery unit	12.9 (130/1010)	22 (83/377)	<0.0005

[a]All results presented as % of n unless otherwise noted; [b]Single group n=1,010; Multiple group n=377, unless otherwise noted.; [c]Age analyzed with independent-groups t test, Length of stay analyzed with Mann-Whitney U test; all other comparisons with Chi-square test.

to home was associated with monotherapy (p<0.0005, Table 1), while both discharge to residential treatment or a crisis recovery unit was associated with polypharmacy (p=0.004 and p<0.0005, respectively, Table 1). Taken as a whole, these cohorts display expected differences in socio-demographic and clinical variables due to the nature of the study. However, even for variables that displayed significant differences across the cohorts, such as age, the diagnosis of Schizophrenia, Schizoaffective Disorder, Psychotic Disorder, or discharge disposition, the differences between the groups did not exceed fourteen percent (or three years or days, for mean age or length of stay, respectively), except for the diagnoses of Psychotic Disorder and discharge to home (20.1% and 15.9%. respectively, Table 1).

Reduced inpatient psychiatric readmission rates are not associated with APP

After assessing the socio-demographic and clinical variables of the cohorts, we sought to determine if the polypharmacy group had either increased or decreased incidence of readmission following discharge. Given the n of the two cohorts, the study yielded sufficient statistical power to detect a difference in rate of readmission of 5.8% with 95% confidence and 80% power. The overall incidence of readmission was 14.3% of patients. Readmission occurred at a rate of 13.7% for those on a single antipsychotic compared to 15.9% for the multiple antipsychotic group, a 2.2% difference (Table 1). This small percentage difference tended to favor the use of a single antipsychotic, but did not meet statistical significance given the scope of the study.

Younger age and concomitant use of a mood stabilizer are predictive of increased risk of readmission

Due to the unequal distribution of socio-demographic and clinical variables between the cohorts, and the potential for these differences to act as cofounders in determining readmission rates amongst the groups, a logistic regression was performed.

Consistent with the univariate analysis (Table 1) demonstrating no significant difference in readmission rate between cohorts, a predictive logistic regression model did not find concomitant antipsychotic to be associated with either increased or decreased incidence of readmission (Table 2).

Two socio-demographic and clinical variables which were significantly associated with either the mono- or polypharmacy treatment groups were also found to be predictors of readmission. Specifically, increased age was found to predict a decreased incidence of readmission (OR=0.982, 95% Cl=0.968-0.996, p=0.010, Table 2), and the concomitant use of a mood stabilizer in addition to at least one antipsychotic was found to predict increased risk of readmission (OR=1.418, 95% Cl=1.001-2.010, p=0.049, Table 2). Having a personality disorder and discharge to residential treatment (OR=7.596, 95% Cl=0.968-59.591, p=0.054, and OR=1.911, 95% Cl=0.916-3.988, p=0.085, respectively, Table 2) approached statistical significance favoring increased readmission risk. Based upon this logistic regression model, for each additional year of age, the odds of being readmitted decreased by 12%. However, if a mood stabilizer was prescribed, the odds of being readmitted increased by 42%.

It should be noted that the types of mood stabilizers recorded for this study were lithium and divalproex sodium.

We also sought to correlate our analysis for the total study duration with a Cox regression

Table 2 Socio-demographic and clinical variables as predictors of readmission (Logistic regression analysis of readmission incidence)[a]

Predictor	Odds Ratio	95% CI	p
Increased age	0.982	0.968-0.996	0.010
Gender, male	0.784	0.556-1.105	0.164
Ethnicity, non-white	0.836	0.596-1.173	0.301
Substance use	0.906	0.635-1.293	0.587
Schizophrenia	1.141	0.722-1.803	0.573
Schizoaffective disorder	1.263	0.813-1.964	0.299
Psychotic disorder	3.160	0.420-23.795	0.264
Personality disorder	7.596	0.968-59.591	0.054
Intellectual disability	1.708	0.192-15.230	0.632
Mood stabilizers	1.418	1.001-2.010	0.049
Anxiolytic	1.175	0.832-1.659	0.360
Antidepressant	1.080	0.764-1.528	0.663
Increased length of stay	1.004	0.997-1.010	0.280
Discharge home	1.518	0.754-3.059	0.243
Discharge to residential treatment	1.911	0.916-3.988	0.085
Discharge to crisis recovery unit	1.077	0.486-2.387	0.856
Multiple antipsychotics at discharge	1.098	0.767-1.572	0.609

[a]Total cases analyzed=1,329. A Hosmer-Lemeshow test indicated that model fit was good (chi-square with 8 df=10.12, p=0.257).

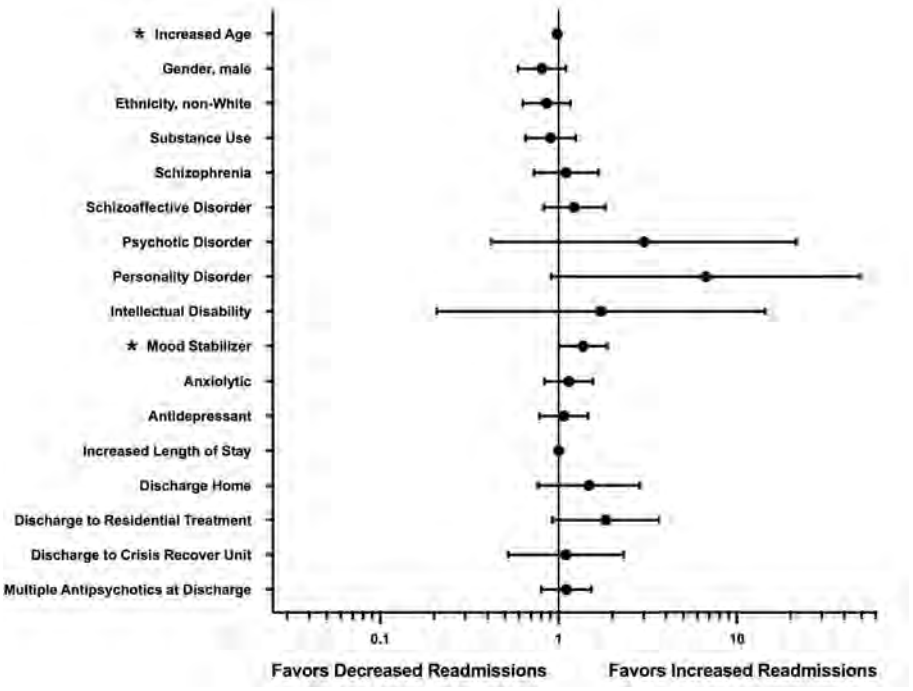

Figure 1 Forest Plot of Cox regression socio-demographic and clinical variables as predictors of survival days until readmission: Total cases analyzed=1,329. – 2Log Likelihood=2434.30, indicating that model fit was good. Mean Hazard Ratios shown with 95% confidence intervals are displayed. Statistically significant predictors are indicated by *, where p<0.05.

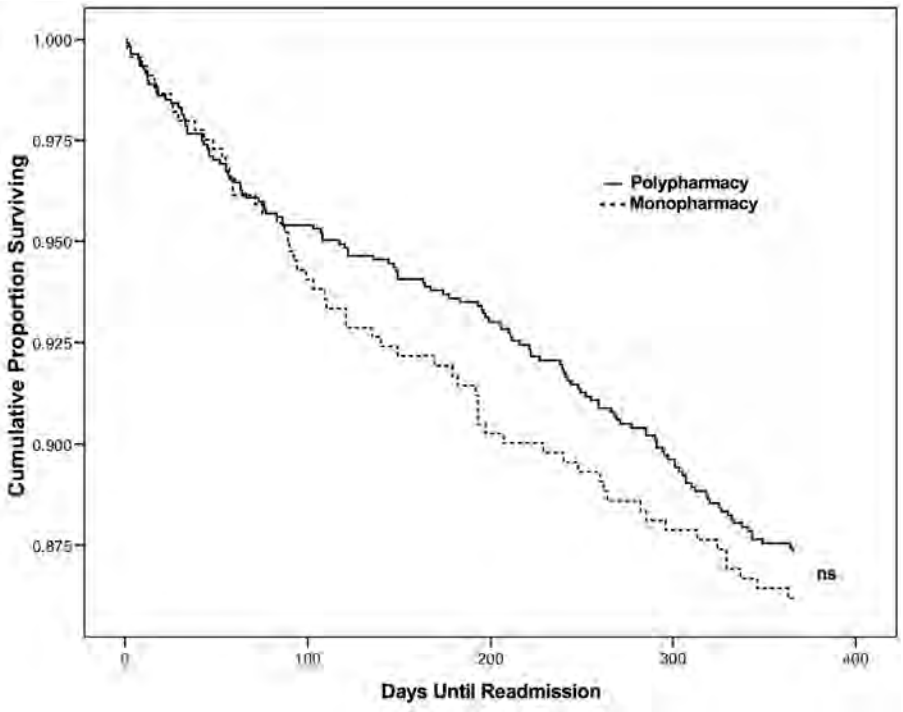

Figure 2 Cox regression days until readmission for mono or polypharmacy cohorts (adjusted for covariates): Total cases analyzed=1,329. – 2Log Likelihood=2434.30, indicating that model fit was good. The difference between the two groups was not statistically significant, p=0.542.

Table 3 Cox regression of socio-demographic and clinical variables as predictors of survival days until readmission[a]

Predictor	Hazard ratio	95% CI	p
Increased age	0.983	0.971-0.996	0.010
Gender, male	0.808	0.593-1.101	0.177
Ethnicity, non-white	0.857	0.630-1.167	0.328
Substance use	0.901	0.652-1.245	0.527
Schizophrenia	1.103	0.724-1.681	0.648
Schizoaffective disorder	1.224	0.822-1.824	0.319
Psychotic disorder	3.019	0.421-21.646	0.272
Personality disorder	6.714	0.909-49.597	0.062
Intellectual disability	1.729	0.207-14.472	0.613
Mood stabilizer	1.372	1.003-1.878	0.048
Anxiolytic	1.141	0.837-1.557	0.405
Antidepressant	1.072	0.784-1.464	0.663
Increased length of stay	1.003	0.997-1.008	0.325
Discharge home	1.480	0.766-2.859	0.244
Discharge to residential treatment	1.839	0.926-3.653	0.082
Discharge to crisis recovery unit	1.097	0.520-2.313	0.809
Multiple antipsychotics at discharge	1.106	0.800-1.528	0.542

[a]Total cases analyzed = 1,329. -2Log Likelihood = 2664.20, indicating that model fit was good.

(survival analysis) model that predicts the variables that modify daily risk of readmission. For each subject readmitted, the number of days between discharge and readmission was calculated. If a subject was not readmitted within the one-year period, the number of days were treated as censored observations with survival times of 365 days (Figure 1).

As with all prior analyses, there was no significant difference between mono- or polypharmacy cohorts (HR of polypharmacy=1.106, 95% Cl=0.800-1.528, p=0.542, Figure 1, Figure 2, and Table 3). Consistent with the logistic regression analysis only increased age (HR=0.983, 95% Cl=0.971-0.996, p=0.010, Figure 1 and Table 3) and the concomitant use of a mood stabilizer (HR=1.372, 95% Cl=1.003-1.878, p=0.048, Figure 1 and Table 3) were statistically significant predictors of days until readmission. Having a personality disorder and discharge to residential treatment again approached significance (HR=6.714, 95% Cl=0.909-49.597, p=0.062, and HR=1.839, 95% Cl=0.926-3.653, p=0.082, respectively, Figure 1 and Table 3). The Cox regression analysis found that for each additional year of age, the risk for readmission on any given day decreased by 12% and if the subject was prescribed a mood stabilizer, the odds of being readmitted on any given day increased by 37%.

Discussion

In spite of evidence-based guidelines limiting the use of APP to specific situations, multiple antipsychotics were prescribed for 27.2% of patients at discharge in the study. The rate of prescription of multiple antipsychotic medications was similar to that for a sample of patients with schizophrenia discharged from a Canadian tertiary care psychiatric facility (8). The rate of polypharmacy in the study was surprising, given that the sample was obtained from a psychiatry residency training program where it is ex-

pected that evidence-based medicine will be followed more closely. However, it is theoretically possible that every prescription for multiple antipsychotics followed evidence-based recommendations, though that is unlikely given the previously reported clinical factors which are associated with polypharmacy are also likely to exist in an academic setting (1, 9, 14-17).

Multiple studies indicate that APP is related to variables that can be proxies for greater illness burden, such as diagnosis of schizophrenia or schizoaffective disorder, COT, longer length of inpatient hospitalization, prescription of additional psychiatric medications, and comorbidities such as substance use (15). This study found areas of overlap with previously reported proxy variables of illness severity, such as the diagnosis of schizophrenia and prescription of additional psychiatric medications. In contrast, in this study a shorter duration of hospitalization was associated with APP. This discrepancy may be related to the overall increased length of stay in the study compared to nationwide hospital statistics for similarly diagnosed patients (28). It is unclear why patients in this study had an overall increased LOS, but this may be due to the legal process for commitment in Arizona where length of inpatient treatment is not factored into whether someone is ordered to receive COT (27). Given that shortened time constraints for rapid stabilization are associated with APP, an increased LOS may in turn lead to less hurried treatment decisions, and therefore also reduce other forms of polypharmacy.

Another illness severity variable, aggression, is difficult to assess via a specific quantifiable measure, and the effect of this variable on prescribing tendencies or readmission risk could not be assessed. However, the prescription of a mood stabilizer, which may be used to treat aggression, was associated with APP. These patients may be more emotionally and/or behaviorally dysregulated, and present with more challenging behavior on inpatient units. The study did not include other measures to quantify behavior, such as mania, preventing a comparison of the presence and severity of these symptoms between the two cohorts.

While APP may be associated with proxy measures for greater disease burden, it is critical to determine if APP effectively treats the increased disease burden by preventing the risk of inpatient readmission, given APP's greater cost and increased risk for side effects. In this study, there was no statistically significant difference in the readmission rates over a one-year period following discharge for patients prescribed APP. Inpatient readmission functioned as a proxy measure for acute relapse, though the nature of COT in Arizona requires that relapsing patients receive an appropriate higher level of care, which would include inpatient admission. It is possible that patients discharged on multiple antipsychotics may be switched to a single antipsychotic, and vise-versa, in the outpatient setting. Given the nature of the study, as an intention to treat study based upon polypharmacy at discharge, these outpatient treatment changes could dilute the differences between the groups. However, the historical trend of patients remaining on a polypharmacy regimen over time, even when the polypharmacy regimen is first initiated as a short-term treatment plan, makes it unlikely that cohort crossover was a significant variable in the study (1, 4, 9, 15).

Critically, when evaluating the factors predicting increased risk of readmission, very few factors were identified. These findings suggest that it is challenging to risk-stratify which patients are likely to relapse: a task that has proven difficult and is of urgent clinical need to reduce morbidity from psychiatric illness (29). This study further reinforces that many proxy measures for disease severity that have been associated with APP are not associated with relapse risk, and

should alter perceived risk of relapse in patients thought to have greater illness severity. It is possible that with a larger study size, other variables, such as discharge to a residential treatment facility, which approached statistical significance, may become statistically significant. Interestingly, discharge to a sub-acute facility, which was the highest discharge level of care in the study, was not predictive of relapse, indicating that disease burden may not predict relapse if treated in the appropriate setting.

Concomitant use of a mood stabilizer has previously been reported as an indicator of disease severity, as is the concomitant use of an antidepressant. Why mood stabilizer use was predictive in this study is unclear, as is whether these medications were discontinued in the outpatient setting. Further studies are needed to identify why mood stabilizers were unique amongst the additional treatment medication classes and if relapse was associated with discontinuation of these medications in the outpatient setting. Additionally, it is known that younger patients are more likely to be prescribed a polypharmacy regimen, but it is not clear why younger patients are at increased risk of relapse. It is possible that elderly patients at an increased risk of relapse were placed in state hospital facilities at a younger age due to perceived risk of relapse or greater disease burden, thus presenting an age based selection bias. However, given the relatively difficult task of predicting risk of readmission, it seems unlikely that identification of these higher risk patients occurred at a younger age. Since acute relapse is associated with an inability to cope with life stressors, it may be that older patients have learned coping mechanisms to address stressors and reduce relapse risk (30).

Clinical implications

This study showed no added benefit from prescribing multiple antipsychotics over a single antipsychotic medication with regard to preventing relapse. Specifically, seen in our logistic regression data and Figures 1 and 2, our findings further support various guidelines and have the ability to protect patients from exposure to unnecessary medications by potentially changing prescriber behavior. Additionally, less medication prescribed has potentially positive financial implications.

Limitations of the study

The main outcome measure used is not a direct measure of relapse, but a proxy measure. Aggression was not assessed via a specific measure and the effect of aggressive behavior can therefore not be assessed. Additionally, the study did not take into consideration a few factors that should be addressed. For example, the study did not survey patient factors such as level of education, marital status, family status, employment, and inheritance psychotic overload. The purpose of the study was to consider the various clinical conditions patients suffered and how they responded to a single or multiple antipsychotic discharge. However, future studies would greatly benefit and bring a wider understanding by considering such other patient factors that this study did not account for.

Conclusion

APP at discharge was not found to prevent readmission to acute psychiatric inpatient facilities, supporting current guidelines recommending antipsychotic monotherapy. The study did not examine the increased risk of side effects due to exposure of higher amounts of antipsychotic medications seen with APP. This should be carefully evaluated in a future study given the lack of evidence for relapse prevention. Strikingly, many variables which are proxies for disease bur-

den were not predictive of readmission risk in statistical models. These conclusions further demonstrate that improved prediction of relapse risk is a pressing clinical need to reduce morbidity from psychiatric disease.

What is already known on this topic

Antipsychotic polypharmacy has been recorded as a routinely utilized treatment modality in a psychiatrist's armamentarium and is backed by current data from the Clinical Antipsychotic Trial of Intervention Effectiveness (CATIE) study. CATIE showed that 6 percent of patients took two or more antipsychotics upon entry to the study. High rates of APP are further confirmed from one Canadian study indicating that 27.5% of patients were placed on a polypharmacy treatment protocol upon discharge. Additionally, antipsychotic polypharmacy has shown to be associated with risk for side effects, adverse reactions and drug-drug interactions. These associations have shown the need for ongoing empirical research to place the implied benefits and drawbacks of Antipsychotic polypharmacy into perspective.

What this study adds

This investigation did not detect any difference between patients on single and multiple antipsychotic regimens at discharge and their one year readmission rates. Given the risks of side effects and financial costs of antipsychotic polypharmacy, those discharging on such prescriptions should not be considered at any less risk of relapse. However, as always, further studies would be beneficial to better identify those at higher readmission risk and the factors that may impact their pathway from stabilization to relapse.

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Original article

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The effects of interprofessional diabetes education on the knowledge of medical, dentistry and nursing students

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Objectives. Interprofessional teamwork is best attained through education that promotes mutual trust and effective communication. The primary aim of the study was to evaluate the impact of interprofessional learning on knowledge about diabetes. **Methods**. The cross-sectional study included students of medicine, dentistry and nursing at the Faculty of Medicine Foča, Bosnia and Herzegovina. The students were randomly allocated into one of two groups. Group 1 attended an interprofessional course on diabetes while group 2 was divided into three subgroups and each of the subgroups attended an uniprofessional diabetes course. The measuring instrument used in the course in order to assess the participants' knowledge about diabetic care was a test containing multiple-choice questions about diabetes. The Interprofessional Questionnaire was used to explore the attitudes, views, values and beliefs of students regarding interprofessional education (IPE). **Results**. No statistically significant difference in total score on the test was found between the groups at baseline, but at follow-up the difference was highly statistically significant (F=10.87; p=0.002). The students from Group 1 had better results (21.82 points), compared to Group 2 (18.77 points). The statistically significant difference was observed in mean values (t=-3.997; p=0.001), between Groups 1 and 2; the students from Group 1 obtained 20.42 points, which is considered to indicate a respectively positive self-assessment of communication and teamwork skills. However, Group 2 indicated a negative self-assessment of communication and teamwork skills. **Conclusion**. The findings suggest that IPE activities may provide health profession students with valuable collaborative learning opportunities.

Introduction

Each profession has distinct profession-based behavior, viewpoint, and values, even before commencing undergraduate training (1, 2). Prior work has found entrenched in-group ratings of academic ability and professional competence among students across 10 professional and social care programs surveyed at the outset of their training; first-year nursing, pharmacy, and medical students all perceive themselves as more "caring" than members of the other disciplines (3, 4). Such health-professional stereotypes can adversely impact teamwork and, ultimately, patient care (5, 6).

West et al. (7) concluded that clear professional roles are essential, and that team members could benefit from a comprehensive understanding of both their own professional role and the professional roles of their colleagues. Damour and Oansen (8) concluded that educational efforts should be introduced early in the curriculum, prior to the developing of professional identities and the formation of stereotypes. Saroo et al. (9) argued that successful interprofessional training should take advantage of the students' psycho-sociological determinants, such as professional role behavior, hierarchy, and power relations. According to the Centre for the Advancement of Interprofessional Education (CAIPE), interprofessional learning involves "educators and learners from 2 or more health professions and their foundational disciplines who jointly create and foster a collaborative learning environment. The goal of these efforts is to develop knowledge, skills and attitudes that result in interprofessional team behaviors and competence" (10, 11).

Different studies noted that the students are not sufficiently exposed to interprofessional teamwork during their clinical training (12, 13). Aase et al. (14) found that theoretical lectures on interprofessional teamwork were not followed-up in clinical training, especially in nursing schools. Medical schools exposed their students to more interprofessional education (IPE), but, still they are not very compliant with the WHO recommendations, calling for strengthening interprofessional teamwork in educational programs (14). The reasons for this are partly because of faculty and students' attitudes (15).

On the other hand, diversity in the implementation of IPE has made it difficult to determine best practice models, optimal IPE delivery model or reliable measurement tools (16, 17). Examples of reliable measurement tools to measure IPE are Interdisciplinary Education Perception Scale (IEPS) and University of the West of England Entry Level Interprofessional Questionnaire (UWE-ELIQ) (18). It has been suggested that IPE courses should have defined the core competences as their curricular components (19). A comprehensive assessment of those competences is needed for effective medical and health care education programs. A clear differentiation between the identification of essential components (knowledge, skills, and attitudes) and of criteria to assess the behavioral performance of health care workers is crucial, as in the performance criteria several components are integrated or used in combination (20).

Numerous studies of the effectiveness on interprofessional education compared IPE to education in which the same professions were learning independently from each other (21, 22). The analyses suggested that IPE seemed to be more effective in relation to reaction and learning, with much less evidence of impact on behavior and results. Based on the findings from other studies evaluating the use of interprofessional education, the staff of Medical School Eastern Sarajevo decided to conduct an interprofessional course on diabetes. The lecturers focused on four interprofessional competency domains outlined by the Interprofessional Education Collaborative: ethics, roles and responsibilities, communication and teamwork.

The primary aim of the study was to evaluate the impact of interprofessional learning on knowledge about diabetes, the roles, and responsibilities of other health professions. The secondary aim was to analyze the attitudes, views, values and beliefs of health profession students who are learning together, regarding IPE, compared to the students who are learning independently from each other.

Methods

Data collection and participant characteristics

The cross-sectional study was conducted during the winter semester at the Faculty of Medicine, University of East Sarajevo, Bosnia and Herzegovina, in January 2017. Participants were all students enrolled in sixth-year of medicine (29 students), fifth-year of dentistry (21 student) and third-year of nursing (16 students), as the teaching curricula for these years and study programs integrated obligatory lectures in diabetes. The list with students' names, provided by the Student Administrative Service, was torn into separate strips. The strips were put in a hat and were mixed. Each name was pulled out and put into one of two groups alternately. Group 1 attended interprofessional course on diabetes. Group 2 was divided into three subgroups: medical, dental and nursing.

Intervention design

The interprofessional diabetes course was designed to provide students with in-depth knowledge of diabetes mellitus. The course was a one-day mandatory class and consisted of four blocks. Block 1 focused didactically on the therapeutic management of Type 1 and Type 2 Diabetes, blood glucose monitoring, patient education and evidence-base for diabetes care and decision making in interprofessional practice. Block 2 didactics focused on the ethics, roles, responsibilities and scope of medical, dental and nursing practices, and introduced the characteristics of an effective team. Block 3 analyzed government strategies for diabetes and examined their benefits for practice within an interprofessional context and evaluated the physical, psycho-social and cultural impact of diabetes on the patient and family. The last block focused on a paper-based case scenario in which a newly established patient with diabetes was presented. The scenario incorporated the history provided by family practitioner. Students included in IPE were placed in eight mixed-profession teams, consisting of 3-4 medicine, 2-3 dentistry and 2 nursing students that remained together throughout the whole course.

After each didactic block, team members met to discuss personal values, teamwork, leadership, consensus building and the ability to identify and achieve joint goals in care for patients related to presented topic. Vision of team-working, decision making processes, shared responsibility for team actions, own role and the roles of others, role boundaries, team skills and knowledge were explored. At the end of course, each team reviewed the activities that occurred at the process of interprofessional learning.

The course content for group 2 was identical except for the interprofessional group work. Students received the same learning experience, but interaction with students of different health profession was lacking. Didactic blocks were handled by three faculty members, with background in family medicine, parodontology and nursing.

The measuring instruments

The measuring instrument used during the course to assess the participants' knowledge about diabetic care was a test containing multiple-choice questions about diabetes. The multiple-choice test contained 20 questions, divided into two scales, about diabetic patient care, with each question carrying between 1 to 4 points, and with the total score being 33. The first subscale consisted of 13 questions about the general knowledge about diabetes (definition, diagnosis, screening, self-control, complication, treatment and care for diabetic patients). To explore if IPE contributes to nursing and medicine students' knowledge regarding oral health in

diabetic patients, the second subscale consisted of 10 questions was also included. The test was distributed at the beginning of the course, and after completion of the course. The large amphitheater was provided to allow students to sit in every third seat and every second row. The students were assigned to specific seat, and seating arrangement was changed for follow-up test. To prevent cheating, mobile phone signals were blocked. Prior to the test, the principal researcher presented student behavior rules. The students were instructed to conduct themselves honestly. They were not permitted to ask the questions of invigilators (except in the case of ambiguities) or to copy someone else's answers. Four invigilators practiced constant observation, watching the students all the time. Test-taking time was 30 minutes long.

To explore the attitudes, views, values, and beliefs of study participants regarding IPE, the Interprofessional Questionnaire, based on the University of the West of England Entry Level Interprofessional Questionnaire (UWE-ELIQ) (23) was used. The Questionnaire included 33 multiple- choices questions, divided into 4 scales: interprofessional learning, communication and teamwork scale, interprofessional interaction and interprofessional relationships. The answers were rated according to Likert-type scale.

The Interprofessional Learning Scale assessed attitudes towards learning in an interprofessional setting, whilst the Interprofessional Interaction scale analyzed perceptions of other interprofessional colleagues and interactions. The perceptions of students' own relationships with colleagues were evaluated with the Interprofessional relationships scale. The answers were scored from 1 (strongly agree) to 5 (strongly disagree), the neutral point being included. The scores for these three scales were predetermined for the questionnaire, indicating positive (8 to 20 points), neutral (21 to 27 points) or negative (28 to 40 points) attitudes or perception.

The Communication and Teamwork Scale provided self-assessment of skills. The statements were scored from 1 (strongly agree) to 4 (strongly disagree). Positive self-assessment was defined as meeting expectation, and unsatisfactory communication/ teamwork skills were transmitted to negative self-assessment. The minimum score was 8, maximum 32. The score from 8–20 represented positive, 21–25 neutral, and 26–32 negative self-assessment of skills. The reliability analysis was conducted by calculating a Cronbach's alpha coefficient and its value of 0.724 was found to be satisfactory.

Ethical considerations

The study is conducted with the approval of the Ethical Committee of the Medical Faculty of Foča, University of East Sarajevo, Bosnia and Herzegovina.

Statistical analysis

Statistical analyses were carried out using SPSS 20 (SPSS Inc., Chicago, IL, USA). The p values of less than 0.05 were considered as statistically significant. Data were described using percentages, mean values, standard deviations, and frequency distributions. The Paired-Samples T test was used to compare mean values before and after intervention on students. ANOVA with repeated measures was used to show possible statistically significant differences between study programs and groups of students at baseline and at follow up. The independent *t-test* was used to show statistically significant differences between study programs and groups of students in mean values of the Interprofessional Questionnaire. To determine correlations between the knowledge of students and the IPE Questionnaire answers, we used Pearson's coefficient for correlations.

Results

The study included 66 undergraduate students divided in two groups, first, interprofessional (Group 1) and second, uniprofessional (Group 2). Mean ±SD age in study population was 23.1±2.8 years, and 57.6% of participants were female. Majority of respondents in both groups study medicine (44.2%), 31.8% dentistry and 25.8% nursing school. The statistically significant differences between baseline and follow-up knowledge about diabetes (t=–8.166; p=0.001) and impact of diabetes on oral health were found (Table 1).

Gradient improvement of knowledge was detected in both groups, but at follow-up, the scores were significantly higher among interprofessional group of students (Table 2).

Table 3 shows the comparisons of mean values of test scores between the students of medicine, nursing and dentistry. Although baseline and follow up general knowledge on diabetes were the greatest among medicine, and oral health among dentistry students, significant differences in total score at follow-up were not found (F=0.179, p=0.836).

The Communication and Teamwork scale was used to evaluate students' communication and teamwork skills. Self-assessment of skills in interprofessional group was positive, and in uniprofessional group negative (t=–3.997; p=0.001). The perceptions of students' own relationships were positive in both groups, conversely, attitudes towards learning in an interprofessional setting and perceptions of interprofessional interactions were positive in group 1 and neutral in group 2 (Table 4).

Dentistry students reported positive self-assessment of communication and teamwork skills (20.38 points), compared to medical (26.03 points) and nursing students (27.23 points), whose self-assessment was neutral and negative, respectively (t=-3.270; p=0.005). The attitude of medicine students

Table 1 Comparisons of test score mean values before and after the course

Test score	Mean values (± SD) of test scores		t - test	p
	Baseline	Follow up		
Subscale 1	5.83 (2.47)	9.13 (3.80)	-8.166	0.001
Subscale 2	7.54 (2.03)	11.34 (2.78)	-11.317	0.001
Total score	13.37 (2.76)	20.39 (4.30)	-12.510	0.001

Table 2 Comparisons of the test score mean values between interprofessional and uniprofessional groups at baseline and at follow up

Test score	Mean values (± SD) of test scores		t - test	p
	Group 1	Group 2		
Subscale 1				
Baseline	6.11 (2.31)	5.51 (2.64)	0.246	0.622
Follow up	10.31 (3.66)	7.80 (3.54)	7.04	0.010
Subscale 2				
Baseline	7.08 (1.54)	8.06 (2.39)	7.25	0.009
Follow up	11.68 (1.23)	10.96 (3.85)	4.26	0.043
Total score				
Baseline	13.20 (2.51)	13.58 (3.05)	1.77	0.188
Follow up	21.82 (4.54)	18.77 (3.41)	10.87	0.002

Table 3 Comparisons of the test scores mean values according to the study programs at baseline and at follow up

Test score	Mean values (± SD) of test scores			F	p
	Medical students	Dentistry students	Nursing students		
Subscale 1					
Baseline	7.64 (1.70)	3.28 (1.67)	6.00 (1.45)	23.22	0.001
Follow up	11.21 (2.42)	5.95 (3.90)	9.64 (2.89)	5.073	0.009
Subscale 2					
Baseline	7.57 (1.16)	9.09 (1.84)	5.58 (1.73)	15.10	0.001
Follow up	10.32 (2.40)	14 (1.76)	9.76 (1.04)	15.48	0.001
Total score					
Baseline	15.21 (2.25)	12.38 (2.51)	11.58 (2.00)	14.57	0.001
Follow up	21.32 (4.75)	19.95 (3.72)	19.41 (4.12)	0.179	0.836

F=Variation between sample means, ANOVA.

Table 4 Comparisons of mean values of Interprofessional Questionnaire in three study programs

Groups of students	Interprofessional Questionnaire scales			
	CETS	ILS	IIS	IRRS
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Group 1	20.42 (5.12)	18.77 (9.58)	20.25 (5.72)	16.65 (7.17)
Group 2	28.06 (7.88)	21.54 (8.84)	22.67 (4.74)	18.45 (7.81)
t - test/p	-3.997 / 0.001	-1,128 / 0.226	-1.857 / 0.068	-0.972 / 0.335
Medical	26.03 (8.01)	22.96 (9,97)	21.60 (5.46)	18.85 (7.90)
Dentistry	20.38 (3.82)	18.47 (5.92)	20.04 (4.37)	16.71 (4.86)
Nursing	27.23 (7,28)	17.29 (10.72)	22.7 (6.33)	16.41 (7.59)
t - test/p	3.270 / 0.005	1.982 / 0.058	1.115 / 0.271	1.019 / 0.314

CETS=Communication, Ethics and Teamwork scale; ILS=Interprofessional Learning scale; IIS=Interprofessional Interaction Scale; IRRS=Interprofessional Roles and Responsibilities Scale.

Table 5 Correlation between students' knowledge and Interprofessional Questionnaire scales

Test score	Interprofessional Questionnaire Scales			
	CETS	ILS	IIS	IRRS
	r / p	r / p	r / p	r / p
Subscale 1	0.084 / 0.501	0.013 / 0.919	0.030 / 0.813	-0.047 / 0.705
Subscale 2	-0.673 /0.001	-0.284 / 0.021	-0.397 / 0.001	-0.247 / 0.046
Total score	-0.327 /0.007	-.137 / 0.274	-0.189 / 0.128	-0.175 / 0.159

CETS= Communication, Ethics and Teamwork scale; ILS=Interprofessional Learning scale; IIS=Interprofessional Interaction Scale; IRRS=Interprofessional Roles and Responsibilities Scale; r=correlation coefficient.

related to inteprofessional learning was neutral contrary to (22.96) positive attitudes of dentistry (18.47) and nursing students (17.29). All three groups had a positive perception about interprofessional interaction and their own interprofessional relationships. However, statistical significant differences in attitudes and perceptions by study program and intervention were not found. The correlation between test subscales and Interprofessional Questionnaire' scales are shown in Table 5.

Anecdotal comments included Group 1 students remarking how much more informed they were now about the role of health care of professions other than their own, and that there should be more of such learning and teaching activities organized during undergraduate studies

Discussion

To evaluate the health profession students' skills, perception and attitudes regarding IPE, the course on diabetes was carried out. The current study showed that health profession students who participated in IPE course had greater overall knowledge of diabetes as well as more positive assessment of their communication and teamwork skill compared to the students involved in uniprofessional course. The findings are in line with other studies showing the influence of bringing different health professions together to learn on achieving better results and interprofessional collaboration (23-25).

The literature suggests that implementation of IPE into undergraduate curricula could increase the likelihood of future physicians, nurses and dentists engaging in a communicative and team member behavior that promotes and improves the model of multidisciplinary, patient-centered care. Helping students form, and successfully integrate, their professional selves into their multiple identities is a fundamental of medical education (26, 27). Albeit the most students in IPE group rated their communication and teamwork skills positively, a significant difference was found between self-assessed skills of different health profession students. Nursing students had more negative opinion compared with neutral self-assessment of medicine and positive view of dentistry students. The quantity of structured interpersonal learning experiences was but few at Faculty of Medicine Foča, and this was the first course in the medicine, nursing and dentistry undergraduate curricula addressing IPE. As curricula are traditional, understanding of others health professions' roles is limited, nurses being seen as proximate, caring aids to physicians, physicians as main care providers for diabetic patients, and dentists as a profession taking care of oral health only. Available data indicate that collaboration readiness and specifically higher levels of confidence in personal skills could be improved by engaging all health profession students in IPE (27, 28). IPE sustains augmented confidence relating to students' skills and positive attitudes toward interprofessional roles and responsibilities (29).

Differences in attitudes toward interprofessional learning, roles and interaction were not found among the students participating in IPE and non-IPE course. However, the students exposed to IPE were more favorably inclined towards interprofessional learning in addition to dentistry and nursing students generally, what corroborates previous studies (30). They held counteractive opinions about interprofessional interaction. In agreement with prior research (31, 32) all students reported improvement in understanding the roles of other health professionals in the patient care and challenges of learning in interprofessional teams as this was the first time for all students, regardless the study program and intervention, to have the opportunity to attend lectures presented by the professors with different expertise.

An improvement in diabetes knowledge level was identified among all three professions, medical, dentistry and nursing. There was a statistically significant difference in baseline knowledge. As expected, medical students had the highest score in general knowledge and dentistry students in oral health due to the previous training in the specific areas. Observing the subscales responses, the scores per group notably increased with the ratio between the professions being stable, but the difference in to-

tal scores by study program was not found. Both interventions included contents new to students, involving multidisciplinary clinical scenario that might have allowed increased understanding of diabetes and its oral-systemic connections (33). IPE course particularly strengthened the knowledge of the nursing students who started with low scores. Sequential with the findings of others, the authors of the current study believe it is possible that integrating IPE learning activities into undergraduate health profession curricula, within the context of a relevant topic like diabetes management, may augment students' learning abilities and improve their collaboration related competences (29, 34, 35).

Creating the IPE courses requires cooperation from deans, administrators and faculty members. Curricula in each discipline should offer sufficient opportunities for students to first interact, both formally and informally, with their own members, and to explore and even challenge accepted frameworks of established roles (36, 37).

Limitations of the study

The current study has several limitations. The findings could be specific to University of East Sarajevo and didactic approach of the faculty, which was not necessarily the same as at the other faculties of medicine in Bosnia and Herzegovina. Knowledge, attitudes and perception were assessed at one point in time. The study was based on the self-assessment, therefore subjectivity and accountability need be considered. Although this study demonstrates promising findings, it is preliminary, so replication of the study longitudinally, throughout study years, analyzing the comparison of a change in attitudes, and including the higher number of participants should be set up at same and other faculties of medicine in the country. Future research is needed to examine whether IPE could result in a breakdown of negative stereotypes over time and if so, what type of IPE intervention would be the most effective.

Conclusion

The findings suggest that interprofessional education activities may provide health profession students with valuable collaborative learning opportunities in addition to improving specific clinical knowledge, level of confidence in own skills and positive attitude conducive to collaboration with other health professionals. In order to prepare the health professional students for interprofessional collaborative practice following graduation, different IPE interventions are needed. Further longitudinal, multi-site studies exploring the impact of interprofessional learning on knowledge, attitudes and perceptions of students are requisite.

What is already known on this topic

Interprofessional teamwork is best attained through education that promotes mutual trust and effective communication. According to the Centre for the Advancement of Interprofessional Education (CAIPE), interprofessional learning involves educators and learners from 2 or more health professions and their foundational disciplines who jointly create and foster a collaborative learning environment. The goal of these efforts is to develop knowledge, skills and attitudes that result in interprofessional team behaviors and competence. Health professionals as team members could benefit from a comprehensive understanding of both their own professional role and the professional roles of their colleagues.

What this study adds

Interprofessional education activities may provide health profession students with valuable collaborative learning opportunities in addition to improving specific clinical knowledge, level of confidence in own skills and positive attitude conducive to collaboration with other health professionals. In this line, we evaluated impact of interprofessional learning on knowledge about diabetes among medical, dentistry and nursing students. We also explored the attitudes, views, values and beliefs of students regarding interprofessional education.

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MR, BNJ, SC, SK, NI, NH, MK, BM, MM, VRJ and MD; Drafting the article: MR, BNJ, SC, SK, NI, NH, MK, BM, MM, VRJ and MD; Revising it critically for important intellectual content: MR, BNJ, SC, SK, NI, NH, MK, BM, MM, VRJ and MD; Approved final version of the manuscript: MR, BNJ, SC, SK, NI, NH, MK, BM, MM, VRJ and MD.

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Case report

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The chondrocoracoideus muscle: A rare anatomical variant of the pectoral area

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Key words: Pectoralis major ▪ Variation ▪ Accessory muscle ▪ Chondrocoracoideus ▪ Thoracic wall muscles.

Objective. The study adds important information regarding the descriptive anatomy of a very rarely reported unilateral chondrocoracoideus muscle (of Wood). Additionally it highlights the concomitant muscular and neural alterations. **Case report.** The current case presents the occurrence of a chondrocoracoideus muscle situated left-sided, as an extension of the abdominal portion of the pectoralis major muscle (PM). The chondrocoracoideus coexisted with a contralateral atypical PM, partially blended with the clavicular fibers of the deltoid muscle. There was an accessory head of the biceps brachii while the palmaris longus was absent on the right side of a 78-year-old Greek male cadaver. **Conclusion**. The above mentioned muscular abnormalities are shown as disturbances of embryological pectoral muscle development, and their documentation is essential in order to increase awareness among clinicians of their potential impact on the diagnosis and treatment of several pathologies.

Introduction

The pectoralis major and minor muscles (PM and Pm) are liable to many slight transmutations and are associated with other muscles of the pectoral, axillary and arm region under variable forms. The PM, the largest and most superficial pectoral muscle, typically originates from the medial half of the clavicle, the anterior surface of the sternum, the 2nd to 6th costal cartilages and the external oblique muscle (EOM) aponeurosis, and inserts into the lateral lip of the intertubercular humeral sulcus (1). Atypical, supernumerary or accessory muscles originating from the anterior thoracic wall (sternalis, chondro or costoepitrochlearis, chondro or costohumeralis, pectoralis quartus and axillary arch) join various parts of the pectoral muscles with the medial intermuscular septum, the axillary or brachial fascia, the greater or lesser humeral tubercle, the medial humeral epichondyle, the coracoid process (CP), the shoulder joint capsule and the biceps brachii muscle (BB) (2, 3), via epigastric, muscular or tendinous slips (4-6). Rarely, a distinct portion of the inferior fibers of the PM forms a muscular slip and inserts into the CP, superficial to the coracobrachialis muscle (CB), medial to the short head of the BB and external to the Pm. This muscle is known as the chondro- or costocoracoideus muscle (of Wood) (6).

Pectoral muscle aberrations are usually asymptomatic and are discovered incidentally during surgery or dissection. Their ectopic insertions may provoke neurovascular entrapment, functionally limited abduction of the humerus, and cosmetic deformities (7, 8). They also implicate breast and axilla reconstruction. When they produce symptoms, they pose a surgical problem, as it is difficult to differentiate them from soft-tissue tumors (9).

The current report presents the occurrence of a very rarely reported unilateral muscular variant, the chondrocoracoideus muscle, situated on the left side, as an extension of the abdominal portion of the PM. Contralaterally, the muscle coexisted with an atypical PM, an accessory head of the BB, and with an absent palmaris longus. Concomitant neural alterations were also recorded. The detected muscular abnormalities are mentioned as disturbances of the pectoral muscles' development, and their documentation is essential in order to increase awareness among clinicians of their potential impact in the diagnosis and treatment of several pathological entities.

Case report

During dissection of the thoracic wall, axilla and arm of a 78-year-old Greek male cadaver (death from cardiac arrest), an accessory left-sided chondrocoracoideus muscle (of Wood) was detected, as an extension of the abdominal portion of the PM. On the ipsilateral side, the chondrocoracoideus coexisted with a CB of one head, arising from the medial border of the tendon of the short head of the BB. The musculocutaneous nerve did not pierce the CB, but it coursed medial to it (Figure 1C). On the contralateral side, an atypical PM coexisted with an accessory head of the BB, while the palmaris longus was absent. On both sides, the PM was divided into a clavicular and a sternocostal portion, with a prominent cleft between them (Figure 1A, and 1D). Both portions inserted via a superficial and a deep flat tendon on the crest of the greater humeral tubercle, the lateral lip of the intertubercular groove, to the point of deltoid muscle insertion. On the right side, the clavicular portion of the PM partially blended with the clavicular fibers of the deltoid muscle. No cephalic vein was found (Figure 1D). The abdominal portion of the right PM was fused to the sternocostal portion, while on the left side the abdominal portion of the PM was represented by the accessory chondrocoracoideus, emerging as three slips from the 6^{th}-8^{th} ribs and the EOM aponeurosis (Figure 1A, and 1B). At the level of the 5^{th} and 6^{th} ribs, the accessory muscle was fused to the sternocostal portion of the PM and inserted into the CP after its fusion with the tendon of the short head of the BB. The chondrocoracoideus was innervated by the medial pectoral nerve, and supplied by the lateral thoracic artery. The short head of the right BB originated together with the CB from the tip of the CP, the long head from the supraglenoid tubercle and the posterior part of the glenoid labrum, and the accessory head from the articular humeral capsule (Figure 1E). The accessory head of the BB joined the long head. On the left side, a communication between the musculocutaneous and the median nerve distal to the CB, and the common trunk of the medial brachial and the medial antebrachial cutaneous nerves, arising from the medial cord of the brachial plexus, were observed.

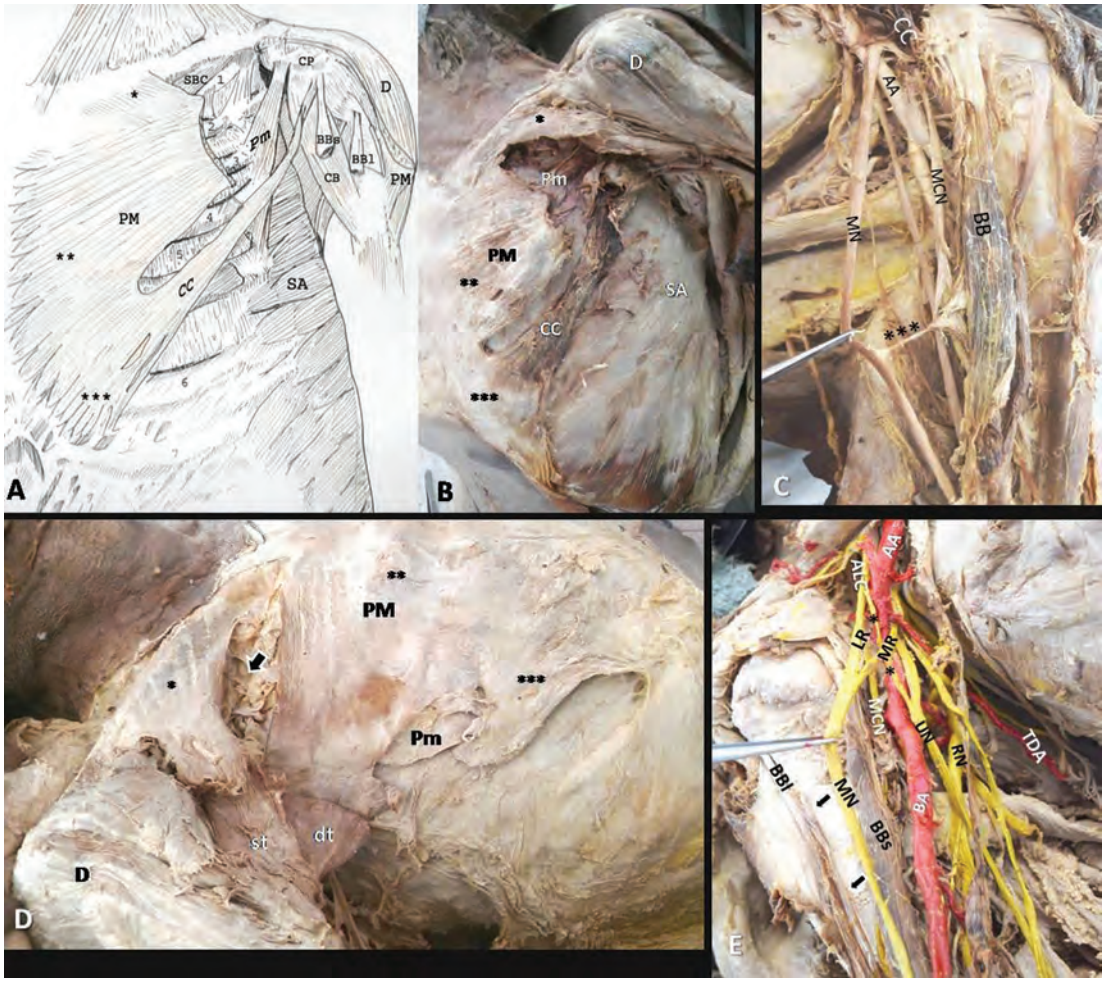

Figure 1 A. Schema and B. Photograph of the left axillopectoral area. The clavicular (*) and sternocostal portion (**) of the pectoralis major (PM). The abdominal portion (***) is an accessory pectoral muscle named the chondrocoracoideus muscle (CC) emerging by three slips from the 6th-8th ribs and the external oblique muscle aponeurosis, and inserted onto the coracoid process (CP) after its fusion with the tendon of the short head of the biceps brachii (BBs). C. Anastomosis (***) between the musculocutaneous (MCN) and median (MN) nerves distal to the coracobrachialis (CB). The MCN coursed medially to the CB. D. The right PM is divided into a clavicular (*), a sternocostal (**) and an abdominal (***) portion. Muscular fibers of the clavicular portion originated from the medial half of the clavicle and were separated from the sternocostal portion by a large interval (arrow). Both sternocostal and clavicular portions inserted via a superficial (st) and a flat tendon (dt) on the crest of the greater humeral tubercle. The abdominal portion was fused to the sternocostal portion. E. Anastomosis (**) between the anterior lateral cord (ALC) of the brachial plexus and the ulnar nerve (UN). BBl-long head of the biceps brachii, D-deltoid, Pm-pectoralis minor, SA-serratus anterior, SBC-subclavius muscle, RN-radial nerve, LR-lateral root, MR-medial root, AA-axillary artery, BA- brachial artery and black arrow- accessory head of the biceps brachii, TDA-thoracodorsal artery, BB-biceps brachii, AA- axillary artery.

Discussion

Pectoral muscles present a wide variability in type and number of attachments and supernumerary slips (1, 9), and may be accompanied by neural variations (10). They also fuse with other muscles of the adjacent (axillary shoulder and arm) area. The presence of aberrant muscular slips may affect the size and origin of the pectoral muscles (7). PM and Pm congenital malformations have an incidence ranging from 0.009% to

0.061% (11). Developmentally, the pectoral muscles derives from a primitive muscle (5th to 7th cervical myotomes) during the 5th fetal week. A combination of migration, fusion and apoptosis of myogenic cell precursors gives the muscles their final shape. Concerning the abdominal portion of the PM, it is considered to be a derivate of the "panniculus carnosus" in mammals (12). In higher primates and humans, the "panniculus carnosus" regresses during evolution in favor of wide upper limb mobility, and remnants are found as: i) extra muscular slips from the EOM aponeurosis attached to the serratus anterior fascia, ii) separate bundles arising below the PM and inserted at the axillary fascia, the pectoral humeral ridge, the fascia between the CB and Pm, or onto the CP, iii) slips from the latissimus dorsi (LD) border extended to the axillary or brachialis fascia, the pectoral region, the humerus, or the CP (1, 13). The chondrocoracoideus may also be considered as an atavistic anomaly, demonstrating the extent of PM insertion in ancestral forms. Concerning PM variants, the fusion of the PM with the deltoid muscle is the simplest deviation, while other unions with the rectus abdominis muscle, the BB, the LD (1), the contralateral PM (5) and the sternocleidomastoid muscle (as the rectus thoracis bifurcalis) (14) are more uncommon. A deltopectoral complex is created when the lateral border of the PM clavicular portion is totally fused with the medial border of the deltoid (15). In such cases, the cephalic vein is either aborted, or reaches the axillary vein. More rarely, the PM may be unilaterally or bilaterally partially (sternal segment or clavicular head) or totally absent (5). Alternatively, the PM may be divided into two slips, with the external slip coursing behind the long head of the BB and inserted into the lateral lip of the intertubercular sulcus, or may be inserted into the CP and CB fascia, or into the glenohumeral joint capsule via an accessory slip, the chondrocoracoideus muscle, so called by Wood and also described by Macalister and Perrin, dating back to the 19th century. The chondrocoracoideus is one of the rarest remnants of the panniculus (of skin-associated musculature), arising only on the left side from the 5th and 6th ribs and the EOM aponeurosis, coursing between the PM and LD, and separating the lateral from the posterior cord of the brachial plexus (1, 5, 6). In our case, the left chondrocoracoideus originated in three slips from the 6th -8th ribs and the EOM aponeurosis, and inserted with the Pm into the CP. Bannur et al. (9) reported a right-sided accessory muscle that originated from the 5th and 6th ribs at the costochondral junction, fused laterally with the Pm and inserted into the CP. It is unknown if the chondrocoracoideus is a developmental variant of the PM or the Pm, since Tountas and Bergman (1) described the aberrant muscle in Pm variations, probably following its insertion into the CP, while Testut (5) and Le Double (4) reported the muscle as a PM variation, considering its origin from the lower ribs and the EOM aponeurosis. In our opinion, the chondrocoracoideus is an extension of the abdominal PM portion. Other variable insertions of the PM include the articular humeral capsule, the medial humeral epicondyle (*chondro or costohumeralis muscle*), the internal brachial ligament or the brachial fascia (*chondrofascialis*) (5), the medial intermuscular septum, and the epitrochlea (*chondro or costo and sterno-costo-epitrochlearis*) via muscular, tendinous or epigastric slips (11).

The Pm may present variations at its costal origins (appearing as *pectoralis minimus,* extending from the 1st rib to the CP, as *sternochondrocoracoideus,* extending from the 3rd cartilage, the border of the sternum to the CP, and as *pectoralis intermedius,* arising from the 3rd and 4th ribs, between the PM and the Pm) and insertions (scapular, capsular, humeral and clavicular) (1, 5). The

Pm may blend with the PM, may be divided into two portions, or may be unilaterally or bilaterally absent (1).

PM anomalies are of paramount importance for interventional clinicians and plastic surgeons, in cases where the muscle is used as a graft during turnover flap removal, for coverage of life-threatening sternal wound infections after sternotomy, in order to avoid sternal osteomyelitis, mediastinitis and systemic sepsis development (16), as well as sternoclavicular joint septic arthritis (17). PM total or segmental flap removal may be used in local mediastinal wounds (18), and in head and neck surgery (19) for pharyngoesophageal and oromandibular reconstruction (20). PM deficits frequently coexist with Pm absence, as part of Poland's syndrome. These deficits make breast reconstruction and final symmetry extremely challenging (21). PM absence also precludes breast implant insertion, and may increase the risk of the direct spread of breast cancer into the thoracic cavity, worsening the disease prognosis (22, 23).

The chondrocoracoideus, similarly to the axillary arch, may have the potential to compress the axillary artery and the brachial plexus branches, thus mimicking clinical manifestations of the thoracic outlet syndrome (11). Pm anomalous insertions may cause (24) neurovascular obstruction due to the muscle's close proximity to axillary vessels and brachial plexus. Other clinical consequences include: impingement syndrome, superior labrum anterior to posterior lesions, shoulder pain, snapping sensation and clicking. An ectopic Pm tendon, inserting onto the supraspinatus muscle, may cause pain, stiffness and diminished range in external rotation (25). A Pm flap may be used in facial palsy, in breast, axilla (21, 22) and glenohumeral joint reconstruction (26). Knowledge of pectoral muscle variants allows surgeons to be better prepared when performing axillary lymphadenectomy. In such cases, the procedure is carried out with extreme difficulty due to the limited surgical field, and if surgeons navigate one level above the axillary vein, the neurovascular axillary bundle may be injured (11).

Conclusion

Accessory thoracic wall muscles present a wide variability, and may coexist with other muscular variations in the axillary, shoulder and arm area, within a disturbance in pectoral muscle development. Neural variations may also coexist. The described left chondrocoracoideus muscle is a very rare muscular variant with an uncertain clinical background. Documentation of such rare variants, with their embryological origin, is essential in order to increase awareness among clinicians of their potential impact in diagnosis of several pathologic entities, and of possible complications during surgical manipulation of the affected area. Knowledge of the variable anatomy of the axilla is necessary for surgeons who perform axillary lymphadenectomy and reconstruct the breast area, in order to achieve symmetry and a favorable cosmetic outcome. Cases of neurovascular disorders, limited abduction of the humerus and cosmetic deformities can be explained if these muscular aberrations are kept in mind.

What is already known on this topic

The pectoralis major and minor (PM and Pm) are associated with variable forms of muscles of the pectoral, axillary and arm region. Atypical, supernumerary or accessory muscles (sternalis, chondro or costoepitrochlearis, chondro or costohumeralis and axillary arch) are described in the literature. A rare muscle known as the chondro- or costocoracoid muscle was described by Wood, as a muscular formation originating from the inferior fibers of the PM and inserting into the coracoid process, superficial to the coracobrachialis muscle, medial to the short head of biceps brachii and external to the Pm.

What this study adds

The study adds important information regarding the descriptive anatomy of a very rarely reported unilateral chondrocoracoideus muscle (of Wood) situated on the left side, as an

extension of the abdominal portion of the PM. Additionally, it highlights the concomitant muscular (atypical contralateral PM, accessory head of contralateral biceps brachii and palmaris longus absence) and neural alterations. These muscular abnormalities are mentioned as disturbances of pectoral muscle development, and their documentation is essential in order to increase awareness among clinicians of their potential impact in the diagnosis and treatment of several pathologic entities.

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Case report

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Variable skeletal anatomical features of acromegaly in the skull and craniocervical junction

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Key words: Acromegaly ▪ Skull base ▪ Craniocervical junction ▪ Paranasal sinuses ▪ Wormian bones.

Objective. This study adds important information regarding the morphological alterations caused by growth hormone hypersecretion in the skull and craniocervical junction (CCJ). A variably asymmetric skull due to acromegaly coexists with expansion of the paranasal sinuses and multiple Wormian bones. **Case report.** A pathologically asymmetric dry skull of a European male, aged 38 years at death, with cranial vault and skull base thickening is described. The extensive paranasal sinus pneumatization caused a generalized thinning of the bony walls. The sphenoid sinus expanded intraorbitally, leading to sella enlargement. The orbital asymmetry coexisted with platybasia and hypoplasia of the occipital condyles and the odontoid process. Facial skeleton elongation and mandibular overgrowth were combined with prognathism, malocclusion and overbite. **Conclusion.** Skull and CCJ alterations are of paramount importance when selecting the surgical approach, if surgery is indicated. Consecutively, detailed preoperative evaluation and planning is essential. During surgery, skilled and experienced neurosurgeons recognize anatomical landmarks, use neuronavigation and micro-instrumentation in order to remain on the midline avoiding any potential lethal vascular injury.

Introduction

Acromegaly is a chronic endocrinopathy caused by growth hormone (GH) hypersecretion and stimulation of periosteal new bone formation, bone remodeling (1) and resorption (2). The subsequent articular chondrocyte replication and hyperfunction lead to cartilage thickening, joint widening and hypermobility (3). The periarticular structures begin to grow and synovial hypertrophy further exacerbates the abnormal mechanical loading of the joints. Disease progression ends with fibrocartilage calcification and osteophyte formation. In advanced cases, the articular cartilage thickening and the joint space narrowing share many features with osteoarthritis (2). Pathognomonic signs in the neurocranium include cranial vault thickening, frontal skull bossing, prominent supraorbital ridges and large external occipital protuberance. In the viscerocranium, nasal bone hypertrophy, maxillary widening, mandibular overgrowth and prognathism with malocclusion and overbite may occur (4).

The current report emphasizes the morphological and morphometric features of acromegaly in the skull and CCJ. These alterations are of special neurosurgical interest and demand a detailed preoperative evaluation and planning. During surgery, the use

of anatomical landmarks, neuronavigation and micro-instrumentation is necessary.

Case report

A pathologically asymmetric skull, with enlarged paranasal sinuses was found among 440 European adult human skulls from the osseous collections of the Department of Anatomy of the National and Kapodistrian University (Athens) and the Department of Anatomy and Surgical Anatomy of Aristotle University (Thessaloniki). The megalocephalic skull belonged to a European male aged 38 years at death, with a known history of acromegaly, according to his medical records. The male subject was a body donator, after giving written informed consent, before his death. The extensive paranasal sinuses pneumatization resulted in thinning of the bony walls, and thickening of the cranial vault and skull base. The anterior cranial fossa was shallower than usual due to the considerable expansion of the frontal sinus. The most impressive intracranial finding was the excessive enlargement of the sella turcica (anteroposterior diameter 33.8 mm) and its porosity due to a pituitary adenoma, and the consequent extreme sphenoid sinus expansion. The deep and wide pituitary fossa was perforated by numerous minute apertures. The thickness and density of the outer and inner tables of the skull were decreased due to the over-pneumatization and expansion of the frontal sinus. Remarkable posterior cranial fossa side asymmetry was easily identified (Figure 1A). The massive periorbital expansion of the paranasal sinuses formed asymmetric orbital cavities and particularly pronounced supraorbital ridges bilaterally, associated with facial skeleton elongation. The maxillary sinuses extended to the palate and the alveolar process (Figure 1B). Fifty-two Wormian bones (WBs) had developed from extra ossification centers within the cranium, 34 of them extracranially, 12 intracranially and 6 intraorbitally (Table 1).

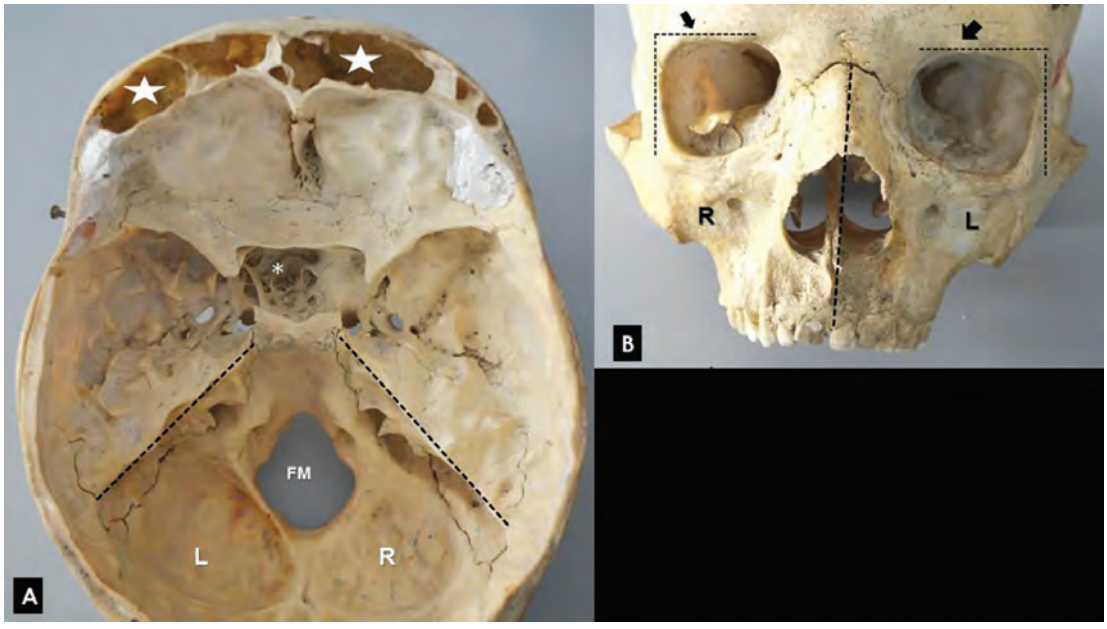

Figure 1A. Posterior cranial fossa side asymmetry indicated by the black dotted lines on the petrous bone crest. The asterisk (*) indicates sella turcica enlargement and the double asterisks show extensive frontal sinus pneumatization, FM – foramen magnum, L-left side and R-right side. B. Black arrows indicate the particularly pronounced supraorbital ridges. Orbital cavity asymmetry is indicated by the dotted vertical lines.

Table 1 Topographical distribution and frequency of Wormian bones according to side

Wormian bones' positions	Side			Number
	Right	Left	Bilaterally	
Extracranially				
Lambdoid suture	-	-	11	11
Coronal suture	-	-	12	12
Metopic suture	-	-	1	1
Zygomatomaxillary suture	2	-	-	2
Sphenofrontal suture	2	-	-	2
Frontozygomatic suture	1	-	-	1
Parietomastoid suture	1	-	-	1
Occipitomastoid suture	1	1	-	2
Squamosal suture	-	1	-	1
Pterion	-	1	-	1
Intraorbitally				
Frontal bone	-	1	-	1
Sphenofrontal suture	5	-	-	5
Intracranially				
Asterion	-	1	-	1
Sphenoid bone	-		8	8
Frontal bone	-	2	-	2
Coronal suture	1		-	1
Total	13	7	32	52

Skull base flattening (platybasia) was detected, taking into consideration the increased basal angle (159°) formed between the clival plane and the sphenoid bone (normal range 121-148°) (2). The wider basal angle in the current case confirms the basilar impression. The foramen magnum (FM), irregular in shape, was surrounded by abnormal protuberances of spongy bone around the right and left occipital condyles (OCs) and the posterior rim of the FM. The mastoid processes were large and bulky and the external occipital protuberance was extremely prominent (bathrocephaly skull) (Figure 2A). Marked side asymmetry was observed in the middle and posterior cranial fossae. The posterior fossa was filled with dental impression (Figure 2B) which was later bisected along the midline and an additional 0.5 cm thick slice was removed from both sides. This way the depth was measured bilaterally taking into consideration the most anterior, middle and posterior points of the FM rim, known as the prosthion, FM middle and opisthion respectively (5). The relative values were 37.98, 37.01 and 38.91 mm on the left side and 32.87, 32.72 and 32.94 mm on the right side (Figures 2C, D).

The dominance off the left hemicranium was confirmed. The length and width of the right OC were 29.07 mm and 21.88 mm, while those of the left were 28.25 mm and 22.35 mm, respectively. The OCs were hypoplastic and completely flattened. New bone formation along the OC margin and the superior articular facets of the atlas was detected. Changes of the normal contour were accompanied by widening and flattening of the articular facets. An increased concavity existed on the right inferior ar-

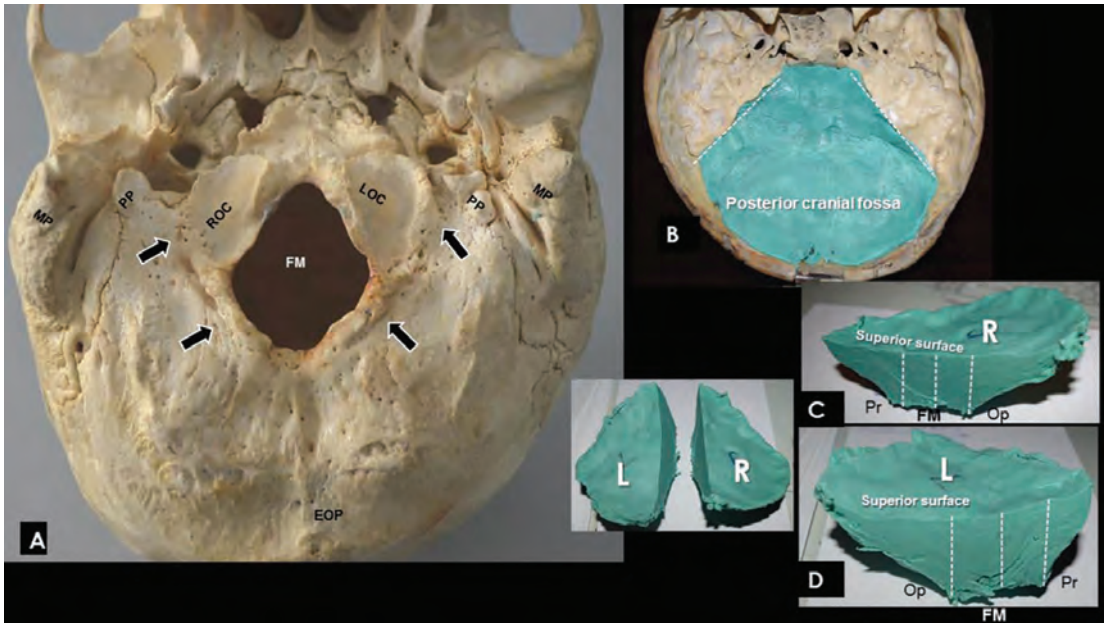

Figure 2A Spongy bone around the right and left occipital condyles (ROC and LOC) and the posterior foramen magnum (FM) rim (arrows). Extremely prominent mastoid processes (MP) and external occipital protuberance (EOP), PP- paracondylar process. B. Posterior fossa filled with dental impression. C, D Left and right half of the impression. Depth measurements at prosthion-Pr, opisthion-Op and the midline of the FM to the superior surface of the impression.

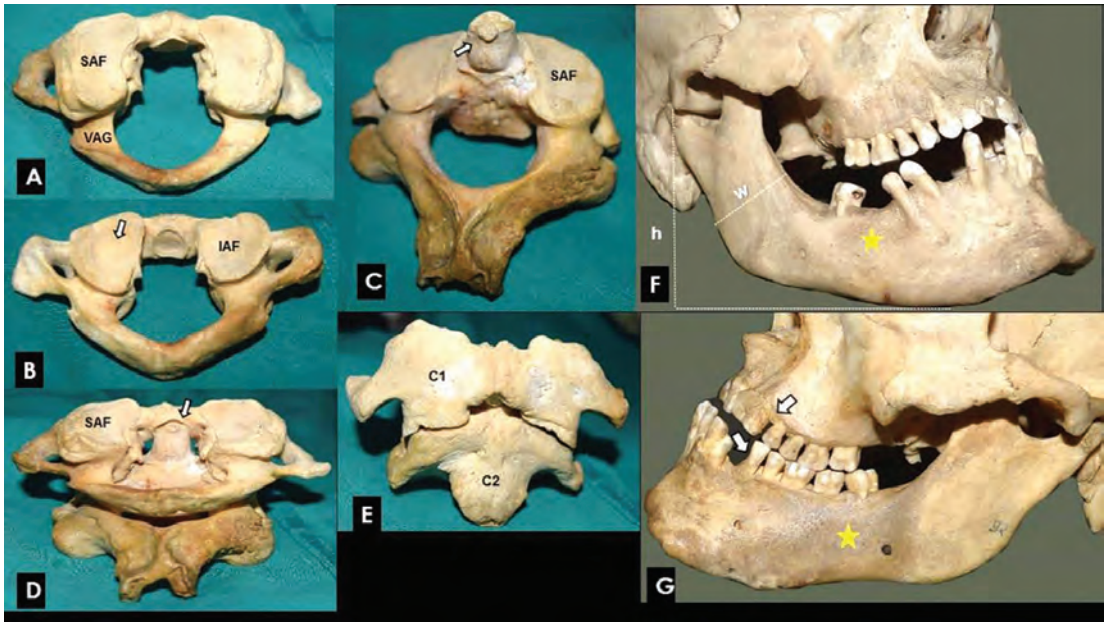

Figure 3A The superior articular facets of the atlas (SAF) and vertebral artery groove (VAG). B. The asymmetric inferior articular facets of the atlas (IAF), arrow indicates right more concave SAF. C. Hypoplastic odontoid process of the axis (arrow), osteophyte formation and asymmetric SAF bilaterally. D. E. The atlanto-axial joint, C_1- atlas, and C_2- axis vertebra. Axis vertebral body overgrowth. F. Increased mandibular rami vertical diameter (height-h) and anteroposterior diameter (width-w). G. Periodontal disease inflammation (yellow asterisk) and mandibular incisors enamel hypoplasia (white arrows).

ticular facets of the atlas (Figures 3A, B). The atypical OCs, in combination with the odontoid process hypoplasia and its slight inclination (Figures 3C, D) were responsible for the atlanto-occipital and atlantoaxial instability (5, 6). The gap present between the OCs and the superior articular facets of the atlas indicates atlanto-occipital dislocation. Both atlanto-occipital and atlantoaxial dislocations were associated with a right-sided torticollis.

The mandible was large and elongated, and the dental arches yielded wider interdental spaces. Symphyseal height was markedly increased. The mandibular rami showed a significantly greater vertical diameter (58.40 mm on the right and 59.14 mm on the left) in comparison to the anteroposterior one (41.73 mm on the right and 42.25 mm on the left). Both mandibular condyles were unusually wide. Mandibular overgrowth coexisted with prognathism, malocclusion and overbite. Temporomandibular joint showed arthritic lipping. Mandible bears 14 teeth; no dental occlusion existed in the right dental hemiarch, while traces of abrasion existed in the left hemiarch due to teeth contact. Enamel hypoplasia was present at the mandibular incisors (Figures 3F, G). All measurements were performed by a single investigator by using a digital sliding caliper (accuracy 0.01 mm) and they were taken twice and the average of the two values was taken as the final measurement.

Discussion

Acromegaly affects both genders equally and occurs with an incidence ranging from 5 to 15 cases per million (7, 8). The true prevalence is higher due to under diagnosis. The excessive skeleton growth depends on the age the disorder becomes active. If the onset of the disease begins early in life (9), as reported in the current case, the ensuing growth is extreme. A benign pituitary adenoma may cause GH hypersecretion in 90% of cases, while in the remaining 10%, tumors of the pancreas, lungs or adrenal glands may produce and release GH (10).

Changes in soft tissue and facial bones related to acromegaly have been extensively published and present with a plethora of symptoms. Hydrocephalus may develop due to the pressure exerted on the cerebral aqueduct and the third ventricle. In the reported case, extensive alterations were found in the neurocranium and viscerocranium, as a consequence of the endochondral and periosteal bone growth. We report, for the first time, the coexistence of acromegaly with CCJ deformities, paranasal sinuses expansion and WBs development. The presence of WBs correlates with metabolic bone disease, GH deficiency, connective tissue disorders, skull deformities, bone softening, aplastic sinuses and platybasia (7, 11, 12). Moreover, cartilage overgrowth may provoke mechanical injuries to the TMJ. Apart from the extreme skull and CCJ skeletal alterations, the abnormal growth of the upper and lower extremities in our individual strongly indicates acromegaly. Other clinical manifestations include: arthritis, osteopenia, vertebral fractures, carpal tunnel syndrome, hyperhidrosis, headache, paresthesia, sexual dysfunction, goiter, visual field defects, cardiomyopathy, arrhythmias, valvulopathy, heart failure, insulin resistance, diabetes, hypogonadism, colon anomalies and visceromegaly. Macroglossia, laryngeal and pharyngeal mucosa hypertrophies may lead to upper airway obstruction, hypoventilation, snoring and sleep apnea, which have been reported in 50% of acromegaly patients (7). Unfortunately, no medical records were available about the clinical manifestations of acromegaly in our subject. Regarding differential diagnosis, several syndromes are associated with a skeletal overgrowth, including Sotos, Beckwith-Wiedemann, Weaver, Simpson-Golabi- Behmel, Fragile X, Mc-

Cune Albright, Marfan, Klinefelter, Beals, and Lujan-Fryns syndromes, homocystinuria and pseudoacromegaly. Classic acromegaly is difficult to differentiate from cases combined with McCune-Albright syndrome and fibrous dysplasia (12), or complicated cases of fibrous dysplasia associated with myxomas, such as Mazabraud's syndrome (13).

The reported case focuses on a pathological skull and CCJ aberrations caused by acromegaly. It is important to recognize these alterations, combined with paranasal sinus enlargement, preoperatively. Variants of the frontal sinuses and their expansion into the orbit, optic canal and clinoid process may play a role in the correlation between the frontal sinusitis and orbital complications. Moreover, known frontal sinus over-pneumatization may influence the treatment plan and radiation dose when treating malignancies in the area, to prevent severe complications, such as cerebrospinal fluid fistula and secondary infections (14). In particular the expansion of the sphenoid sinus towards the sella turcica may lead to compression of the optic chiasm. Pituitary adenoma resection is technically challenging due to the anatomic inaccessibility of the pituitary gland and the proximity of the adjacent vital anatomical structures. Therefore, it is of immense significance to know the exact extent of the sphenoid sinus in order preoperatively to locate the exact position of the internal carotid arteries (ICAs), and the limits of the narrow intercarotid space which ultimately makes the trans-sphenoidal approach impossible. Moreover, coexisting ICA abnormalities (elongation, tortuosity, and especially dilatation) warrant particular intraoperative attention. Various complications may occur, including ICA perforation and laceration (0.2-0.4%), iatrogenic formation of pseudoaneurysms, caroticocavernous fistulae, life threatening post-operative hemorrhage, and vasospasm, causing cerebral ischemia (11). CCJ degenerative changes may cause instability, chronic subluxation and focal atrophy of the upper cervical cord due to the repetitive compression. The OC hypoplasia observed in the current case, as a consequence of atlantoaxial rotation, may lead to transient compression of the vertebral artery, secondary to posterior subluxation of the occiput (15). Hypoplastic OCs, in combination with the odontoid process hypoplasia and its slight inclination are often associated with paramedian type basilar invagination and medullary compression (15, 16).

Conclusions

The paper reports a case of acromegaly, focusing on the coexistence of the variable and asymmetric skeletal background of the skull and craniocervical area, combined with extensive paranasal sinus pneumatization, significant enlargement of the sella and multiple WBs development. Skull base flattening caused an increased basal angle leading to basilar impression. The atypical OCs, in combination with the odontoid process hypoplasia and its slight inclination, were responsible for atlanto-occipital and atlantoaxial instability. The gap between the OCs and the superior articular facets of the atlas indicate atlanto-occipital dislocation. Both the atlanto-occipital and the atlantoaxial dislocations were associated with torticollis. Skull bone alterations are of paramount importance in choosing the preferred surgical approach. Consecutively, detailed preoperative evaluation and planning, the use of anatomical andmarks, neuronavigation and micro-instrumentation may help skilled and experienced neurosurgeons to remain on the midline and avoid potential vascular injury.

What is already known on this topic

Acromegaly is a chronic endocrinopathy caused by growth hormone hypersecretion and stimulation of periosteal new bone formation and bone remodeling. Bone resorption is also stimulated. The consequent articular chondrocyte replication

and hyperfunction lead to cartilage thickening yielding joint widening and hypermobility. Pathognomonic features in the neurocranium include cranial vault thickening, frontal skull bossing, prominent supraorbital ridges and a large external occipital protuberance. In the viscerocranium, nasal bone hypertrophy, maxillary widening, mandibular overgrowth and prognathism, with malocclusion and overbite, may occur.

What this study adds

The study adds important information about the morphometric alterations in a megalocephalic skull and its craniocervical area in a case of acromegaly. The coexistence of an extensive pneumatization of the paranasal sinuses with significant enlargement of the sella and multiple WBs development are highlighted. Skull base flattening (platybasia) led to an increased basal angle leading to basilar impression. The atypical OCs in combination with the odontoid process hypoplasia and its slight inclination were responsible for atlanto-occipital and atlantoaxial instability. Atlanto-axial dislocation existed due to the gap between the OCs and the superior articular facets of the atlas. Both atlanto-occipital and atlantoaxial dislocations were associated with torticollis. Skull bone alterations are of paramount importance in choosing the appropriate surgical approach. Consecutively, detailed preoperative evaluation and planning, the use of anatomical landmarks, neuronavigation and micro-instrumentation may help skilled and experienced neurosurgeons to remain on the midline avoiding potential vascular injury.

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Severe vertebral erosion by chronic contained rupture of an abdominal aortic aneurysm

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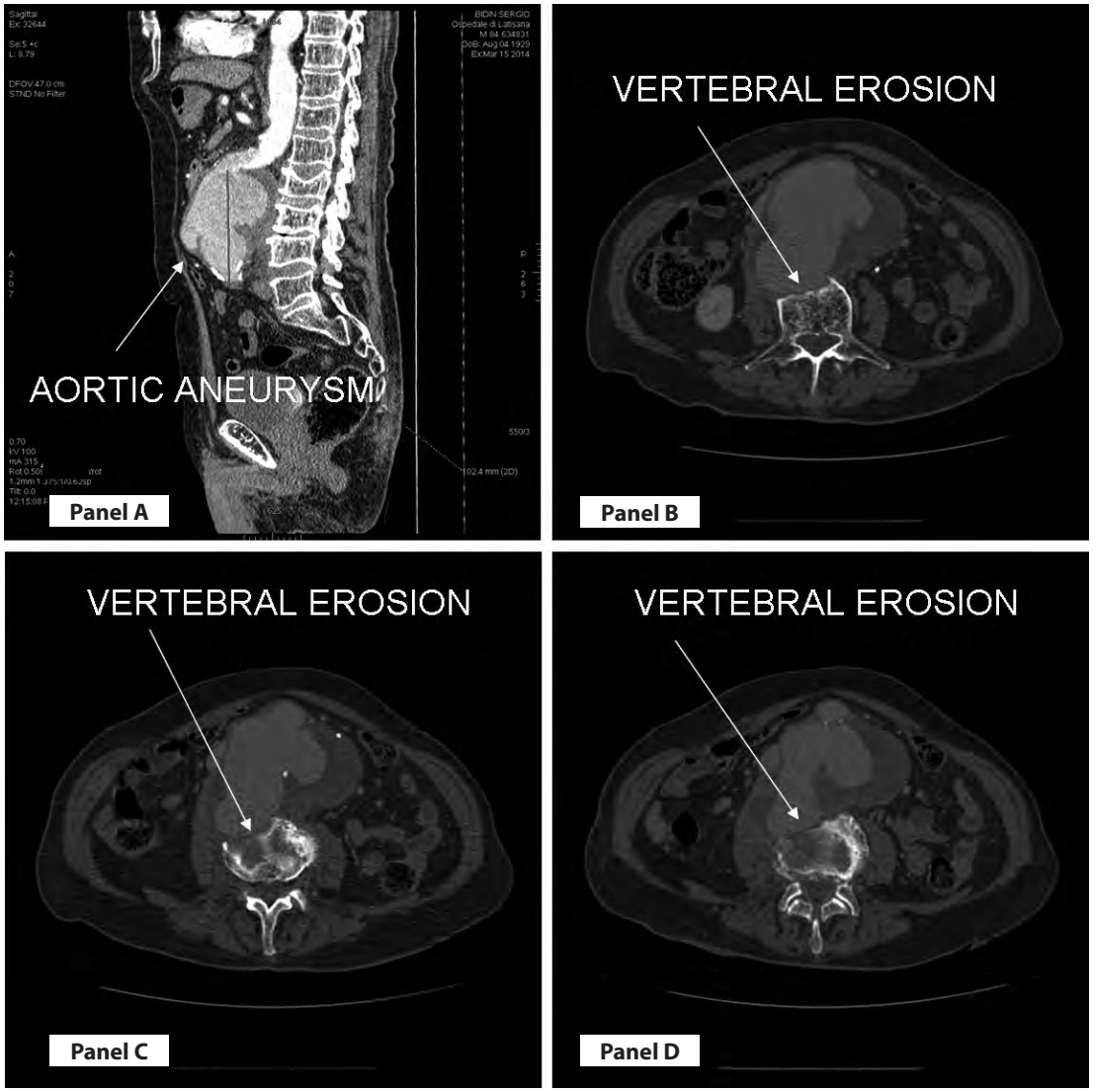

An 85-year-old man, with a history of smoking and hypertension, was admitted to the emergency room for severe back pain. The back pain had been progressive and disabling over the preceding 6 months. Moving his spine was very painful, and abdominal examination revealed a pulsatile, abdominal mass. Ultrasonography of the abdomen showed a giant aortic aneurysm. Abdominal computed tomography (CT) with intravenous administration of contrast revealed a chronic contained rupture of an abdominal aortic aneurysm (Panel A). Chronic contained rupture was suggested by the non-circular shape of the aneurysm in the transverse CT planes (Panel B, C, D), and by the presence of calcifications in the middle of the "aneurysm" (Panel C). Multiple severe vertebral erosions were detected in the anterior part of the lumbar vertebrae (Panel B, C, D). The patient rejected any hypothesis of treatment. It is well known that aortic aneurysm should be included in the differential diagnosis of chronic back pain. The great majority of chronic low back pain is related to vertebral degenerative pathology, but other possible reasons, even unusual, have to been considered, such as abdominal pathology (pancreatic for instance) and aortic abdominal aneurysms, with or without chronic contained rupture. Vertebral body erosion due to a primary chronic ruptured aortic aneurysm is an uncommon but important cause of low back pain. Vertebral erosion happens in approximately 3% of cases (1). A contained rupture of an abdominal aortic aneurysm is established progressively by haematoma expansion on the posterior aneurysm wall that triggers vertebral erosion. The specific mechanism for the vertebral erosion is not clear, and there are several hypotheses implicating arterial pulse, aneurysm or haematoma infection, inflammatory processes, or simply an unspecific reason (2, 3). Open or endovascular surgery and subsequent lumbar arthrodesis are generally performed in these cases.

Key words: Abdominal aortic aneurysm ▪ Vertebral erosion.

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Spontaneous closure of a full thickness macular hole

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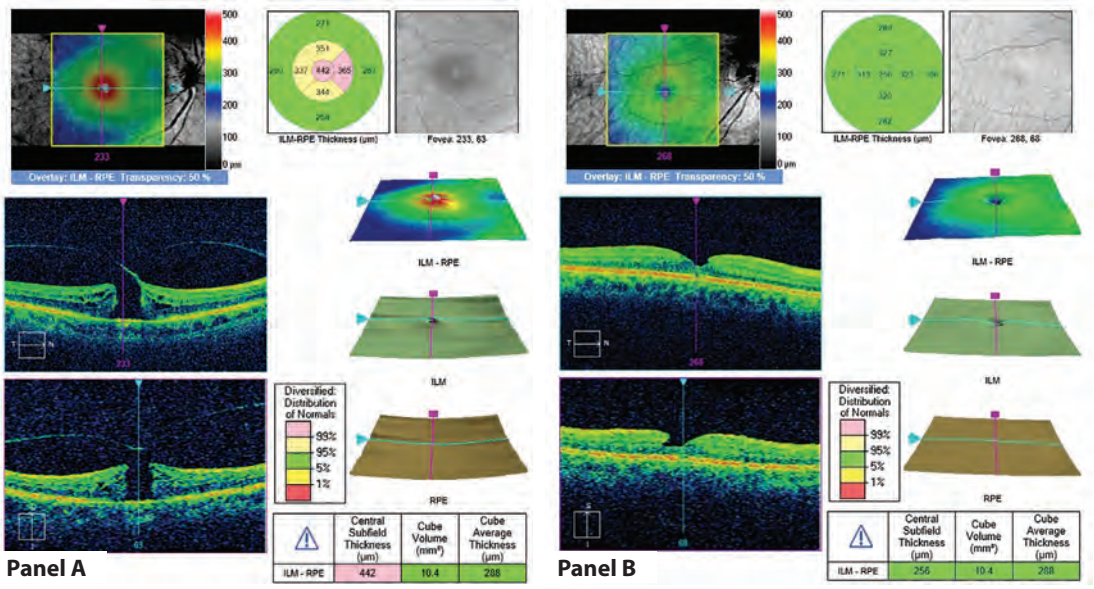

A primary full-thickness macular hole (FTMH) is defined as an idiopathic disruption of all the retinal layers in the fovea (1). Optical coherence tomography (OCT) enables better understanding of various macular disorders, and provides new FTMH classification based on two OCT features: the size, and the presence of vitreomacular traction (VMT) (2). The presence of VMT can be a significant prognostic factor, since FTMH, with the presence of VMT, tends to be smaller in diameter (1). Treatment options include: observation, pars plana vitrectomy and intravitreal ocriplasmin application (1, 2). A 64-year old man was referred with low vision in his right eye, noted five days earlier. Clinical examination revealed visual acuity (VA) of 20/80 in the right eye and 20/20 in the left. Anterior segment examination of both eyes was unremarkable. The measured intraocular pressure was 15 mmHg bilaterally. Fundus examination of the right eye revealed the presence of FTMH, while the left eye was normal. Optical coher-

ence tomography showed FTMH of 213μm diameter and significant VMT in the right eye, and a normal OCT finding in the left (Panel A). The patient was scheduled for surgical treatment. Fifty-six days after the initial examination, and twelve days before the scheduled surgery, the patient noted significant spontaneous visual improvement. A control examination revealed VA of 20/20 in both eyes. Fundus examination of the right eye presented complete vitreous detachment (PVD) and a small residual foveal depression, while the left eye remained normal. Control OCT examination showed complete spontaneous closure of the FTMH, with a small residual superficial defect and without VMT (Panel B). At the six-month follow up examination, he presented with preserved VA and stable OCT findings. The presence of PVD is reported to be related to the increased size of the FTMH (1). However, a small early FTMH has the capacity to repair itself after complete VMT separation via proliferation of retinal glia cells (2). Spontaneous FTMH closure has previously been reported in up to 3.5% of cases (3). Therefore, it is important to undertake a detailed preoperative examination in all patients with FTMH, even with persistent VMT.

Key words: Vitreomacular traction syndrome ▪ OCT ▪ Vitreoretinal surgery.

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

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Intussuscepted Meckel's diverticulum within its own lumen

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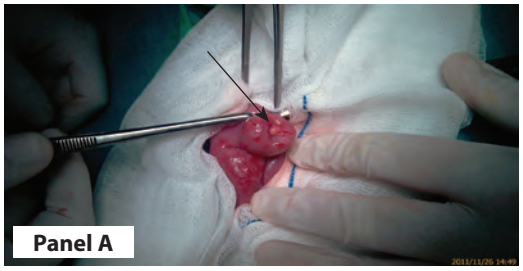
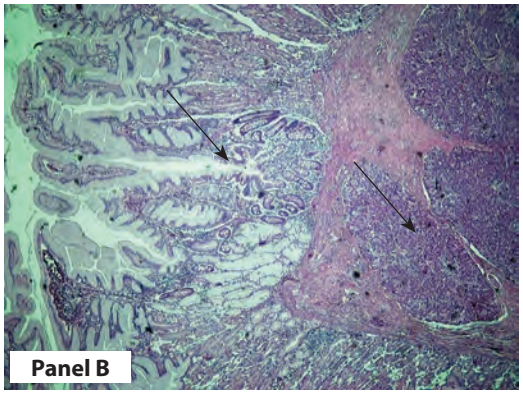

Panel A


Panel B

A 8-year old boy with negative medical history presented to the Emergency Department (ED) complaining of colicky, abdominal pain over two days. This pain progressed to being generalized in nature, while a bloody, mucoid stool was noted prior to ED presentation. Physical examination revealed tenderness and mild rigidity in the right abdominal wall. Laboratory tests were indicative of inflammation. Abdominal x-ray findings were unremarkable. Performance of ultrasonography excluded the presence of pathology of the intra-abdominal solid organs, the presence of free fluid within the peritoneal cavity, or enlarged intra-abdominal lymph nodes, whereas the typical target (or doughnut) and pseudokidney signs for intussusception were absent.

The patient underwent urgent exploratory laparotomy, after a Lanz incision. The appendix was not inflamed, but preventive appendectomy was performed. Further, a thorough investigation of the peritoneal cavity followed. Seventy centimeters from the ileocecal valve, a Meckel's diverticulum was found intussuscepted within its own lumen (Panel A).

Intraoperatively we observed the hemorrhagic infiltration of its wall, along with the presence of ectopic tissue (Panel A, black arrow). We then performed excision of the Meckel's diverticulum. The postoperative course was uneventful and the patient was discharged home in good general condition on the second postoperative day. Histological examination (Panel B, Hematoxylin eosin stain, X40) revealed that the Meckel's diverticulum was lined with gastric and in-

testinal epithelium (red arrow), while inside the muscular layer, islands of ectopic pancreatic tissue were observed (black arrow). The wide base or the small dimensions of the diverticulum have been reported as precipitating factors for the development of intussusception of Meckel's diverticulum within its lumen, but these were not observed in the present case (1, 2). Histological findings indicative for the presence of an active ulcer at the luminal orifice on the antimesenteric bowel wall could constitute a predisposing factor for intussuscepted Meckel's diverticulum (1, 3).

Key words: Meckel's diverticulum ▪ Intussusception ▪ Lumen ▪ Inflammation.

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Healing invisible wounds – have we done enough to help the victims of wartime rape?

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

The Balkan wars had dramatic consequences for all the republics of the former Yugoslavia, and more specifically Bosnia and Herzegovina (BH), where the most hostile action and violent fighting took place. Over 20,000 girls, women and men were raped (1).

Raped females were often separated from the children they gave birth to, because many were detained for a long time after multiple rape (2). The rape resulted in physical injuries, psychological turmoil, and significant impairment in general functioning. Faced with difficulties in accessing appropriate health care, social stigma and the slow pace of justice, women victims were not offered adequate and effective psychological assistance, or legal, economic and social protection. When the victims of rape, both male and female, talk about what happened, they often feel rejection and condemnation instead of support. The same victims often feel guilty and choose to remain silent and carry their own pain. There are only a few who deal with or who are even slightly interested in how the victims of rape live today (1, 2).

In the research we undertook to assess the prevalence and gender characteristics of the psychological health problems of individuals raped during the BH war, sixteen years after the war ended, we assessed 95 (81 females) Bosniac (Muslim) war survivors who experienced rape as a tool of genocide. In terms of the DSM-IV criteria, raped women were significantly more often anxious than men, and raped men consumed alcohol significantly more often than women, without significant gender differences between them in the prevalence of PTSD, depression, somatic symptoms, anxiety and insomnia, social dysfunction, severe depression and smoking cigarettes (3).

Today's public dialogue is aimed at drawing attention to women who are marginalized in order to help them. They are there but others do not see them. Sweeping these problems under the carpet results in far greater trauma, both for these people and society (1, 2). One important aspect of the Islamic tradition for Bosniac woman, in both their spiritual and everyday life in the family, in the relationship with their fathers, mothers, husbands, children and the

broader family, is shame. Shame, which is highly important, is considered to be "half the faith" in the Islamic tradition. If somebody wants to destroy an individual, it is necessary to do something violently to them against that person's will.

In his book "Healing invisible wounds: paths to hope and recovery in a violent world" Richard Mollica (4) emphasized that in most societies, institutional religious structures have difficulty helping women who have been sexually violated, both because of the theological limits on women's role in the religion and because of conservative teachings on sexuality and sexual purity. But he quoted the example of the Dervish, Ahmed Nurudin Mešić (5), a highly regarded 90-year old cleric who was a judge under Sharia law – the Islamic law based on the Qur'an and the traditions of the Prophet Muhammad. In March 1993 he issued a decree that Bosnian Muslim women who had been sexually abused should be given the status of martyrs. In his fatwa he declared that, as a human being and as a scholar, he needed to implement the truth and restore those women to their rightful place within society: "Is there anyone who can say that these women are guilty and sinful because of what they have been through? Thus they may be guilty for some other reason but for this – no way. Therefore, no one should talk about them like that. We as Muslims, and especially those closest to them, should accept them as heroines, as martyrs, and support them both morally and materially. We recommend especially to men, the husbands of the women who experienced this tragedy, to be sufficiently strong and to embrace their wives, in both a literal and figurative sense. Thus they will show that they really sympathize with their wives' pain and are willing to make it easier for them to endure (5)." The healing of Bosnian women thus found expression through a wise old cleric. This is not always the case however. Ordinarily the traumatized person is challenged to establish a more direct line to God without the help of religious institutions or clergy (4).

This should be talked about constantly because this is a crime, not the woman's attitude, a woman is not guilty of being raped. It is a very complex trauma, very few women have come to our clinic looking for help; most of them have left the country and most have remained silent. It is certain that the public health system of Bosnia-Herzegovina never expected, before the war, to have to deal with such a large number of consequences of the war, on such a vast scale. The increasing shortage of resources and the lack of a multi-sectorial integrative approach, as well as the lack of cultural and gender sensitivity, all contribute to the "conspiracy of silence" even 20 years later, and need to be discussed.

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Response to whether the definition of the term "children born of war" and vulnerabilities of children from recent conflict and post-conflict settings should be broadened

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Key words: Children born of war ▪ Post-conflict settings ▪ Bosnia and Herzegovina.

Dear Editor,

I am writing in response to the Letter to the editor submitted by Amra Delić et al. to Acta Medica Academica (1). The letter proposes that the current definition of "children born of war" be broadened to include children from "various homogenous and heterogeneous non-integrated post-conflict communities" on the basis of the authors' hypothesis that other groups of children from recent conflict and post-conflict settings are comparable with respect to certain "core psychosocial issues". Based on my own ongoing work in the area of mothering children born of war rape in Bosnia and Herzegovina, I have good reasons to assume that there is truth to this hypothesis. Therefore, I would like to suggest that broadening the definition would be advantageous from a theoretical as well as a strategic perspective. I would also like to point out that there are further issues to consider with regard to how this new definition is formulated.

Issues related to children born of war on the territories of the former Yugoslavia, as well as in other post-conflict settings, remain under-researched and understudied due to poorly-kept records and continued social stigma associated with the children and their mothers. From the point of view of human rights law, it has been argued that even the 1989 Convention on the Rights of the Child, and the Fourth Geneva Convention and Its Additional Protocols, whose goal is to protect children as a vulnerable population in general as well as to provide specific treatment for war-affected children, "might be said to be inapplicable" when it comes to the collective and individual protection of those affected, since they do "not outlaw discrimination against children born out of wedlock as a social category" (2). So, much more work is needed — as Delić et al. (1) rightly observe--to begin to adequately study children born of war, with the goal of providing effective collective and individual interventions, rehabilitation, empowerment, and social integration.

In a theoretical and academic sense, having a good working definition of the con-

cept "children born of war" would be a good starting point from which to begin to more broadly theorize issues relating to this population. If it is to be relevant and applicable to various contexts, this theorizing needs to be done in a multi-disciplinary and interdisciplinary manner in order to make evident the links among conceptual, psycho-social, cultural, economic, political, and legal frameworks pertaining to children born of war in homogenous and heterogenous global contexts. An encompassing working definition is a solid basis for sustained, informed and systematic exploration and analysis of multitude of factors that affect all those groups that may be considered children born of war from a variety of disciplinary perspectives. Establishing the points of comparison and commonality among those groups would then provide a solid starting point from which to examine, explore and analyze in greater detail and within diverse local contexts the divergence among the groups, and the establishment of their unique analytical features.

There is also a strategic benefit to adopting a broad definition of the category of children born of war in terms of an advocacy agenda as it relates to peace-building efforts, development of shared historical narratives and delivery of support programs in post-conflict societies. A significant problem facing present-day Bosnia and Herzegovina, for example, is the "widespread marginalization and highly selective reading of Tribunal verdicts and of the related historical record by ethno-national historians" (3). Conflicting historical interpretations are particularly detrimental to the daily lives of children born of war who continue to be subjected to forms of prejudice and discrimination and who are politically marginalized. Starting to build a body of knowledge relating to a broad category of children born of war will provide local and global institutions with the language and conceptual apparatus necessary to produce historical syntheses of past conflicts, offering a more encompassing alternative to nationalist histories, and building a more unified civil framework for understanding the past. This approach would assist with the development of a "shared narrative concept as a tool of rebuilding trust between communities and engaging the past" (3), and a peace-building process in which children born of war are well positioned to take an active part by virtue of their "mixed" identity. The benefits of developing a shared narrative would also be seen in the context of educational policy and the potential adoption of common textbooks.

A related issue is the work of advocacy networks and civil society groups that is for the most part still sectarian in nature. This is true in Bosnia and Herzegovina, but also in other recent post-conflict societies. In Bosnia, due to the lack of uniform legislation, many organizations have articulated an operative framework based on ethnic affiliation, rather than on survivors as individual citizens. In human rights literature it has been acknowledged that significant disagreement and animosity may exist among different advocacy groups who either oppose one another's causes or how those causes are framed (4). Having access to literature that draws upon a broader definition of children born of war as a population that is affected by conflict in particularly adverse ways would help civil society groups in Bosnia and Herzegovina whose efforts have been focused on bridging ethnic divides. The strategic deployment of that definition in the context of their own advocacy work would provide them with a balanced shared narrative that stresses the commonalities among affected groups. This approach would in turn assist with the development of "multisectoral collaboration and interorganisational partnerships based on mutual respect" (5).

If, however, the definition of children born of war is to be broadened so as to ac-

quire foundational theoretical and practical value in the future study of the category "children born of war," it would need to be further refined and articulated in a way that gestures toward completeness. In this case it would mean formulating it in such a way that the definition can apply to the same groups in any recent conflict setting, and any post-conflict society. While the Bosnian context does contain its own specific features and specific local factors that impact in unique ways on the lives of those we may refer to as children born of war, research shows that many other issues, such as those relating to the psychology of war-related trauma and resilience, ethnicity and human rights, the politics of identity and gender and social justice can be studied effectively as possessing certain common attributes across various global locations. **Conclusion**. So while the broadening of the definition as suggested by Delic et.al. (1) is advantageous from a theoretical and a strategic perspective, the definition itself would need to be formulated in an even broader way, so as not to make reference to any specific geographical context, but to encompass the broad category of children born of war wherever that population is found. This reformulation would ensure that the definition is applicable to the category of children born of war in any global context, including Bosnia and Herzegovina.

Conflict of interest: The author declares that she has no conflict of interest.

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Reflections on the definition and categorization of "Children Born of War"

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In their "letter to the editor" in issue 46 (1) Amra Delić, Philipp Kuwert, and Heide Glaesmer (1) address whether the term "Children Born of War" should be broadened to include also other groups of children. As examples they address various groups of children in Bosnia and Herzegovina, such as children whose mothers were victims of international trafficking, children whose parents belong to opposing sides in the fratricidal war that took place in 1993-1995 in the Autonomous Province of Western Bosnia, and finally children of inter-ethnic marriages. They argue that these children also have severe psycho-social issues and should also be included in the definition of "Children Born of War".

The challenges of defining new research fields include the establishment of definitions and concepts, categories, measurement instruments etc. which can be applied across all research disciplines, where the research field is of relevance. Furthermore, when the topic has a comparative and longitudinal aspect this needs to be taken into account as well. The research field of "Children Born of War" has evolved over a time period of more than ten years (2) and some of the basic questions have been:

- Which concept can be used to describe this particular group of children born to local mothers and foreign soldiers in conflict and post-conflict situations? The concept "Children Born of War" was considered to be the most appropriate one.
- Which categories of "Children Born of War" can be identified? Based on the empirical evidence available, four categories of children were suggested by Mochmann (3), i.e. children fathered by enemy soldiers, by occupation soldiers, by peacekeeping soldiers and children of female child soldiers whose fathers were members of e.g. rebel groups.

These categories may not be exhaustive and many of the groups of children, that may fall into one and the same category, can seem quite different. Furthermore, concepts and categories need to be adjusted to the changing patterns of warfare. This process

is under constant revision and has recently been adjusted to cover the consequences of modern warfare. Along these lines, Delić, Kuwert, and Glaesmer suggest broadening the definition by referring to specific groups in Bosnia and Herzegovina. On the basis of the rather limited presentation of these groups of children, it is difficult to provide a clear answer to their question whether an extension of the definition may be useful. Before attempting do so, I would nevertheless like to point out two aspects which have guided our research on "Children Born of War" so far (2):

- "Children Born of War" is a group at risk because its rights are challenged in multiple dimensions, such as the medical, political/juridical, socio-economic and psychological aspects of life. Many groups of children are exposed to risks in one or several of these dimensions, but empirical evidence on "Children Born of War" indicates that this group is exposed to most of the risks simultaneously and over a longer period of time, sometimes even their whole lives.
- This exposure to risk is closely related to the (former) military position of their biological father, and the (enemy) perception of him in the family, community and society.

The focus of Delić, Kuwert, and Glaesmer (1) was mostly on psychosocial issues. From the disciplinary focus of psychiatry and psychology, it may seem beneficial to broaden the definition to include several other groups of children that have similar "*psychosocial issues*". However, this ignores the fact that the original definition of "Children Born of War" addresses very complex and multifaceted dimensions. It is the combination of all those issues that impacts the lives of these children in very specific ways. Having said this, it seems as if some of the groups addressed by the authors fit into the already existing definition and categories applied in the research field of "Children Born of War".

Conclusion

From a methodological perspective, concepts, definitions and categories always face the challenge of being *too narrow* or *too broad*. This is also the case with "Children Born of War" and it is important to continuously probe its validity and reliability, keeping in mind the aim it is intended to serve. As we move on with expanding the evidence on "Children Born of War" the use of control groups to test discipline specific hypotheses is becoming increasingly necessary and relevant. At present my suggestion to the authors Delić, Kuwert, and Glaesmer (1) is to analyze more thoroughly whether the suggested groups may already fit into the existing definition and categories, or whether they may serve as control groups in an analysis of the impact of psycho-social issues.

Conflict of interest: The author declares that she has no conflict of interest.

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by Nerma Tanović

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More than six authors:

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Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin

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No author given:

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

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Dorland's illustrated medical dictionary. 29th ed. Philadelphia: W.B. Saunders; 2000. Filamin; p. 675.

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