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AIMS AND SCOPE

Acta Medica Academica is a biannual, peer-reviewed journal that publishes: (1) reports of original research, (2) original clinical observations accompanied by analysis and discussion, (3) analysis of philosophical, ethical, or social aspects of the health profession or biomedical sciences, (4) critical reviews, (5) statistical compilations, (6) descriptions of evaluation of methods or procedures, (7) case reports, and (8) images in clinical medicine. The fields covered include basic biomedical research, clinical and laboratory medicine, veterinary medicine, clinical research, epidemiology, phramacology, public health, oral health, and medical information.

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The Cover Picture: Franjo Likar (1928-), "Female bust", 1988, installation. Courtesy of Art Gallery of Bosnia and Herzegovina, Sarajevo.

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Periodontal disease and dental caries from Krapina Neanderthal to contemporary man – skeletal studies

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Objective. The aim of this study was the quantification of alveolar bone resorption as well as the number and percentage of teeth with dental caries. **Materials and Methods**. Four samples of jaws and single teeth were studied from four time periods, i.e. from the Krapina Neanderthals (KN) who reportedly lived over 130,000 years ago, and groups of humans from the 1st, 10th and 20th centuries. Resorption of the alveolar bone of the jaws was quantified by the tooth-cervical-height (TCH) index. Diagnosis of dental caries was made by inspection and with a dental probe. TCH-index was calculated for a total of 1097 teeth from 135 jaws. Decay was calculated for a total of 3579 teeth. **Results.** Resorptive changes of the alveolar bone in KN and 1st century man were more pronounced on the vestibular surface than interdentally ($p<0.05$), while no significant difference could be confirmed for 10th and 20th century man ($p=0.1$). The number (percentage) of decayed teeth was 0 (0%, n=281 teeth) in KN, 15 (1.7%; n=860 teeth) in 1st century, 24 (3.4%; n=697 teeth) in 10th century, and 207 (11.9%, n=1741 teeth) in 20th century. **Conclusion**. On the basis of our results it may be postulated that in contemporary man in relation to KN, the accumulation of plaque pathogens in the interdental space is substantially greater than on the vestibular side. These findings have practical, educational and preventive value for oral hygiene improvement, especially of the interdental space, which should help decrease the prevalence of periodontal disease and dental caries, and improve oral as well as general health.

Key words: Periodontium, Alveolar resorption, Neanderthal, TCH-index, Dental caries.

Introduction

The hominids are zoological groups who had some characteristics of Homo sapiens. Hillson (1) describes the family Hominidae (Table 1).

Paleoanthropological and archeological materials have been frequently used in medicine, not only because they can help us understand a biomedical problem of human nature, but also because they are convenient for investigation. Paleoanthropology and archeology are fields of high interest for many branches of science, dentistry being no exception, in fact dentistry has special interest in mutual collaboration. A general survey

Table 1 The family Hominidae (1)

Species	Sites	Stratigraphic division	Date ranges
Australopithecines			
Ardipithecus ramidus	East Africa	Pliocene	c. 4.4 Ma BP
Australopithecus anamensis	East Africa	Pliocene	4.2-3.9 Ma BP
Australopithecus afarensis	East Africa	Pliocene	3.75-2.8 Ma BP
Australopithecus africanus	South Africa	Pliocene	3-2.5 Ma BP
Paranthropus robustus	South Africa	Lower Pleistocene	1.8-1.5 Ma BP
Paranthropus boisei	East Africa	Pliocene, Lower Pleistocene	c. 2.6-1.2 Ma BP
Hominines			
Homo habilis	Africa (+ ?)	Pliocene, Lower Pleistocene	2.2-1.6 Ma BP
Homo erectus	Asia (+ Africa earlier dates)	Middle Pleistocene	700-125 ka BP (1.9 Ma BPm 1.6 Ma BP)
Homo sapiens (archaic)	Africa + Europe	Middle Pleistocene	700-125 ka BP
Homo sapiens (Neanderthal)	Europe + West Asia	Upper Pleistocene	100-35 ka BP
Homo sapiens (anatomically modern)	Worldwide	Upper Pleistocene + Holocene	90 ka BP, 50 ka BP - present

BP - years before present; Ma - millions of years; ka - thousands of years; Holocene - 10 ka BP – present; Pleistocene - 2 Ma-10ka BP; Pliocene - 5,1 Ma-2 Ma BP.

of dental variability in human groups would be incomplete without some consideration of pathologic differences. These may reflect variations in the genetic, dietary, bacteriologic and physiologic aspects of man's development.

Two major problems in dentistry, i.e. periodontal disease and dental caries (decay), can be thoroughly examined by use of paleoanthropological and archeological materials. There are more literature data on decay than on periodontal disease in the past in the human race (2, 3). This is so because caries lesions have been preserved on skeletons from the very beginning of their occurrence, whereas the only evidence for periodontal disease is when the pathologic events involve the calcified periodontal structure (alveolar bone and cementum).

In this study there were two aims: First, the loss of alveolar bone was assessed by the tooth-cervical height index (TCH-index) on the vestibular and interdental side of the alveolus (4) in Krapina Neanderthals (KN) who lived 130,000 years ago (5), and in groups of contemporary humans from the 1st, 10th and 20th centuries, and second, the number and percentage of caries lesions on all samples of teeth in these four groups was diagnosed.

Material and methods

For alveolar bone resorption, all samples of jaws, except for KN, were subdivided into two age groups: 20-29 and >50 years. This grouping was not applicable in the KN group, as none of KN individuals was older than 23 years at the time of death.

Group 1 - KN

The alveolar bone included 54 KN teeth, i.e. 10 teeth from three maxillae and 44 teeth from six mandibles. Examples of a KN maxilla and a KN mandible are presented in Figures 1 and 2.

The age of the KNs whose jaws were examined was reported to be 14 – 23 years. Caries lesions included 281 teeth, 91 belong-

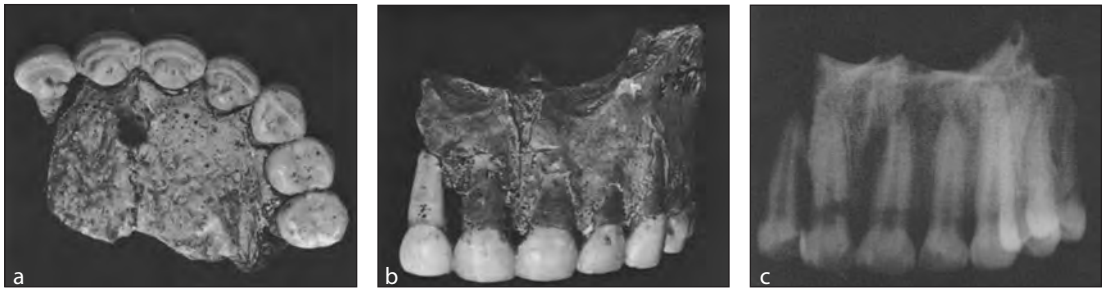

Figure 1 Maxilla of Krapina Neanderthal (6, 7): a - occlusal aspect; b – vestibular aspect; c – radiographic image.

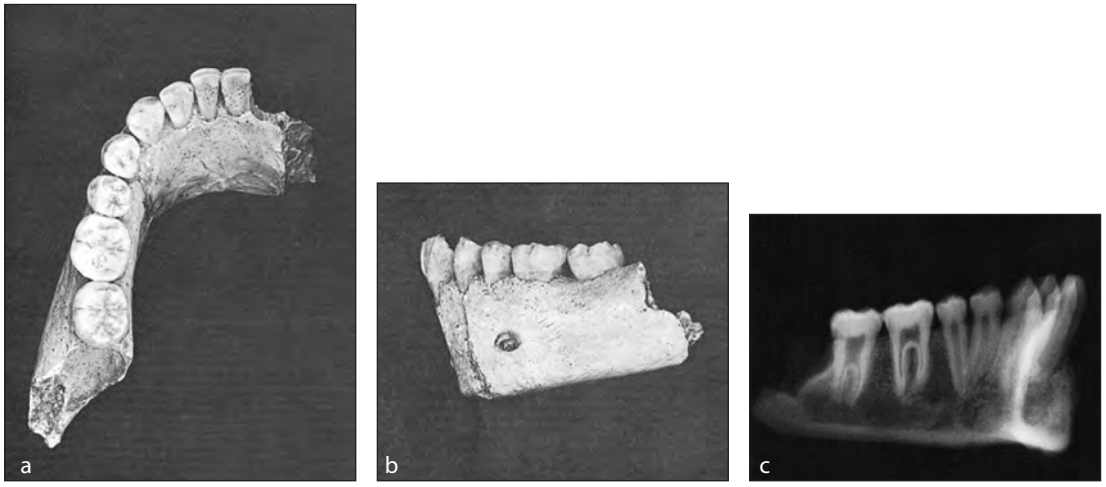

Figure 2 Mandible of Krapina Neanderthal No. 55 (6, 7): a – occlusal aspect; b - vestibular aspect; c – radiographic image.

ing to the jaws, and 190 teeth were outside the jaws, as a single tooth. The age of the KNs whose teeth were checked was 3 – 27 years. These jaws and teeth are in the possession of the Natural History Museum in Zagreb, Croatia.

Group 2 – Contemporary humans from the 1st century

The alveolar bones included teeth belonging to human skulls from the 1st century, divided into two subgroups according to age:

- 20-29 age subgroup of 14 skulls with 28 jaws and 295 teeth, and
- >50 age subgroup of 8 skulls with 16 jaws and 94 teeth.

Caries lesions included 860 teeth which belonged to the 1st century jaws.

Group 3 – Contemporary humans from the 10th century

The alveolar bones included teeth belonging to human skulls of the 10th century, also divided into subgroups according to age:

- 20-29 age subgroup of 8 skulls with 16 jaws and 135 teeth, and
- >50 age subgroup of 6 skulls with 12 jaws and 60 teeth.

Caries lesions included 697 teeth belonging to the 10th century jaws.

The skulls from the 1st and 10th century belong to the National Museum in Sarajevo, Bosnia and Herzegovina.

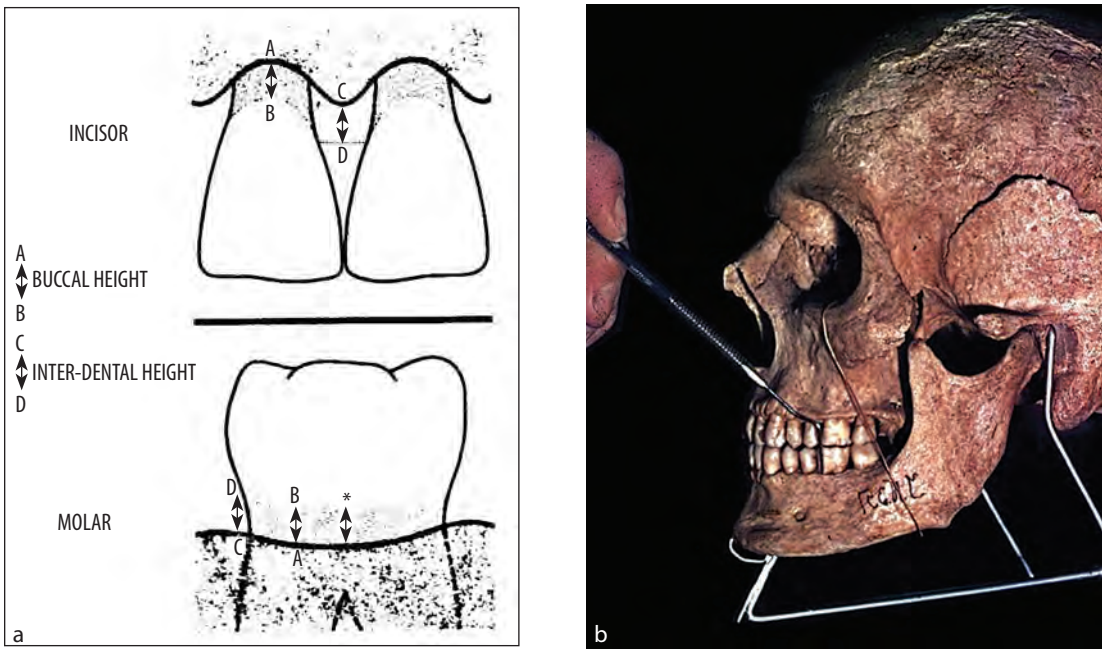

Figure 3 Measurement of Tooth Cervical Height (TCH-index). (a) Vestibular TCH – mean distance between A (at alveolar crest) and B (at cementoenamel junction) per all teeth in the jaw; interdental TCH – mean distance between C (at alveolar crest) and D (at cementoenamel junction) per all teeth in the jaw (figure from Davies et al. (4). Buccal height – vestibular height. (b) Measuring alveolar bone resorption with periodontal probe.

Group 4 – Contemporary humans from the 20th century

The alveolar bones included teeth belonging to human skulls from the 20th century, divided into two subgroups:

- 20-29 age subgroup of 13 skulls with 26 jaws and 281 teeth, and
- >50 age subgroup of 18 skulls with 36 jaws and 178 teeth.

Caries lesions included 1741 teeth belonging to the 20th century jaws.

The skulls from the 20th century belong to the Institute of Anatomy, School of Medicine, University of Sarajevo, Bosnia and Herzegovina. The age of the skulls from the 1st and 10th centuries was determined by the Vallois method (8). This method is based on measurements of the obliteration of the suture skulls. The resorption of the alveolar bone was measured by the TCH-index according to Davies et al. (4), using a compass and a periodontal probe. TCH-index was defined as the mean distance from the cementoenamel junction to the alveolar crest measured per all teeth in the jaw (Figure 3). The measurement was done on two sides, vestibular and interdental and expressed in millimeters, with a precision of 0.5 mm. Diagnosis of caries lesion was done by inspection and with a dental probe by a single evaluator.

Statistical analysis

Data are presented as mean and standard deviation (SD) and as proportions if they are nominal. Comparisons between mean vestibular and interdental TCH-index were done using paired t-tests, while comparisons of the mean TCH-index between study groups were done using unpaired t-tests or one-way analysis of variance. The relation between the mean vestibular and interdental TCH-index was expressed as the V/I or I/V ratio and its 95% confidence interval (CI).

For dental caries, differences between observed and expected frequencies in the four study groups were analysed by the χ^2 test, followed by post-hoc comparisons with the Keppel modification of the Bonferroni correction of type I error. The level of significance was defined as $p<0.05$. Statistical analysis was performed using the R language for statistical computing (9).

Results

The results for alveolar bone resorption assessed as mean TCH-index are given in Table 2.

Comparison of the mean TCH-index between the vestibular and interdental tooth sides shows that there is a significantly greater resorption of alveolar bone vestibularly than interdentally in the KN group (p=0.001) and also in the 1st century group (p=0.001 or p=0.05). On the other hand, no statistically significant difference could be confirmed between the vestibular TCH-index and the interdental TCH-index in contemporary man from the 10th and 20th century groups (p=0.1). Results expressed as a V/I ratio show that in the subgroup of >50 year old contemporary man of the 20th century the interdental resorption is almost equal to the vestibular resorption (V/I=1.06, 95% CI: 1.03, 1.10). Changes in interdental resorption increase in contemporary man in the subgroups of >50 years old ($p<0.001$), and its increase from KN to 20th century is almost two-fold (1.94, $p<0.001$).

Interdental and vestibular values of TCH-index for KN were taken as reference values and expressed as 100% (Table 3).

For the other three study groups, the interdental and vestibular TCH-index was calculated in respect to the KN reference and expressed as a percentage. Dynamics of interdental resorption was already higher in the subgroup of >50 years old from the 10th century (123.3%), while in the subgroup of >50 years old from the 20th century the interdental resorption is almost twice as high

Table 2 Mean TCH–index and vestibular-interdental ratio (V/I) values according to the study group (KN, 1st, 10th and 20th century)

Study group	No. of jaws	No. of teeth	Vestibular TCH, mean (SD)	Interdental TCH, mean (SD)	p-value[1]	V/I ratio (95% CI)
KN (14-23 years)						
Maxilla	3	10	8.15 (1.36)	2.10 (0.22)	0.01	–
Mandible	6	44	5.33 (0.46)	2.68 (0.21)	0.001	–
Total	9	54	6.12 (0.44)	2.57 (0.17)	0.001	2.38 (2.32, 2.45)
1st century						
20-29 years	28	295	2.80 (0.29)	1.79 (0.10)	0.001	1.56 (1.54, 1.59)
>50 years	16	94	4.36 (1.17)	2.34 (0.22)	0.05	1.86 (1.76, 1.97)
10th century						
20-29 years	12	135 60	2.50 (0.28)	1.82 (0.38)	0.1	1.37 (1.32, 1.43)
>50 years	8		3.78 (0.32)	3.17 (0.44)	0.1	1.19 (1.14, 1.24)
20th century						
20-29 years	26	281	3.67 (0.17)	2.99 (0.17)	0.1	1.23 (1.22, 1.24)
>50 years	36	178	5.31 (0.95)	4.99 (0.73)	0.1	1.06 (1.03, 1.10)

[1]p-values derived from a paired t-test comparing vestibular and interdental TCH-index. CI – confidence interval; KN - Krapina Neanderthals; SD - standard deviation; TCH - tooth-cervical height index.

Table 3 Percentual TCH–index and interdental-vestibular ratio (I/V) values according to the study group (KN, 1st, 10th and 20th century)

Study group	Age subgroup	Interdental TCH, (%)	Vestibular TCH, (%)	I/V ratio (95% CI)
KN	14-23	100.0	100.0	1.00
1st century	20-29	69.6	45.8	1.52 (1.51, 1.53)
	>50	91.1	71.2	1.28 (1.24, 1.32)
10th century	20-29	70.8	40.8	1.74 (1.71, 1.75)
	>50	123.3	61.8	2.00 (1.98, 2.02)
20th century	20-29	116.3	60.0	1.94 (1.93, 1.95)
	>50	194.2	86.8	2.24 (2.23, 2.25)

CI – confidence interval; KN - Krapina Neanderthals; TCH - tooth-cervical height index.

Table 4 Dental caries according to the study group (KN, 1st, 10th and 20th century), for all age groups together

Study group	Number of teeth examined	Number (%) of teeth with caries
KN	281	0 (0.0)
1st century	860	15 (1.7)
10th century	697	24 (3.4)
20th century	1741	207 (11.9)

KN - Krapina Neanderthals

(194.2%) in respect to the KN, whereas vestibular resorption in contemporary humans did not reach the resorption values of KN. Results for caries lesions are shown in Table 4.

The proportion of decay varied significantly between the study groups ($p<0.001$). In teeth from the 1st century, the proportion of caries lesions was significantly higher than in KN teeth (1.7% vs. 0%, $p=0.029$), while the highest proportion of decay was observed in teeth from the 20th century (11.9% vs. 0%, $p<0.001$).

Discussion

The first skeletal remains, after which the Neanderthal hominid was named, were found in 1856 in Neanderthal, a valley in Germany. In 1899, the first remains of Neanderthal hominids were found by Dragutin Kramberger-Gorjanović in Krapina, Croatia. Krapina is a town in the north-west Croatia, some 60 km from Zagreb. Most recent studies on tooth enamel (electron spin resonance) ESR showed that KN lived 130,000 years ago (5). The Krapina diluvium collection contains about 900 bone remains of KN. A very important segment of this collection are the remains of 281 teeth (190 single teeth and 91 teeth still attached to the alveolar bone). These measurements were made on 54 attached teeth aged ≥14 years where the alveolar bones were not destroyed post mortem.

All skeletons were aged between 3 and 27 years. Alveolar resorption was examined on the jaws belonging to the 14-23 years group. This is why the groups from the 1st, 10th and 20th century are divided into two age subgroups of 20-29 and >50 years each. Considering the KN life expectancy of 23 years, it may have been more prudent to compare KN with the >50 age group of contemporary man. The samples of jaws from KN, 1st and 10th centuries were accidental rather than representative or stratified. All skulls from the 1st, 10th and 20th centuries had both jaws well preserved.

The results of the TCH-index for the 1st, 10th and 20th century humans showed the alveolar resorption to be greater on the interdental septum and smaller on the vestibular side, as compared with KN. The vestibular to interdental resorption ratio expressed as a factor ranged from 2.38 in KN to 1.23 in the 20-29 year group or 1.06 in the age group of >50 years in the 20th century jaws (Table 2). If the interdental vs. vestibular values are expressed as 100%, then the interdental to vestibular (I/V) factor KN expressed as a factor is 1.00. The factor was 1.52, 1.74 and 1.94 for the 20-29 year group, and 1.28, 2.00 and 2.24 for the >50 year group from the 1st, 10th and 20th centuries, respectively (Table 3).

What do these results imply? Without the presence of the gingiva, it is possible to determine to the nearest millimeter the amount of bone loss from the cementoenamel junction tothe alveolar crest much more easily than it is possible in patients. Various methods have been proposed for recording the amount of alveolar bone loss in skeletal material by measuring the distance from the cementoenamel junction to the alveolar crest for each tooth and averaging them for the arch, arch quadrant, or individual (4, 10, 11).

The loss of alveolar bone by resorption is the critical event in the pathogenesis of periodontal disease. Bone resorption is a complex process that is morphologically manifested by erosion of the bone surface (Howship's lacunes) and large multinuclear cells, osteoclasts. Another mechanism of bone resorption is the formation of an acidosis environment, leading to dissolution of the bone constituent minerals. The mediators of bone resorption include prostaglandins, osteoclast activating factor (OAF), lipopolysaccharides (LPS), complement system, interleukins (IL-1, IL-3, IL-6), parathormone (PTH), macrophage colony stimulating factor (M-CSF), tumor necrosis factor alpha (TNF-alpha), tumor necrosis factor beta (TNF-beta) and vitamin D-3 (12).

Severson et al. (13) demonstrated on human autopsy material that the cell count in the osteogenic part of the periodontium decreased with age. Sarajlić et al. (14) showed that the process of alveolar resorption on the labial aspect of anterior monoradicular 845 teeth from 198 male bodies aged 23-69 years at death, increased with age.

We did not study the morphological relationship of proximal areas in any of the four groups. The results confirmed the well-known opinion on plaque retention to be greater in the interdental space than on the vestibular side

Löe et al. (15) proved that plaque was the initial etiopathological agent for the onset of gingivitis, as known from Müller's theory of decay.

Mandel (16) divides the history of periodontology into a number of eras:

- era of calculus (from Hippocrates to 1955.) and after
- era of plaque
- era of host response
- era of bacterial specificity
- era of host – bacterium interaction
- era of transition
- era of regulation and
- era of genetics.

There are several hypotheses on the role of plaque in the etiology of gingivitis:

- non-specific plaque hypothesis
- specific plaque hypothesis and
- opportunistic plaque hypothesis.

Plaque quantification according to teeth area was investigation by Lang (17). He found the highest plaque accumulation on the distal side of molars, followed by premolars and canine, and least on incisors. The lower distal surfaces of the teeth were more severely involved than the upper ones.

Plaqueology is a new field of dentistry, established in 1969, dealing with the origin,

development, structure, bacteriology, biochemistry, immunology, control and prevention of plaque (18). During evolution, the composition of plaque and its quantity have been changing, particularly in the interdental area, as also demonstrated in the present study. The recognition of the increasing rate of interdental alveolar bone resorption during the evolution of man has a practical, educational as well as preventive value for oral hygiene improvement and in the struggle against periodontal disease and decay. In 1998, a symposium on interdental space hygiene was held in Florence, Italy (17, 19–23). The papers presented were focused on the role of patient cooperation, including knowledge, skills, willingness, information – cognitive, instruction – psychomotor, motivation – affective, methods and techniques.

The method of the TCH index (4) used in the study is based on linear measurements taken from a fixed point of the cementoenamel junction to the alveolar bone margin, and this measurement was equated with inflammatory bone loss (10, 24–27). The method should be additionally controlled in groups or individuals with severe occlusal attrition, as compensatory tooth eruption has been shown to have likely occurred in such a condition (28, 29–32). This subsequent tooth eruption appears to be compensation for the lost tooth height and an attempt to maintain vertical dimension of the masticator apparatus. Attrition was not examined in the present study, so it could not be related to compensatory tooth eruption and root exposure.

In the 20-29 year groups, the values of the TCH-index on the vestibular side were slightly lower in the 10th than in the 1st century sample. The difference was not statistically significant (Table 2). The values of TCH-index obtained in the 20th century sample were significantly higher than those recorded in the 1st and 10th century samples, indicating that periodontal disease and dental caries have an increasing trend in modern man.

The results of this study for teeth caries are shown in Table 4. Decay was not diagnosed on KN teeth whereas on teeth samples from the 1st, 10th and 20th centuries it is

Table 5 Dental caries in some earlier human population (adults only) (33)

Time	Series	Author	Number of teeth examined	Number (%) of teeth with caries
70.,000-35,000 B.C.	European Neanderthal	Brothwell	259	0 (0.0)
35,000-10,000 B.C	M. Carmel (Skhul)	Brothwell	523	5 (1.0)
3,000-1,000 B.C.	French Neolitic	Hartweg (1945)	11717	379 (3.2)
	German Neolitic	Brinch (1949)	1589	27 (1.8)
	Swedish Neolitic	Holmer (1956)	6402	91 (1.4)
	Danish Neolitic	Pedersen (1939)	3612	56 (1.2)
	British Neolitic	Brothwell (1962)	1151	36 (3.1)
	Predinastic Egyptian	Brothwell (1963)	1742	40 (2.3)
	China	Mao Yen (1959)	884	38 (4.3)
3,000-1,000 B.C.	Total:		27879	674 (2.4)

B.C. – Before Christ.

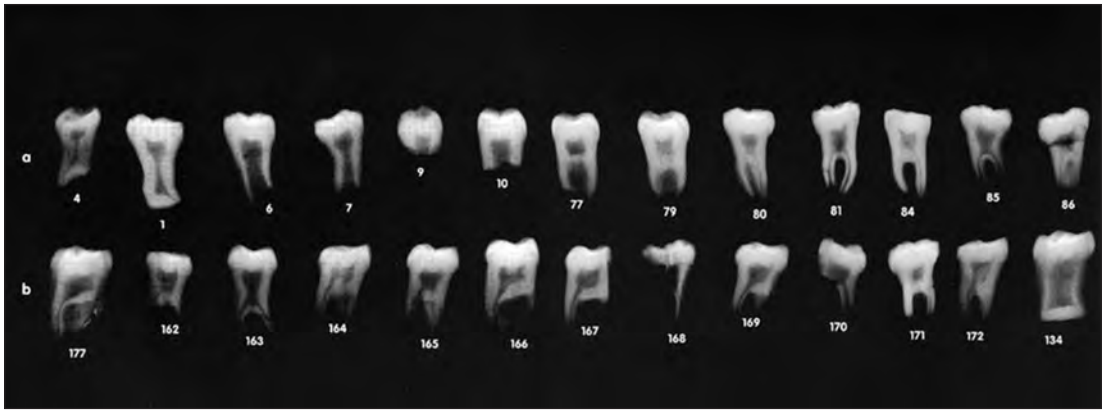

Figure 4 Hypertaurodontism permanent molar teeth of Krapina Neanderthals (7).

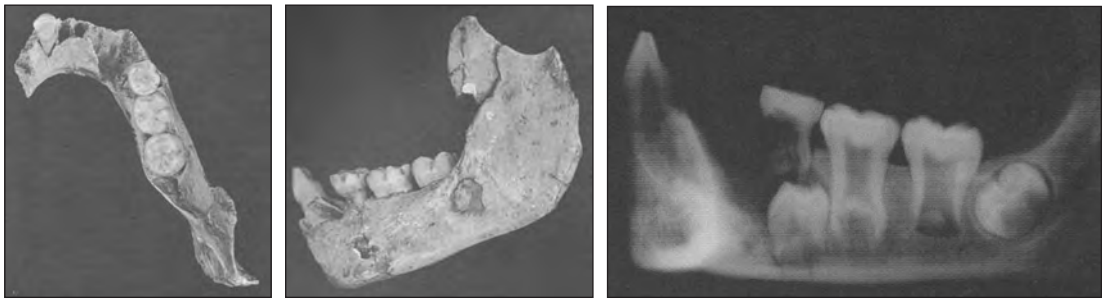

Figure 5 Mandible, age 10-11 years, position crown wisdom tooth (6, 7).

present in 1.74%, 3.44% and 11.89% respectively. When these results for KN and persons from the 1st century are compared to the results of Brothwell (33), they show very good complementarity (Table 5).

In Brothwell there was no caries diagnosed on 259 teeth of European Neanderthals, just as there was no caries diagnosed on the 281 teeth of the KN. Brothwell indicates the life expectancy of European Neanderthal 70,000 – 35,000 years B.C. His results (33) were published in 1963. At that time life expectancy of KN was determined by radioactive carbon (C-15) and it was considered that KN lived earlier than 40,000 – 30,000 years B.C. but the newer method ESR from 1995, showed that KN lived 130,000 years B.C. (5).

The frequency and distribution of caries in the mediaeval population (34), from the 10th to 11th century in Croatia on a sample of 979 teeth, showed recorded caries as interproximal 3.9% (76 teeth), buccal/lingual 1.3% (25 teeth), and on the occlusal surfaces of a sample of 645 teeth, caries was found in 2.9% (19 teeth).

Some specificities of KN teeth: The term „taurodont" was coined by Arthur Keith 1913 (35) to describe the condition of free roots. Taurodontism is best measured in radiographs and, whilst it may affect all permanent or deciduous cheek teeth, it is most pronounced in molars. The whole dentition may be affected, or just a few teeth (1, 36). Taurodontism is prominent amongst the Krapina Neandertal specimens (37-39).

Mandible 53, age 10-11, x-ray findings, if KN had lived some years more, he would have had the diagnosis – dentitio difficilis (Figure 5).

Like other Neanderthals, the KN teeth showed multiple evidence of manipulations and processing activities in the anterior teeth. The permanent and deciduous teeth

showed numerous scratches of the incisors and canines, especially in the upper jaws. Out of seven specimens predominantly showing oblique scratches, six exhibited a right-handed pattern. Combined with other Neanderthal data, KN showed the frequency of handedness similar to contemporary humans (40).

Conclusions

A skeletal study was undertaken on the skulls of KN and 1st, 10th and 20th century contemporary man of the connection between alveolar bone resorption and dental caries. Alveolar resorption changes in KN, 1st, 10th and 20th century human tooth samples were assessed by use of the TCH-index (4). Diagnosis of dental caries was made by inspection and with a dental probe. The results obtained pointed to the following conclusions:

1. Resorption changes were greater on the vestibular than interdental side in all jaws from the four study periods. The greatest resorption of 6.12 mm was recorded on the vestibular side in the jaws of KN.
2. During evolution, alveolar bone resorption on the vestibular side was in decrease, as differentiated from resorption of the interdental septum, which showed an increasing tendency. In the KN and 20th century samples, this ratio was 2.38 and 1.23 in the 20-29 years subgroup, respectively, and 1.06 in the 20th century >50 year subgroup (Table 2).
3. When the ratio of interdental to vestibular (I/V) resorption was expressed as a factor, it was 1.00 in KN and 1.94 in the 20th century 20-29 year subgroup, and 2.24 in the >50 year subgroup (Table 3).
4. The dynamics of the two more pronounced resorption patterns of the interdental septum relative to the vestibular side of the alveoli in contemporary man is a consequence of greater plaque deposition and its pathogenesis in the interdental space than on the vestibular side.
5. Four samples of teeth from four time periods, i.e. the KN who reportedly lived over 130,000 years ago showed no caries (281 teeth), whereas the teeth from the 1st century showed 1.74% caries (860 teeth), the 10th century 3.44% caries (697 teeth) and the 20th century 11.87% caries (1,741 teeth).
6. These findings have a practical, educational and preventive value for oral hygiene improvement, especially of the interdental space, which should help decrease the prevalence of periodontal disease and dental caries, and improve oral as well as general health.

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References

1. Hillson S. Dental anthropology. Cambridge: University press; 2003.
2. Brothwell DR. Dental anthropology. Oxford-London: Pergamon press; 1963.
3. Kelly MA, Larsen CS. Advances in dental anthropology. New York: Wiley Lis Inc; 1991.
4. Davies DM, Picton DCA, Alexander AG. An objective method of assessing the periodonta condition in human skulls. J Periodont Res. 1969;4:74-7.
5. Rink WJ, Schwartz HP, Smith FH, Radovčić J. ESR ages for Krapina hominids. Nature. 1995;378:24.
6. Radovčić J, Smith FH, Trinkaus E, Wolpoff MH. The Krapina hominides – an illustrated catalog of skeletal collection. Zagreb: Croatian natural history museum – Mladost; 1988.
7. Kricun M, Monge J, Mann A, Finkel G, Lampl M, Radovčić J. The Krapina hominides – a radiographic atlas of the skeletal collection. Zagreb: Croatian natural history museum; 1999.

8. Martin R, Saller K. Lehrbuch der Anthropologie. Band 2: Kraniologie, Osteologie. Jena: Gustav Fischer Verlag; 1928.
9. R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: http://www.R-project.org
10. Goldberg HJ, Weintraub JA, Roghman KJ, Cornwall WS. Measuring periodontal disease in ancient populations: Root and wear indices in study of American Indian skulls. J Periodont. 1976;47:348-51.
11. Muller D, Perizonius WR. The scoring of defects of the alveolar process in human crania. J Hum Evol. 1980;9:113-6.
12. Manson Jd, Eley BM. Outline of periodontics. Oxford: Wright; 2000.
13. Severson JA, Moffett BC, Kokoich V, Selipsky H. A histologic study of age changes in the adukts humans periodontal joint (ligament). J Periodontol. 1978;49:189-200.
14. Sarajlić N, Topić B, Brkić H, Alajbeg ŽI. Aging quantification on alveolar bone loss. Coll Anthropol. 2009;33:1165-70.
15. Löe H, Theilade E, Jensen J. Experimental gingivitis in man. J Periodont. 1965;36:177-87.
16. Mandel ID. Summary of conference and perspectives for the future. In: Genco RJ, Mergenhagen SE, editors. Host – parasite interactions in periodontal diseases. Washington, D.C.: American Sosiety for Microbiology; 1988. p. 404-9.
17. Lang NP. The basis for mechanical plaque control. In: Osborne D, Doherty F, editors. The art and science of interdental cleaning. New York – London: The Parthenon publishing group; 1998. p. 15-22.
18. Mc Hugh WD. Dental plaque. Edinbourgh: Livingstone; 1969.
19. Osborne D, Doherty F. The art and science of interdental cleaning. New York – London: The Parthenon publishing group; 1998.
20. Kinane DF. The role of interdental cleaning in primary and secondary prevention. In: Osborne D, Doherty F, editors. The art and science of interdental cleaning. New York – London: The Parthenon publishing group; 1998. p. 23-32.
21. Prato PG, Cattabriga M, Rotundo R. Interdental cleaning for post-periodontal surgery patients and other special groups. In: Osborne D, Doherty F, editors. The art and science of interdental cleaning. New York – London: The Parthenon publishing group; 1998. p. 33-9.
22. Schou L. Behavioural aspects of interdental cleaning. In: Osborne D, Doherty F, editors. The art and science of interdental cleaning. New York – London: The Parthenon publishing group; 1998. p. 41-7.
23. Vialle L. Interdental cleaning – patient motivation and compliance: practical advice and recommendations for the dental hygienist. In: Osborne D, Doherty F, editors. The art and science of interdental cleaning. New York – London: The Parthenon publishing group; 1998. p. 49-57.
24. Lavelle CLB, Moore WJ. Alveolar bone resumption in Anglo-Saxon seventeen mandibles. J Periodont Res. 1969;4:70-3.
25. Kerr NW. The periodontal status of a Scotish mediaeval cohort. J Paleopathol. 1989;2:119-28.
26. Topić B, Cekić-Arambašin A, Jorgić-Srdjak K. Alveolar bone resorption in the skulls. Coll Anthropol. 1998;22(Suppl):117-22.
27. Topić B, Papo A. Alveolar bone resorption in jaws of the Krapina Neandertals. II Internacional conference: "The Krapina Neandertals and human evolution in the Central Europe". August 23-26 1999. Zagreb-Krapina. Book of Abstracts. 1999;46.
28. Whittaker DK, Molleson T, Daniel AT, Williams JT, Rose P, Resteghini R. Quantitative assessment of tooth wear, alveolar crest height and continuing eruption in a Romano-British population. Arch Oral Biol. 1985;30:493-501.
29. Clarke GN, Hirsch SR. Physiological, pulpal, and periodontal factors influencing alveolar bone. In: Kelley MA, Larsen CS. Advances in Dental anthropology. New York: Wiley-Lis; 1991;241-66.
30. Kerr NW. Prevalence and natural history of periodontal disease in Scotland – the mediaeval period (900 – 1600 AD). Arch Oral Biol. 1991;26:346-54.
31. Kerr NW. Prevalence and natural history of periodontal disease in a London, Spitalfields, population (1645 – 1852 AD). Arch Oral Biol. 1994;29:581-8.
32. Kerr NW. Prevalence and natural history of periodontal disease in prehistoric Scots (pre-900 AD). J Periodont Res. 1998;33:131-7.
33. Brothwell DR. The macroscopic dental pathology of some earlier populations. In: Brothwell DR, editor. Dental anthropology. Oxford-London: Pergamon press; 1963. p. 271-88.
34. Vodanović M, Brkić H, Šlaus M, Demo Ž. The frequency and distribution of caries in the mediaeval population of Bijelo Brdo in Croatia (10-th – 11th century). Arch Oral Biol. 2005;50:669-80.
35. Keith A. Problems relating to the teeth of the ealier forms of prehistoric man. Proc R Soc Med (Odontol sec). 1913;6:103-19.

36. Konjhodžić-Raščić H, Malokas-Čuvalo J. Taurodontism – exotaurodontism, endotaurodontism. Stomatol Vjes. 1982;11:35-8.
37. Kallay J. Osobitosti zubi krapinskih neandertalaca. In: M. Malez, editor. Krapina 1899–1969. Zagreb: Jugoslavenska akademija znanosti i umjetnosti; 1970. p. 165-76.
38. Kallay J. Dentalna antropologija, Svezak I. Zagreb: Izdavački zavod Jugoslavenske akademije; 1974.
39. Dumančić J, Kaić Z, Brkić H, Petrovečki M. Tooth pulp dimensions and taurodontism in Krapina Neanderthals. II International conference "The Krapina Neandertals and human evolution in Central Europe". August 23-26 1999. Zagreb-Krapina. Book of Abstracts. 1999;21-22.
40. Frayer DW, Fox CL. Labial scratches, dental manipulation and handedness at Krapina. II International conference "The Krapina Neandertals and human evolution in Central Europe". August 23-26 1999. Zagreb-Krapina. Book of Abstracts.1999;24.

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The relationship between difficulties in psychological adjustment in young adulthood and exposure to bullying behaviour in childhood and adolescence

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Objective. This study investigates the relationship between involvement in bullying in childhood and adolescence and psychological difficulties in young adulthood. **Materials and method**. A total of 249 college students completed the Retrospective Bullying Questionnaire and Trauma Symptom Checklist. **Results.** The results showed significant differences in psychological adjustment among respondents who were exposed to bullying compared to respondents who were not exposed to bullying. Those exposed to bullying had significantly higher levels of anxiety, depression, sleeping problems, and dissociative and traumatic symptoms compared to those who were not exposed to bullying. Respondents who were exposed to bullying in all three examined periods (the period from the first to fourth grade, the period from the fifth to eighth grade and the high school period) had higher scores on the subscale of dissociative symptoms and sexual trauma symptoms compared to respondents who were exposed through one or two periods. Victims abused in all three periods have more symptoms of anxiety and sleeping problems compared to the subjects exposed to bullying during one examination period. There were no differences in the level of depressive symptoms and sexual problems regarding the duration of bullying. Also, there were no differences in psychological adjustment between respondents who were bullied during one specific period. **Conclusion.** Bullying experiences in childhood and adolescence are connected with difficulties in psychological adjustment in young adulthood.

Key words: Bullying, Psychological adjustment, Young adulthood, Trauma symptoms, Retrospective research.

Introduction

Bullying is not a new phenomenon. Different forms of bullying have existed as long as schools. Almost all adults have childhood memories or experiences of bullying in different situations, whether they acted aggressively towards other children, experienced bullying themselves or witnessed bullying. Many adults, regardless of age, are still able to recall details of these events, such as the name of the school bully, classes they attended, duration of exposure to bullying and so on. In a study that was conducted by Crozier and Skliopidou (1), among 220 adults, who were asked about their memo-

ries related to the period when they were in primary school, it was established that two thirds of adults recalled that they were exposed to mockery or derogatory names.

Respondents who were exposed to bullying, stated that these events were accompanied by feelings of anger, shame and unhappiness. Recollection of these events and the derogatory names they were called are still painful for one fourth of the questioned participants and they believe that these experiences caused long-lasting negative effects on the development of their personalities and attitudes.

There is no single common definition of bullying, but most agree that there are factors that should be taken into consideration such as: firstly, different patterns of behavior that are repeated over time with the intent to hurt or disturb one or more students by one or more other students; secondly, there must be a perceived imbalance of power between the bully and the victim, which allows one student to dominate over others (2). Situations where there is a conflict between individuals of equal or similar power are not considered bullying. This distinction is important because the consequences of repeated attacks or threats by individuals or groups more powerful than themselves, most likely differ from the effects of exposure to threats or attacks by someone of the same strength. In the first case individuals feel helpless (3).

In different ways, psychologists tried to determine the varieties of forms of bullying amongst children. In certain studies, bullying amongst children is conceived globally (4). For example, as an action that aims to threaten someone or to hurt someone who is weaker. In other studies (5) distinctions were made between the different types of aggressive acts or behaviors that may be performed, for example, physically, verbally or indirectly. The distinction can also be made depending on whether a child is abused by one individual or by a group. Someone can be abused because they belong to a group which the bully does not approve of or because of some personal characteristics. Finally, we also distinguish exposure to bullying in one or several situations as opposed to long term exposure to bullying. All these distinctions are very important especially when talking about the consequences of bullying (3).

Numerous studies show that bullying is a worldwide problem. Studies conducted in Australia, Canada, UK, Japan, Scandinavia and Croatia, indicate that 20% to 30% of school children participate in bullying (6-14). When we take into account the frequency of bullying in regard to the role in bullying behavior, the research results show that 7-23% of respondents were identified as bullies, 5-12% as victims and 2-21% of respondents as bully/victims (7, 15-18). Studies in this field have demonstrated that children exposed to bullying are more likely to experience a wide range of adverse psychosocial and behavioral outcomes (19-21).

Most of the studies are focused on short-term consequences of peer victimization (22). Among the wide range of negative consequences associated with bullying, special attention is focused on the development of depressive disorders (22, 23-26), and suicidal thoughts and ideas in children exposed to bullying (27-29). One of the most common emotional responses to prolonged exposure to bullying are different forms of anxiety disorders such as social and chronic anxiety (22, 30-33). In children exposed to violent behavior, symptoms of PTSD were also identified. The above stated symptoms can manifest as behavioral problems, avoiding school, the class and persons associated with bullying, and loss of interest in people, imposed memories of traumatic events, nightmares, frightening memories of one or more traumatic events (34-36). Physical symptoms such as headaches, stomachaches,

back pain, chest tightness, sore throat, sleeping problems, morning fatigue, poor appetite and night urination are often associated with exposure to bullying (23, 29, 37-38).

However, cross-sectional studies are not able to provide evidence for something more than correlations among bullying and psychological difficulties. A small number of studies investigated the long-term consequences of bullying. The results of longitudinal studies are consistent with the results of the intersection of research and suggest that exposure to bullying is correlated with different forms of internalizing and externalizing psychological problems (8, 30, 39-41). A prospective study by Schreier et al. (42) showed associations between adverse experiences in childhood and psychotic symptoms in adulthood. Furthermore, prospective types of studies have shown that history of victimization predicts the onset of emotional problems in the early teen years (43).

However, most longitudinal studies that have been conducted so far have focused on shorter periods of time and still lack longitudinal studies through longer period of time (39). Moreover, a shortcoming of longitudinal and prospective studies is that the data are collected over a specific period of time, and rarely cover the entire childhood (44). Also, this type of research is expensive current and because of that researchers are directed towards the implementation of retrospective studies. Retrospective studies of bullying conducted so far are rare and mainly focus on specific populations (3, 45-46). They suggest a correlation between victimization and difficulties in heterosexual relationships, declaring that they were exposed to bullying behavior in childhood. In a study conducted on 276 adults (aged 15-66) in England who were exposed to child abuse, approximately one half of the participants reported that they had a long-term psychological effects, most commonly in the field of personal relationships (46). In a retrospective study conducted on adult subjects (3) it was discovered that men exposed to victimization at school had interpersonal difficulties, resulting in fear of intimacy and shyness, which limits them in creating a satisfying intimate relationship with the opposite sex. Allison, Roeger and Reinfeld-Kirkman (47) investigated the relationship between past victimization and adult health-related quality of life. In a representative sample of Australian adults experience in bullying was determined by interview. Furthermore, the health-related quality of life was measured using the Medical Outcomes Study 36-item ShortForm Health Survey. The results showed that those who had been bullied experienced significantly poorer mental and physical health compared to those who had not been bullied.

Retrospective studies which researched exposure to bullying in the student population, indicate that the subjects who were exposed to bullying in childhood and adolescence were more likely to develop depressive disorders (39, 48-49), anxiety disorders (49-50, 51-52) and difficulties in interpersonal relationships (53-54) compared to students who did not have such experiences. Furthermore, Ronning et al. (55) found that frequent bullying is a marker of present and later psychopathology.

The duration of exposure to violent behavior is another significant factor in the development of long-term psychological consequences in individuals exposed to violent behavior (54). Students who reported that they were exposed to bullying through the period of both the lower and upper grades of primary school were more likely to develop psychological problems compared to those who were exposed to bullying in one of these two periods (during the lower grades of elementary school or only in the higher grade) (54).

Although these studies provide some evidence of the specific effects of being bullied, most of these studies (42-43) do not extend into later adolescence. It is important to know the effects of being bullied during the period of the lower and upper grades of primary school, as well as high school on later adolescent functioning. To our knowledge this is the first study that includes a retrospective investigation of different periods of bullying on psychological functioning of adolescents. This could have important implications for the possibility of identifying the critical period when bullying has the strongest effects on adolescent behavior. Furthermore, the studies conducted examine specific psychological difficulties that could be the result of being bullied. Studies also need to account for all co-existing difficulties that could appear simultaneously in bullied children. Finally, to explore whether bullying has an impact on adolescent behavior and mental health, it is important to include a not bullied comparison group. Most of these studies examine the association between bullying and some types of psychological difficulties. It is methodologically more appropriate to categorize subjects into different categories with regard to experience in bullying.

The aim of this study was to examine the association between exposure to bullying during childhood and adolescence and difficulties in psychological adjustment amongst young adults.

Methods

Participants

The study includes 249 students from different departments of the Faculty of Humanities and Social Sciences, University of Mostar, (173 females and 76 males). The average age of the participants was 21.3±3.14. These students were in different years of study and academic specialization, including social work, psychology, and philosophy. The participants were provided with an information sheet that outlined the main principles of the research and contact information if they wished to contact the researchers later on. They were given time to read the information sheet and to ask questions. The study was approved by the Ethics Committee of Mostar University and by The Ministry of Science, Education, and Sports of Herzegovina-Neretva Canton, Bosnia and Herzegovina.

Instruments

For the purpose of this study a questionnaire was constructed with sociodemographic data. The questionnaire included data on student sex, age, type and year of study. The exposure to bullying among children in primary and secondary schools was examined through the Retrospective Bullying Questionnaire (RBQ), which was constructed by Schäfer et al. (54). The implementation of the questionnaire in this study was approved by the authors of the questionnaire. For the purpose of this study, the questionnaire was translated into Croatian and adapted through the technique of reverse translation. It was translated from English to Croatian and then afterwards from Croatian into English. After this procedure, the verification of the translated version was done by two independent experts in the field of bullying and after their alignment, the final version was made.

The questionnaire contained 44 questions, mostly multiple choice. It covered experiences of victimization in school (six types of victimization, two physical, two verbal, two indirect), and specifically their frequency, severity, and duration (all 5-point scales), gender, the number of bullies (six options), and the students' participation in active bullying. These questions were asked firstly for primary school and then for secondary school. This was followed by a 5-item trauma subscale of intrusive and recurrent recollections of victimization (each 5-point

scales), and a question on suicidal ideation if bullied (4-point scale). The last part of the questionnaire included questions about the frequency, intensity and duration of abuse in the workplace. However, this part of the questionnaire was not an appropriate form for a group of students, so we decided to modify the questionnaire and to reduce the questions. The questionnaire was introduced by a definition of bullying. The anonymity of the questionnaire was stressed, and a detachable sheet of advice, helplines and some useful websites were given at the end for those who might wish to discuss their experiences further. The criterion for classification of students as bullies was based on the confirmatory answers to questions relating to how they abused their peers and how frequently it occurred. As for the classification of the victims, confirmatory answers in the first part of the questionnaire related to exposure to, as well as experience of frequent abuse, were the criteria for classification. The victim group was further divided into subgroups regarding the period of exposure to bullying: the period from first to fourth grade (approximate age range - 6 to 10 years), the period from the fifth to eighth grade (app. age - 10 to 15 years) and the high school period (app. age - 15 to 19 years).

In previous studies in which this measure instrument was used, the authors did not specify the factor structure (54, 56). This is reasonable because the questionnaire examines different aspects of bullying, in which responses are expressed on a continuous scale. Schäfer et al. (54) reported reliability of 0.88 for testing the scale of bullying in elementary school, 0.87 for the examination of bullying in high school, and 0.77 for exposure to bullying and the strategies used to cope with bullying.

Psychological adjustment in young adulthood was assessed by the Trauma Symptom Checklist 40 (TSC-40) (57). TSC-40 is used to determine the symptomatology in adults who experienced trauma in either childhood or adult age. It measures aspects of post-traumatic stress and other symptoms that occur in traumatized individuals. TSC-40 is a 40-item self-reporting instrument. In addition to yielding a total score, it has six subscales: Anxiety, Depression, Dissociation, Sexual Abuse Trauma Index, Sexual Problems, and Sleep Disturbances. For each item of the scale, the respondents are asked to rate the frequency of symptom occurrence during the preceding two months, using a Likert-type scale from 1 ("never") to 4 ("often"). Briere (58) found that the coefficients of internal consistency subscale range from 0.66 to 0.77 (58). In this study, coefficients of internal consistency range from 0.64 to 0.73, and the reliability of the entire scale is 0.90. Satisfactory levels of reliability justify the use of existing subscales in subsequent analyzes.

Data collection

Data were collected in the summer semester of 2012 during lectures. Questionnaires were group applied (the study units) and were not limited in time. On average they took 15 minutes. In the instructions to the participants, the anonymity of data was emphasized and it was explained how to fill the questionnaire. Each participant first filled out the questionnaire with sociodemographic data, while the sequence of filling out the Retrospective Bullying Questionnaire and the Trauma Symptoms Checklist – 40 was rotated by the principle of ABBA. Thus, half of participants first filled out the Retrospective Bullying Questionnaire, and the Trauma Symptoms Checklist – 40, while the other half of participants worked in the reverse order.

Data analysis

Statistical analysis was performed using Statistica 7.0 (StatSoft, Inc., Tulsa, OK, USA). Depending on the distribution of the vari-

ables, the Student t-test or the Mann-Whitney test was used to determine the difference in psychological adjustment between the neutral group (not exposed to peer abuse) and victims (exposed to peer abuse). Using the one-way ANOVA and Kruskal-Wallis tests, victims` psychological adjustment was additionally analyzed depending on the period of peer abuse. The Bonfferoni correction test for multiple testing and multiple comparisons of mean ranks for all groups were applied where it was necessary. P-values less than 0.05 were considered significant.

Results

In analyzing the results, we first defined the term 'victim' for our sample. We then considered differences of our outcome measures. Our primary interest was in victim/non-victim differences. Victims (N=119) were identified from their responses about the frequency and intensity of reported physical, verbal and indirect bullying. A person was considered a victim when they reported being bullied in one or more ways 'sometimes' or more (frequency) and classified this as 'quite serious' or 'extremely serious' (intensity).

Respondents who were assessed to have never been exposed to bullying or those who indicated that they were exposed to bullying sometimes or rarely were categorized as neutral. Analysis of the results of the Retrospective Bullying Questionnaire showed that 44.1% out of the 119 participants were sometimes or frequently exposed to one or more forms of bullying. Verbal forms of bullying are the most common during elementary school (lower grades- 21.5%; higher grades – 19.9%), while indirect forms of bullying become more frequent in high school (17.9%), compared to periods in elementary school. Physical forms of bullying are somewhat less frequent, ranging from 2.9% at high school, to 8.1% and 11.8% at lower and higher grades of elementary school, respectively. Respondents were most exposed to bullying during one of the examined periods (50.4%). During the two examined periods 48 (40.3%) respondents were exposed to bullying. Throughout all three periods 21 subjects (17.6%) were exposed to bullying.

Significant differences were established between respondents who were exposed to bullying and those who were not exposed to bullying in all measures of psychological adjustment. Respondents exposed to bullying had higher levels of symptoms of anxiety, depression, dissociative disorders, sleeping problems, sexual problems and higher levels of traumatic symptoms associated with sexual abuse (Table 1). The range of average differences in scores on the subscales achieved between the abused and the neutral group was lowest on the subscale of sexual difficulties and highest on the depression subscale.

When we took into account the duration of exposure to bullying, differences in the levels of psychological difficulties between the two groups were established (Table 2).

Respondents who were exposed to bullying in all three periods had higher scores on the subscale of dissociative symptoms (M=7.45; SD=3.531) than respondents exposed to bullying during one (M=4.63; SD=2.863) or two periods (M=5.46;2.887), $F(2, 113)=6.277$, $p=0.003$. Also, being exposed to bullying through all three periods was connected with having more sexual trauma symptoms (M=6.63; SD=3.989) compared to being exposed through one (M=3.36; SD=2.595) or two periods (M=4.44; SD=3.148), $F(2, 113)= 7.469$, $p<0.001$. Victims of bulling through all three periods had more symptoms of anxiety (N=7, range of 4-8) than those bullied through one period (N=4, range of 2-7), $F(2, N=114)=7.128$, $p=0.028$. Likewise, respondents who were bullied through all periods had more sleeping problems (N=6, range of 4-8) than those who were exposed

Table 1 Differences in psychological adjustment among respondents exposed to bullying and those who were not exposed to bullying

Psychological difficulties	Exposure to bullying			
	Exposed	Not exposed	t/Z	P
Dissociation ($\bar{X}$±SD)	5.45±3.13	4.02±2.61	3.867	<0.001†
Anxiety (Median; range)	6 (3-8)	4 (2-7)	2.565	0.01‡
Depression ($\bar{X}$±SD)	7.17±4.14	5.06±3.19	4.362	0.008†
Sexual abuse trauma index ($\bar{X}$±SD)	4.37±3.28	2.91±2.32	3.949	<0.001†
Sleep disturbances (Median; range)	5 (3-8)	3 (2-6)	4.358	<0.001‡
Sexual problems ($\bar{X}$±SD)	3.84±3.27	2.81±2.83	2.550	0.011†

†Student t-test; ‡Mann-Whitney U test.

Table 2 Differences in psychological adjustment in respondents exposed to bullying in regard to the duration of exposure to bullying (all three periods, combination of two periods, one period)

Psychological difficulties	Duration of exposure to bullying				
	One period	Two periods	Three periods	F/H	P
Dissociation ($\bar{X}$±SD)	4.62±2.86	5.46±2.89	7.45±3.53	6.277	0.002†
Anxiety (Median; range)	5 (3-8)	6 (3-8)	9 (5-15)	7.128	0.028‡
Depression ($\bar{X}$±SD)	6.68±3.18	7.13±4.07	8.87±6.39	1.606	0.205†
Sexual abuse trauma index ($\bar{X}$±SD)	3.36±2.59	4.44±3.15	6.63±3.99	7.469	<0.001†
Sleep disturbances (Median; range)	4 (3-6)	5 (3-8.5)	8 (5-12)	7.299	0.001‡
Sexual problems ($\bar{X}$±SD)	3.76±2.70	3.74±2.89	4.38±5.38	0.247	0.781†

†ANOVA; ‡Kruskal-Wallis ANOVA.

Table 3 Differences in psychological adjustment in respondents exposed to bullying during one specific period

Psychological difficulties	Duration of exposure to bullying				
	Lower grades (elementary school)	Higher grades (elementary school)	High school	F/H	P
Dissociation ($\bar{X}$±SD)	4.50±2.39	5.44±3.37	3.75±2.80	1.236	0.300†
Anxiety (Median; range)	5 (1-9)	7 (0-14)	4 (1-11)	1.543	0.462‡
Depression ($\bar{X}$±SD)	6.24±2.41	8.20±3.78	5.45±3.05	2.974	0.061†
Sexual abuse trauma index ($\bar{X}$±SD)	3.1±2.40	4.23±3.00	2.83±2.41	1.083	0.348†
Sleep disturbances (Median; range)	7 (0-10)	6 (1-10)	3 (0-8)	4.237	0.439‡
Sexual problems ($\bar{X}$±SD)	3.71±2.26	4.21±3.74	3.20±1.81	0.406	0.669‡

†ANOVA; ‡Kruskal-Wallis ANOVA.

to bullying during one period (N=3, range of 2-5), F(2, N=114)=10.299, p=0.006. There were no differences in the level of depressive symptoms and sexual difficulties regarding the duration of bullying.

The levels of psychological difficulties of groups who were exposed to bullying during one period (lower/higher grades of elementary school, high school) were compared. This was done in order to try to isolate a possible critical period when more difficulties occurred (Table 3). However, no differences were found.

Discussion

The results of this study support the conclusions of previous studies (8, 48) which established the correlation between exposure to bullying during childhood and adolescence and psychological difficulties in young adulthood. According to the results of this research, 44.1% respondents were exposed occasionally or frequently to bullying during childhood and adolescence. Retrospective studies that have been conducted until now on college students have found that many of them recalled their experiences of bullying during school age. In a survey conducted of 119 college undergraduates, 48.7% of respondents reported that they were bullied at least once or twice; 15.1% reported they were bullied occasionally, and 2.5% stated that they were bullied frequently during high school (59). Newman et al. (60) found a higher rate of occasional and frequent bullying than the percentage found in the study by Chapell et al. (59). In a sample of 853 college students, Newman et al. (60) found that 24% of the respondents reported that they were occasionally bullied during high school and 9.1% recalled that they were frequently bullied during high school. These prevalence figures are higher than those obtained in school-based surveys, which often ask for reports of the last 3 or 6 months (61). This is in line with what might be expected from these school based surveys, if we take into account reports over the whole duration of education.

According to the results of this study, considering the data related to duration of bullying, respondents were most exposed to bullying during one of the indicated periods (42%). 40.3% of respondents were exposed to bullying during two periods examined. In this case, these were the lower and higher grades of elementary school (24.4%). 21 respondents (17.6%) were exposed to bullying throughout all three periods. In this study, the established frequency of exposure to abuse, taking into account the duration of bullying, was significantly higher than in the research of Schäfer et al. (54).

In above mentioned study, 28% of respondents reported being victimized at school. Also, about half of these victims reported relatively extended victimization, lasting for months or longer, and about 8% of them reported being victimized in both primary and secondary school (54). The differences that were found between our study and the already mentioned research are not surprising. The study by Schäfer et al. (54) was conducted in Spain, Britain and Germany and it lasted longer. Furthermore during that study preventive work was systematically implemented. The main aim of these prevention programs was to reduce the occurrence of bullying. In Bosnia and Herzegovina and even in the region, research into bullying is in its initial stages as are the prevention programs. Moreover, in our region, a large number of children are not even aware of behaviors that are considered as bullying and are not aware of the negative consequences of such behavior, which directly affects its frequency. This study has shown that those young people who were exposed to bullying during their years of education reported more depressive symptoms compared to those who did not experience abuse during primary and secondary school.

These results are in line with the results of retrospective studies conducted so far, in which respondents were exposed to bullying during their childhood and/or adolescence and consequently developed a higher risk of depressive disorders (48-49, 62-63).

Other than the symptoms related to depression, young people who were exposed to bullying in childhood and adolescence, reported a greater number of anxiety symptoms compared to those who were not. In studies conducted so far dealing with a retrospective examination of bullying, we found that the students who reported being victims of bullying also had symptoms associated with anxiety (50-52). Similarly, according to the results of Gladstone et al. (52) adult men who were exposed to bullying once a week for five or more years, had their current anxiety symptoms attributed to their abuse experience. In addition, respondents who were exposed to bullying during childhood and/or adolescence reported the symptoms of anxiety disorders earlier than those who did not report the experience of bullying (50, 52).

Cognitive theory gives us an insight into how the process of internalization of bullying events could potentially contribute to eventual long-term effects, such as depression and anxiety. According to this theory, the meaning one ascribes to events determines the affective response (64). If this tendency toward negative internalization persists, then these individuals may be at greater risk for depression, anxiety, and or problems in relationships (65-67). In Beck's example (65) of a young boy being teased by his friends, he provided an illustration of how internal evaluation of an event can be influential in determining emotional responses. In this example, Beck stated that objective meaning might be that his friends were simply joking with him. The boy's internal evaluation might be that he is "a weakling" or "they don't like me" (Beck, p. 48). Because these internal evaluations, or private meanings, are often regarded as embarrassing, the individual is less likely to examine these beliefs with others. Without the opportunity for others to challenge such thoughts, these negative perceptions about the self may persist and continue to influence beliefs about the self. Since children and adolescents who were targets of bullying are more likely to be socially isolated (48, 68-69) these individuals may be particularly unlikely to have such negative perceptions disconfirmed by others. In contrast, those with other opportunities to build social competence may be more likely to demonstrate resilience as they have additional opportunities to have these negative beliefs dispelled by others. Also drawing from cognitive theory (65-67), those low in competence or lacking other sources of developing competence may develop negative schemas associated with social experiences. New social experiences during college that remind one of these earlier, aversive experiences with peers may trigger negative social schemas, eliciting emotions, thoughts, images, and behavioral impulses associated with these earlier, aversive situations (70). Such interpretations may reinforce anxiety associated with social situations. Being the target of bullying also may contribute to a sense of learned helplessness (71), a cognitive pattern often displayed by individuals with depression (72). Those individuals who do not possess opportunities for developing competence may be more prone to learned helplessness. As victims may believe that they are unable to stop the bullying, they may also begin to believe that their efforts to affect the outcomes of other situations will be ineffective (49). If bullying persists for a long period of time, targets of bullying may begin to generalize this sense of incompetence to other areas of their lives, which may lead to low self-esteem and a greater likelihood of developing depression and anxiety during college years (73).

According to the results of some studies (74) there is evidence that suggests that

there is a possible connection between bullying in schools and post-traumatic stress disorder. To our knowledge, this is one of the first studies to investigate the long-term traumatic consequences of exposure to bullying, and also a wide range of traumatic symptoms. The analysis of the obtained results showed that the respondents who were exposed to bullying, besides the symptoms of anxiety and depression, had more pronounced sleeping problems, dissociative symptoms and SATI than those who were not bullied. Whether we focus on the individual symptoms or analyze them together, these symptoms are a measure of the trauma (75-76). A significant correlation was determined between the abuse and trauma symptoms, which indicates that bullying as a form of violent behavior is a traumatic experience for people who are exposed to it (77). The time that elapsed from the first experience of abuse to the moment of the research may complicate the distinctions of the different forms of abuse (physical, verbal or relational), but nevertheless, the obtained results illuminate the potentially traumatic nature of bullying. If we are to truly understand the association between psychological difficulties and experiences of bullying, it is necessary to consider other potential factors that may contribute to the development of long-term psychological difficulties. The duration of such an experience is one of the potential risk factors (54, 60, 63). In this study we found that respondents who were exposed to abuse during all three periods of education had higher scores on the subscale of dissociative symptoms and the subscale for the assessment of traumatic symptoms associated with sexual abuse than those who were exposed to bullying in one or two of the studied periods. Furthermore, respondents who were exposed to bullying in all three periods had higher levels of anxiety and more sleeping problems than those abused in only one period.

Comparison of the results of psychological difficulties in those exposed to bullying in one and two periods showed that the lowest scores on the subscales of dissociation, anxiety, sexual trauma and sleep difficulties were seen in those with the shortest duration of bullying. However, although these differences are significant, they are quite small compared to the group which was bullied through all three periods of interest.

These results are consistent with the results of the retrospective studies by Newman et al. (60) conducted on 853 students. The later one stated that the greater frequency and longer duration of abuse in childhood and adolescence is associated with increased stress symptoms. Schäfer et al. (54) found that the duration of peer victimization is a risk factor that seems to have an impact on the development of long-term consequences. Students who reported that they were exposed to bullying in the lower and higher grades of elementary school had more psychological difficulties than those who were abused in only one of these periods (54). Kochenderfer and Ladd (78) found that the duration of the victimization experience was associated with the magnitude of difficulties in psychological adaptation in persons exposed to bullying. Unlike the results of other studies (8, 22, 48, 63) which showed that the length of time of bullying may relate to the development of depression in later life, in our study we did not distinguish the difference in symptoms of depression caused by different durations of bullying.

In addition to analyzing the duration of bullying, psychological difficulties were analyzed regarding the specific time period in which bullying occurred. However, no differences in levels of psychological difficulties were found. Psychological difficulties are associated with being exposed to bullying, regardless of the period in which it happened. It must be noted, however, that levels of depression in specific bullying periods are near

the critical value of significance (F=2.974, p=0.061). This could imply that there could be some specific critical period when bullying victims are at higher risk of having symptoms of depression in later life. To support this, a meta-analysis of longitudinal studies performed by Ttofi et al. (79) shows that the level of depression is to be expected to be lower, if the student was younger when the bullying happened. Future studies with a larger sample are needed in this country, investigating the relationship between specific periods and the later occurrence of depression to see if the results confirm the findings of Ttofi et al. After this the implications of such results may be found.

The differences between our study and other studies of the symptoms of depression can be justified by the difference in the way we analyzed the data. In our study, the respondents were divided into categories according to the duration of bullying. After that, we made comparisons between categories considering the duration of bullying. In other studies, the length of bullying and its correlation to the symptoms of depression were considered, without making comparisons between the respondents who were exposed to bullying on the basis of the duration of bullying. Rigby and Slee (80) attempted to explain why the length of bullying affects the shape and magnitude of psychological difficulties. They suggest that the attitudes of the environment become less supportive as children become older. It may be that children, who were abused for a longer period of time and were treated in a less friendly manner than their peers while growing up, are consequently at greater risk for later psychological difficulties.

Limitation of research

The limitation of our study is mostly related to its retrospective design. The data collected from students was of a private nature and required them to recall negative experiences from childhood. Some negative experiences may have been repressed and forgotten, whereas others could have been exaggerated. The limitation associated with the retrospective design is that the cause cannot be established from the results. It is possible that those who were bullied may possess other characteristics that made them vulnerable to bullying (e.g., poor social skills, shyness, etc.) and that these characteristics have persisted into adulthood, making them more prone to depression, anxiety, and loneliness (81). Future research should focus on testing mediation effects. The demographic characteristics of the sample should also be taken into consideration when examining the results. There were significantly more females than males. This sample closely reflects the gender distribution at the Faculty of Humanities and Social Sciences at the University of Mostar. These findings may not be representative of other faculties and colleges with different gender distribution.

Conclusion

The results of this study support the results of some previous studies where it was determined that exposure to bullying during childhood and adolescence may have an influence on the development of long-term consequences on mental health, including the increased risk of developing depression, anxiety, post-traumatic stress and difficulties in interpersonal relationships (22, 54, 60, 63, 74). The identified psychological difficulties are similar or identical to the symptoms identified in children who are currently exposed to bullying (33, 73, 81-82). This indicates that the consequences of bullying may persist until young adulthood. These results indicate that bullying is not a separate phenomenon but is associated with psychopathology. Therefore, it is important for college counseling professionals to be aware of the consequences of bullying in late adolescence and young adulthood, as this may

strengthen their understanding of how these early traumatic experiences may influence the current functioning of the individuals.

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References

1. Crozier WR, Skliopidou E. Adult recollections of name-calling at school. Educ Psychol. 2002;22(1):113-24.
2. Rivers I, Duncan N, Besag VE. Bullying: A handbook for educators and parents. Westport: Greenwood/Praeger; 2007.
3. Rigby K. Consequences of bullying in schools. Can J Psychiatry. 2003;48:583-90.
4. Holzbauer JJ, Berven NL. Disability harassment: A new term for a long-standing problems. J Couns Dev. 1996;74(5):478-83.
5. Crick NR, Grotpeter JK. Relational aggression, gender and social psychological adjustment. Child Dev. 1995;66:710-22.
6. Dake JA, Price JH, Telljohann SK. The nature and extent of bullying at school. J Sch Health. 2003;73(5):173–80.
7. Forero R, McLellan L, Rissel C, Bauman A. Bullying behavior and psychosocial health among school students in New SouthWales, Australia: cross sectional survey. BMJ. 1999; 319:344–8.
8. Kumpulainen K, Rasanen E. Children involved in bullying at elementary school age: their psychiatric symptoms and deviance in adolescence. An epidemiological sample. Child Abuse Negl. 2000;24:1567–77.
9. Nansel TR, Overpeck M, Pilla RS, Ruan WJ, Simons-Morton B, Scheidt P. Bullying behaviors among US youth: prevalence and association with psychosocial adjustment. JAMA. 2001;285:2094–100.
10. Wolke D, Woods S, Bloomfield L, Karstadt L. Bullying involvement in primary school and common health problems. Arch Dis Child. 2001;85(3):197–201.
11. Craig WM, Pepler D, Atlas R. Observations of bullying in the playground and in classroom. School Psychol Int. 2000;21:22-36.
12. Rios-Ellis B, Bellamy L, Shoji J. An examination of specific types of ijime and their prevalence within Japanese schools. School Psychol Int. 2000;21(3):227-41.
13. Salmivalli C, Lagerspetz K, Bjőrkqvist K, Ősterman K, Kaukianen A. Bullying as a group process: participant roles and their relations to social status within group. Aggressive Behav. 1996;22:1-15.
14. Radman – Petrušić K. Nasilništvo i strategije suočavanja kod djece osnovnoškolske dobi [diplomski rad]. Zagreb: Odsjek za psihologiju filozofskog fakulteta u Zagrebu; 2005.
15. Juvonen J, Graham S, Schuster MA. Bullying among young adolescents: the strong, the week, and the troubled. Pediatrics. 2003;112(6 Pt 1):1231-7.
16. Mazur J, Malkowska A. Bullies and victims among Polish school-aged children. Med Wieku Rozwoj. 2003;7(1 Pt 2):121-34.
17. Yang SJ, Kim JM, Kim SW, Shin IS, Yoon JS. Bullying and victimization in boys and girls at South Korean primary schools. J Am Acad Child Adolesc Psychiatry. 2006;45(1):69-77.
18. Ivarsson T, Bronberg AG, Arvidsson T, Gillberg C. Bullying in adolescence: Psychiatric problems in victims and bullies as measured by the Youth Self Report (YSR) and the Depression Self-Rating Scale (DSRS). Nord J Psychiatry. 2005;59:365-73.
19. Herrenkohl TI, Sousa C, Tajima EA, Herrenkohl RC, Moylan CA. Intersection of child abuse and children's exposure to domestic violence. Trauma Violence Abus. 2008;9:84-99.
20. Sternberg KJ, Baradaran LP, Abbot CB, Lamb ME, Guterman E. Type of violence, age, and gender differences in the effects of family violence on children's behavior problems: A mega-analysis. Dev Rev. 2006;26:89-112.
21. Wolfe DA, Crooks CV, Lee V, McIntyre-Smith A, Jaffe P. The effects of children's exposure to domestic violence: A meta-analysis and critique. Clin Child Fam Psych. 2003;6:171-87.
22. Hawker DSJ, Boulton MJ. Twenty years' research on peer victimization and psychosocial maladjustment: a meta-analytic review of cross-sectional studies. J Child Psychol Psychiatry. 2000;41:441-55.
23. Fekkes M, Pijpers FI, Verloove-Vanhorick SP. Bullying behavior and associations with psychosomatic complaints and depression in victims. J Pediatr. 2004;144:17-22.
24. Kaltiala-Heino R, Rimpela M, Marttunen M, Rimpela A, Rantanen P. Bullying, depression, and suicidal ideation in Finnish adolescents: school survey. BMJ. 1999;319:348-51.
25. Klomek AB, Marrocco F, Kleinman M, Schonfeld IS, Gould MS. Bullying, depression, and suicidality in adolescents. J Am Acad Child Psych. 2007;46(1):40-9.

26. van der Wal MF, de Wit CA, Hirasing RA. Psychosocial health among young victims and offenders of direct and indirect bullying. Pediatrics. 2003;111:1312-7.
27. Kaltialo-Heino R, Rimpela M, Marttunen M, Rimpela A, Ratenen P. Bullying, depression and suicidal ideation in Finnish adolescents: school survey. BMJ. 1999;319:348-50.
28. Kim YS, Koh YJ, Leventhal B. School bullying and suicidal risk in Korean middle school students. Pediatrics. 2005;115:357-63.
29. Williams K, Chambers M, Logan S, Robinson D. Association of common health symptoms with bullying in primary school children. BMJ. 1996;313:17-9.
30. Bond L, Carlin JB, Thomas L, Rubin K, Patton G. Does bullying cause emotional problems? A prospective study of young teenagers. BMJ. 2001;323(7311):480-4.
31. Kumpulainen K. Psychiatric conditions associated with bullying. Int Journal Adolesc Med Health. 2008;20(2):121-32.
32. O'Moore AM, Hillery B. What do teachers need to know? In: Elliott M, editor. Bullying: A Practical Guide to Coping in Schools. Harlow: David Fulton; 1991. p. 56-69.
33. Craig WM. The relationship among bullying, victimization, depression, anxiety, and aggression in elementary school children. Pers Indiv Differ. 1998;24:123-30.
34. Rivers I, Cowie H. Bullying and homophobia in U.K. schools: A perspective on factors affecting resilience and recovery. JGLED. 2006;3:11-43.
35. Rivers I, Duncan N, Besag VE. Bullying. Handbooks for educators and parents. Westport, Connecticut, London: Praeger Publisher; 2007.
36. Sharp S. How much does bullying hurt? The effects of bullying on personal well-being and educational progress of secondary aged students. Educ Child Psychol. 1995;12:81-8.
37. Due P, Holstein BE, Lynch J, Diderichsen F, Gabhain SN, Scheidt P, et al. Health Behaviour in School-Aged Children Bullying Working Group. Bullying and symptoms among school-aged children: international comparative cross sectional study in 28 countries. Eur J Public Health. 2005;15(2):128-32.
38. Rigby K. The relationship between reported health and involvement in bully/victim problems among male and female secondary school students. J Health Psychol. 1998;3(4):465-76.
39. Storch EA, Ledley DR. Peer victimization and psychosocial adjustment in children: Current knowledge and future directions. Clin Pediatr. 2005;44(1):29-39.
40. Haavisto A, Sourander A, Multimaki P, Parkkola K, Santalahti P, Helenius H, et al. Factors associated with depressive symptoms among 18-year-old boys: a prospective 10-year follow-up study. J Affect Disord. 2004;2-3:143-54.
41. Kim YS, Leventhal BL, Koh YJ, Hubbard A, BoyceWT. School bullying and youth violence: causes or consequences of psychopathologic behavior? Arch Gen Psychiatry. 2006;63:1035-41.
42. Schreier A, Wolke D, Thomas K, Horwood J, Hollis C, Gunnell D, et al. Prospective Study of Peer Victimization in Childhood and Psychotic Symptoms in a Nonclinical Population at Age 12 Years. Arch Gen Psychiatr. 2009;66:527-36.
43. Bond L, Carlin JB, Thomas L, Rubin K, Patton G. Does bullying cause emotional problems? A prospective study of young teenagers. Brit Med J. 2001;323:480-84.
44. Hardt J, Rutter M. Validity of adult retrospective reports of adverse childhood experiences: review of the evidence. J Child Psychology Psyc. 2004;45(2):260-73.
45. Gilmartin BG. Peer group antecedents of severe love-shyness in males. J Pers. 1987;55:467-89.
46. Hugh-Jones S, Smith K. Self-reports of short and long term effects of bullying on children who stammer. Brit J Educ Psychol. 1999;69:141-58.
47. Allison S, Roeger L, Reinfeld-Kirkman N. Does school bullying affect adult health? Population survey of health-related quality of life and past victimization. Aust NZ J Psychiat.2009;43:1163-70.
48. Olweus D. Victimization by peers: antecedents and long term outcomes. In: Rubin KH, Asendorf JB, editors. Social Withdrawal, Inhibition, and Shyness in Children. Erlbaum: Hillsdale, NJ; 1993.
49. Roth DA, Coles ME, Heimberg RG. The relationship between memories for childhood teasing and anxiety and depression in adulthood. J Anxiety Disord. 2002;16:149-64.
50. McCabe RE, Antony M, Summerfeldt L, Liss A, Swinson R. Preliminary examination of the relationship between anxiety disorders in adults and self-reported history of teasing or bullying experiences. CBT. 2003;32:187-93.
51. Dempsey AG, Stroch EA. Relational victimization: The association between recalled adolescent social experiences and emotional adjustment in early adulthood. Psychol Schools. 2008;45(4):310-22.
52. Gladstone GL, Parker GB, Malhi, GS. Do bullied children become anxious and depressed adults? A cross-sectional investigation of the correlates of bullying and anxious depression. J Nerv Ment Dis. 2006;194(1-3):201-8.

53. Ledley DR, Stroch EA, Coles, ME, Heimberg RG, Moser J, Bravata EA. The relationship between childhood teasing and later interpersonal functioning. J Psychopathol Behav. 2006;28:33-40.
54. Schäfer M, Korn S, Smith PK, Hunter SC, Mora-Merchán J, Singer MM, et al. Lonely in the crowd: Recollections of bullying. Brit J Dev Psychol. 2004;22(3):379-94.
55. Ronning JA, Sourander A, Kumpulainen K, Tamminen T, Niemela S, Moilanen I, et al. Cross-informant agreement about bullying and victimization among eight year olds: Whose information best predicts psychiatric caseness 10–15 years later? Soc Psychiatry Psychiatr Epidemiol. 2009;44(1):15-22.
56. Mora-Merchán JA. Coping strategies: mediators in long-term effects of victims of bullying? Annuary of Clinical and Health Psychology. 2006;2:15-25.
57. Briere JN, Runtz, MG. The Trauma Symptom Checklist (TSC-33): Early data on a new scale. J Interpers Violence. 1989;4:151-63.
58. Briere J. Trauma Symptom Checklist for Children (TSCC) Professional Manual. Odessa, FL: Psychological Assessment Resources; 1996.
59. Chapell MS, Hasselman SL, Kitchin T, Lomon SN, Maclver KW, Sarullo PL. Bullying in elementary school, high school, and college. Adolescence. 2006;41:633-48.
60. Newman ML, Holden GW, Delville Y. Isolation and the stress of being bullied. J Adolescence. 2005;45:343-57.
61. Smith PK Madsen K, Moody J. What causes the age decline in reports of being bullies in school? Towards a developmental analysis of risks of being bullied. Educ Res. 1999;41:267-85.
62. Storch EA, Roth DA, Coles ME, Heimburg RG, Bravata EA, Moser J. The measurement and impact of childhood teasing in a sample of young adults. J Anxiety Disord. 2004;18:681-94.
63. Jantzer AM, Hoover JH, Narloch R. The relationship between school-aged bullying and trust, shyness, and quality of friendship in young adulthood: A preliminary research note. School Psychol Int. 2006;27:146-56.
64. Beck AT. Thinking and depression: II. Theory and therapy. Arch Gen Psychiatry. 1964;10:561-71.
65. Beck AT. Cognitive therapy and the emotional disorders. New York: International Universities Press; 1976.
66. Beck AT, Emery G, Greenberg RL. Anxiety disorders and phobias. A cognitive perspective. New York, NY: Basic Books; 1985.
67. Beck AT, Rush JA, Shaw BF, Emery G. Cognitive therapy for depression. New York: Guliford Press; 1979.
68. Boulton MJ, Trueman M, Chau C, Whitehand C, Amataya K. Concurrent and longitudinal links between friendship and peer victimization: Implications for befriending interventions. J Adolesc. 1999;22:461-6.
69. Espelage DL, Swearer SM. Research on school bullying and victimization: what have we learned and where do we go from here? School Psychol Rev. 2003;32(3):365-83.
70. Brewin CR. Cognitive change processes in psychotherapy. Psychol Rev. 1989;96:379-94.
71. Besag V. Bullies and Victims in Schools: A Guide to Understanding and Management. Philadelphia: Open University Press; 1989.
72. Abrahamson LY, Seligman, Teasdale M. Learned Helplessness in Humans: Critique and Reformulation. J Abnorm Psychol. 1978;87:49-74.
73. Smokowski PR, Holland K. Bullying in School: Correlates, Consequences, and Intervention Strategies for School Social Workers. Children & Schools. 2005;27(2):101-10.
74. Rivers I. Recollections of bullying at school and their long-term implications for lesbians, gay men and bisexuals. Crisis. 2004;24:169-75.
75. Briere J, Runtz M. Symptomatology associated with childhood sexual victimization in a nonclinical adult sample. Child Abuse Neglect. 1988;12:51-9.
76. Elliott DM, Briere J. Sexual abuse trauma among professional women: Validating the Trauma Symptom Checklist-40 (TSC-40). Child Abuse Neglect. 1992;16:391-8.
77. Herman JL. Father-daughter incest. Cambridge, MA: Harvard University Press; 1981.
78. Kochenderfer BJ, Ladd GW. Peer victimization: Cause of consequence of school maladjustment? Child Dev. 1996;67:1305-17.
79. Ttofi MM, Farrington DP, Lösel F, Loeber R. Do the victims of school bullies tend to become depressed later in life? A systematic review and meta-analysis of longitudinal studies. J of Aggression, Conflict and Peace Research. 2011;3:63-73.
80. Rigby K, Slee PT. Bullying among Australian school children: reported behaviour and attitudes to victims. J Soc Psychol. 1991;131:615-27.
81. Ledley D, Stroch E, Coles M, Heimberg R, Moser J, Bravata E. The Relationship between childhood teasing and later interpersonal functioning. J Psychopathol Behav. 2006;28:33-40.
82. Andreou E. Bully/victim problems and their association with psychological constructs in 8-to 12-year old Greek school-children. Aggressive Behav. 2000;26:49-56.

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The reliability of transthoracic and transesophageal echocardiography in predicting the size of atrial septal defect

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Objective. To determine the reliability of transthoracic echocardiography (TTE) and transesophageal echocardiography (TEE) in predicting the size of an atrial septal defect (ASD). **Material and methods.** The study included 16 patients who underwent the catheter-based procedures to close an atrial septal defect between February 2008 and December 2011 at the Paediatrics Clinic, CCU Sarajevo, after clinical and TTE and TEE evaluation. In order to determine the assumed diameter of the balloon (A-SBD), we used the formula of quantification A-SBD=TTE defect diameter×1:09 + 3.9 mm and A-SBD=1.1× transesophageal diameter of ASD+2.0 mm**.** The ASD was examined using the long-axis view, the basal short-axis view, the apical four-chamber view and the subcostal view to observe its position, diameter and relation to neighbouring structures. The largest diameter was selected as the reference diameter. **Results.** Of the total number of treated patients, 11 were female. Treatment was conducted by a foreign and local team of invasive cardiologists. The average age of the patients was 8.43 years (2 -17 years). Apart from a transient disturbance of rhythm in the youngest patients, there were no other intra and post-procedural complications. The obtained formulas represent "our" default size of the SBD, based on measurements of TTE and TEE**:** A-SBD (TTE)=6.02+0.86×TTE and A-SBD (TEE)=3.93+0.86×TEE. **Conclusion.** ASD diameter determined by TTE and TEE can reliably determine the appropriate size needed Amplatzer Septal Occluder device.

Key words: Interatrial septal defect, Echocardiography, Streched ballon diameter, Amplatzer septal occluder.

Introduction

Atrial septal defect (ASD) is one of the most common cardiac anomalies in children. ASD accounts for 6–10% of cases of congenital heart disease detected at birth, the incidence being 3.78 per 10,000 livebirths (1). Surgical repair was once considered the best treatment (2, 3), but insertion of a septal occluder has gradually become an alternative (4–6). Accurate measurement and detailed anatomic delineation of ASD are essential for successful transcatheter closure (6–17).

Transthoracic echocardiography (TTE) is the primary tool for detecting ASD but is limited by a small field of view, small acous-

tic window, and operator dependence. Accurate assessment of the size, localization of the defect and surrounding structures is very important in selecting patients for further treatment, the final decision before the implantation of the device is based on the measurement of the Stretched ballon diameter (SBD) during cardiac catheterization. Measuring the SBD is necessary to measure the hard edges of the defect adequately as the actual hole in the interatrial septum.

The aim of this study is to determine the reliability of transthoracic and transesophageal echocardiography in predicting the size of the ASD and to answer the question: Are both methods equally reliable or unreliable in determining the size of ASD? Primary hypothesis: There is no difference in the size of the diameter of ASD measured with TTE and TEE and the actual size obtained by SBD catheterization. TEE and TTE are equally reliable. Alternative hypothesis: There is a statistically significant difference in the size of the ASD diameter measured using TTE and TTE and the actual size obtained during catheterization (SBD). Individually, both methods (TEE and TTE) are equally unreliable, and an "assumed" size must be calculated that is not statistically significant different from the actual size of the ASD.

Patients and methods

Patients

During the period from February 2008 to December 2011, a total of 16 pediatric patients with secundum ASD confirmed at TTE were considered as candidates for transcatheter closure with an Amplatzer septal occluder (AGA Product, St. Jude Company).

Methods

Transthoracic echocardiography

Transthoracic echocardiography (TTE) (GE Vivid) was performed by one of two experienced pediatric cardiologists using a 3- or 10-MHz transducer. Standard TTE evaluation with color and pulsed Doppler examinations was performed in the subxiphoid, parasternal short axis and apical four-chamber views. Because the size of ASD changes during the cardiac cycle and its maximal at end-systole, we chose the temporal window at end-systole to obtain the greatest size of the ASD. The lengths of four rims from the circumference of the ASD to the aortic valve (anterior superior rim), the tricuspid valve (anterior inferior rim), the superior vena cava (posterior superior rim), and the inferior vena cava (posterior inferior rim) were also measured (Figure 1 and Figure 2) (18) .

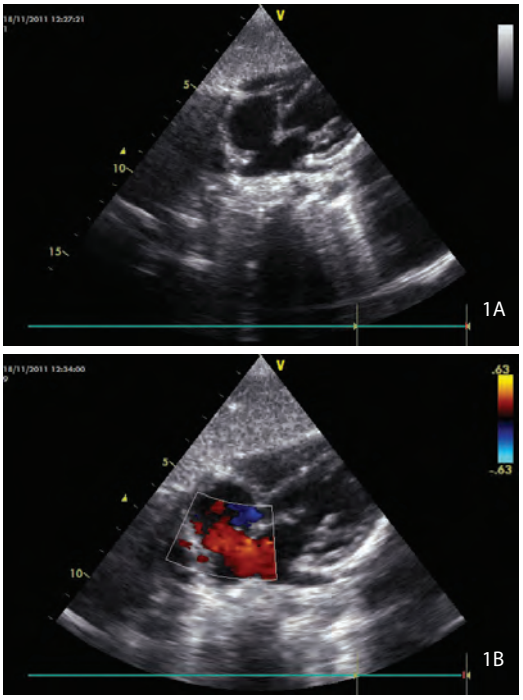

Figure 1 Transthoracis echocardiography in subcostal view to demonstrate ASD, distances between ASD and mitral annulus, without (1A) and with (1B) color Doppler.

Rim deficiency was defined as a maximal rim length less than 3 mm. Left-to-right shunt with a pulmonary-to-systemic blood flow (Qp/Qs) ratio of 1.5 or greater, measured at TTE. The Qp/Qs ratio was calculat-

ed by dividing the product of flow through the pulmonary artery and pulmonary arterial diameter by the product of flow through the aorta and aortic diameter.

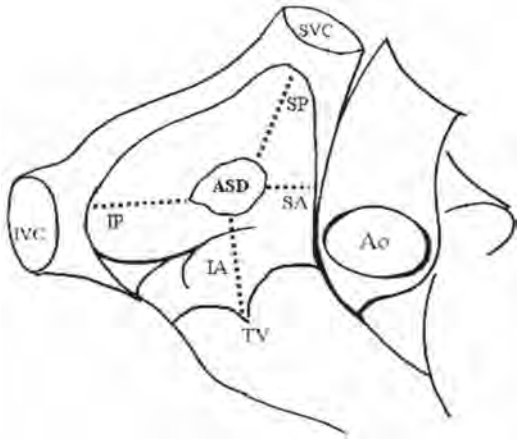

Figure 2 The relationship of the four rims in atrial septal defect (ASD). SVC=superior vena cava, IVC=inferior vena cava, SP=posterior superior, IP=posterior inferior, SA=anterior superior, IA=anterior inferior, Ao=aorta, TV=tricuspid valve (18).

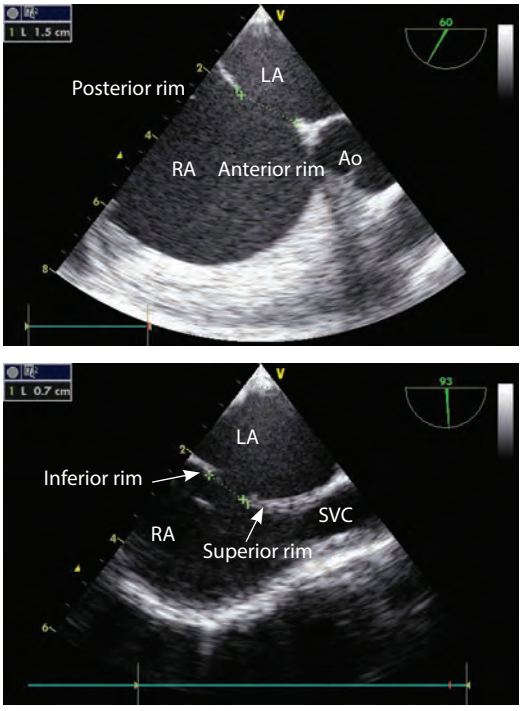

Figure 3 Transesophageal echocardiography. RA=right atrium, LA=left atrium, Ao=Aorta, SVC=superior vena cava.

Transesophageal echocardiography and transcatheter ASD closure with amplatzer aeptal occluder

The implantation of the device was performed under general anesthesia and using fluoroscopic and echocardiographic guidance. Antibiotic prophylaxis with a single dose of cefazolin (25 mg/kg) was administered before the procedure. Heparin (100 U/kg IV) was administered during the procedure. A comprehensive TEE examination (GE Vivid) was performed according to the guidelines of the American Society of Echocardiography and Society of Cardiovascular Anesthesiologists. After appropriate positioning with optimal imaging of the interatrial septum, the maximal lengths of the long and short axes of the ASD, the interatrial septum, and the four rims were measured (Figure 3). Contrast material–enhanced angiography and device delivery were accomplished through a catheterized right femoral vein. Contrast material was injected into the right atrium and right superior pulmonary vein to define the anatomy of the ASD and to confirm that it is a secundum defect. A 7- or 8-F catheter was then placed in the right superior pulmonary vein, and a 0.0035-inch wire is exchanged for the catheter. A sizing balloon was introduced over the guidewire to measure the "stretched" diameter of the defect. Because the unstretched diameter determined at echocardiography often represents an underestimation of the stretched diameter of the ASD, the defect was balloon stretched in order to determine the size of the tissue rim where the device was going to be deployed (figure 4). The method of sizing used was the "pulling technique," in which the balloon is inflated in the left atrial cavity and pulled across the septum into the right atrial cavity. The device is then "oversized" by 2 mm to ensure that it is self-centered and fits the defect completely. The sizing balloon is then exchanged for a long delivery sheath,

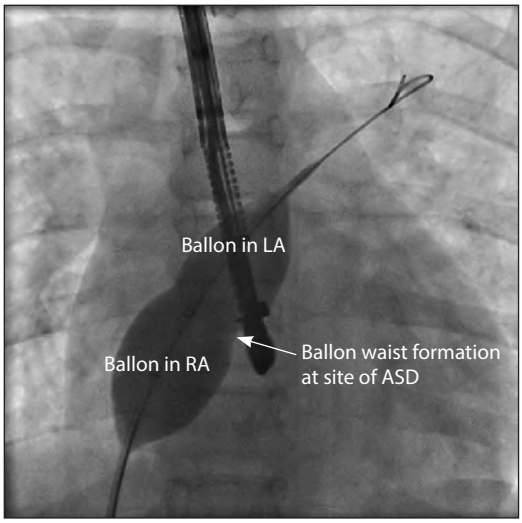

Figure 4 Stretched balloon diameter - measurement of the "stretched" diameter of the defect.

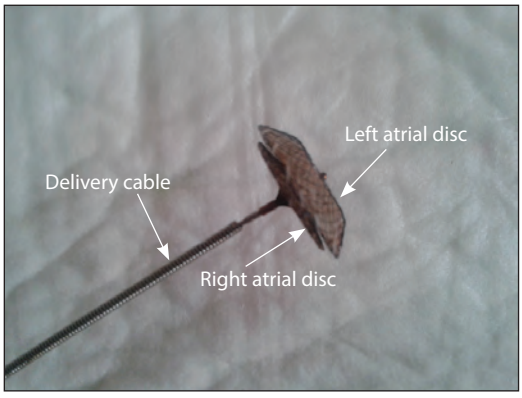

Figure 5 Appearance of the septal occluder. A 90° profile view of the septal occluder shows the left atrial disk, right atrial disk, and the delivery cable which is connected to the right atrial disk.

and the left disk of the device is first deployed by withdrawing the sheath. The device is pulled through the ASD into the right atrium, and the right disk is deployed after further withdrawal of the sheath so that the waist of the device occludes the ASD (Figure 5). Before the Amplatzer septal occluder device was released, TEE was performed to ensure there was no significant compromise of the caval and pulmonary veins or atrioventricular valves (Figure 6). After implantation of the Amplatzer septal occluder, TEE

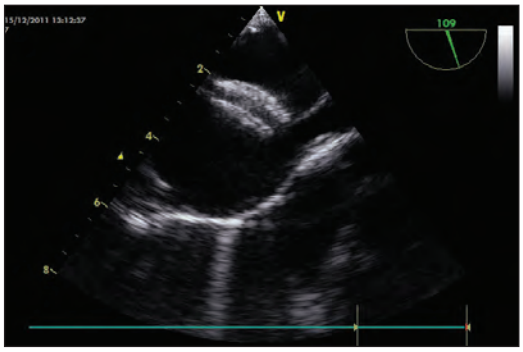

Figure 6 Transesophageal echocardiography. The position of the Amplatzer septal occluder.

was performed again for assessment of the position of the occluder and detection of any residual shunt.

Statistical analysis

Data were analyzed using a SPSS 13.0 (SPSS, Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). A two-tailed P <0.05 was considered statistically significant. Based on a linear regression equation, we made equations with the two new variable A-SBD (TTE) and A-SBD (TEE) and compared with measured SBD (MSBD). Then we could see whether the new formula is better than the formula used in the clinical trial and whether these differences are significant.

Results

The patients' demographic data and indications for ASD closure are reported in Table 1. The youngest respondent was 2 years and the oldest respondent 17 years old. The respondents' average (median) age was 8.43. During the period of the study percutaneous closure was planned for 18 patients and was successfully achieved in 16 of them. Two subjects (11.1%) were taken to the catheterization laboratory but were excluded after transesophageal echocardiography and balloon sizing of the defect because the defect was too large and/or had deficient rims.

Table 1 Patients' demographic characteristics and indications for atrial septal defect closure

Demographic characteristics and indications for atrial septal defect closure	
Number	16
Age [years; Median (range)]	8.43 (2.0-.17.0)
Gender (M/F)	5/11
Indications for closure	
Group A	
Elective closure (n)	12
Group B	
Frequent respiratory infections (n)	3
Failure to thrive (n)	1

In one of the patients in whom implantation was unsuccessful, the device embolized to the right atrium and was removed using the transcatheteral snaring system.

Using the linear regression analysis method, we obtained formulas for calculation of the assumed size of the measured SBD (Figure 7 and Figure 8):

M-SBD(TTE)= 6.02+0.86×TTE and M-SBD(TEE)= 3.93+0.86×TEE

The obtained formulas now actually represent "our" default size of the SBD, based on measurements of TTE and TEE. The paired t test showed that differences in the mean (arithmetic mean) between A-SBD (TTE) and A-SBD (TEE) were not statistically significant, p=0.638. The paired t test showed that differences in the mean (arithmetic mean) between A-SBD (TTE) and M-SBD were not statistically significant, p=0.820. The paired t test showed that differences in the mean (arithmetic mean) between A-SBD (TEE) and M-SBD were not statistically significant p=0.978. The A-SBD (TTE) formula gives more approximated values of M-SBD in relation to the A-SBD (TEE) but this difference was not statistically significant p=0.48

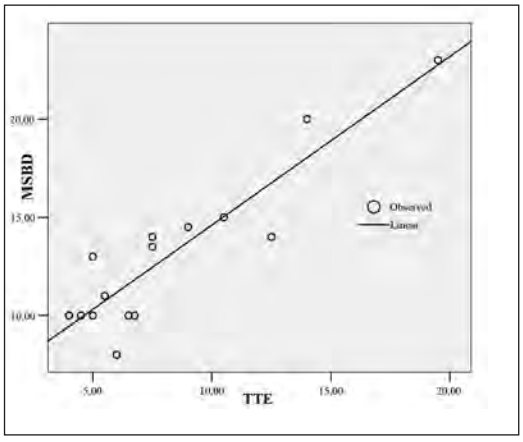

Figure 7 Linear regression analysis graph, comparing transthoracic echocardiography (TTE) and stretched-diameter fluoroscopy (M-SBD).

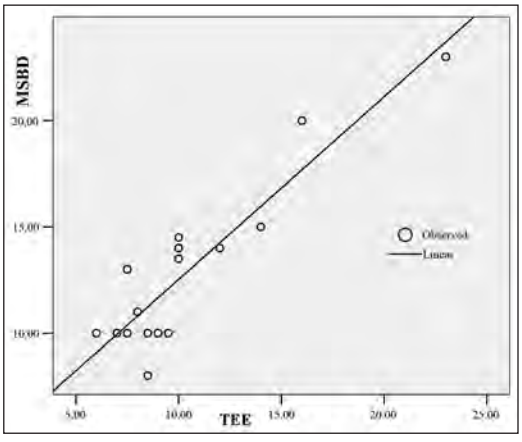

Figure 8 Linear regression analysis graph, comparing transesophageal echocardiography(TEE) and stretched-diameter fluoroscopy (M-SBD).

Discussion

Atrial septal defects as large as 5 to 8 mm may close spontaneously in a significant portion of subjects as old as 2 to 3 years of age (19, 20), so we treated children age <3 years old only when symptoms were present (4 patients). Furthermore, in these subjects the defects were always 8 mm or larger. Rastegari et al. (21) reported a series of 20 subjects aged between 6 months and 20 years who underwent ASD closure with an Amplatzer device. They reported no complications and successful closure was achieved in all subjects. In our series of 10 patients aged ≤5 years, successful closure was achieved in all. Technical problems occurred in two subjects, but these problems were managed percutaneously. Although no major complications occurred in our series, complications are, of course, possible. In our study, only one patient had transient 2nd-degree atrioventricular block, which occurred 2 days after device implantation and resolved spontaneously the next day. All patients in whom a (ASO) device was placed were in sinus rhythm at their 6-month follow up evaluations. Finally, there were no complications during the follow up; patients with frequent respiratory infections had no significant recurrences; and subjects with failure to thrive showed significantly better development.

Rao et al. (22) compared the stretched ASD diameter with 2-D echocardiographic measurements obtained in two subcostal views (long- and short-axis). Rao and colleagues used the following formula to estimate the stretched diameter of ASD from the TTE measurement: 1.05×TTE diameter of ASD in mm+5.49. In their prospective study, this equation was found to accurately predict the stretched diameter ($p<0.001$). However, Rao's group actually pulled the sizing balloon through the septum, rather than just occlude the septum. In the patients whom we studied, the balloons were used for occlusion only.

The echocardiographically measured diameter correlated well with the stretched diameter ($r=0.82$; $p<0.001$). The stretched diameter could be estimated by the following formula: 1.05× echocardiographically obtained diameter in millimeters + 5.49. The differences between measured and predicted values were within 2 mm. Therefore, the stretched ASD diameter could be estimated accurately by 2-D subcostal echocardiographic measurement, which in turn could be used for selection of device size for occlusion of the ASD. Jan et al. (23) obtained a similar formula: the stretched ASD diameter = 1.09 × TTE-measured ASD diameter in millimeters + 3.9.

Zhang (24) found good correlation between the diameter of ASD with soft or hard rims with the selected ASO size, but the authors neither illustrated which ASD diameters were measured from several echocardiographic views as the bases for selecting ASO size nor analyzed the potential influences of different ASD diameter ranges on selecting ASO size. Several studies showed that ASD diameters measured by echocardiography, TTE or TEE, were always 4–6 mm smaller than those measured by balloon (stretched diameter) (25, 26).

Comparing the results of two formulas, "our" A-SBD (TTE) and the results of the formulas used in clinical studies ASBD, the conclusion is that both formulas can be used because the differences in relation to the actual values are not statistically significant $t=0.709$ $p=0.49$.

In the present study, before ASD interventional therapy, we used TTE to detect ASD diameter as a routine process to guide ASO size selection and obtained good results. Multiple views were obtained for the the largest ASD diameter as the reference for selecting ASO size. For small ASD, because the rims were generally intact, an ASO size a little larger than the ASD diameter measured by TTE was sufficient. However, for a

larger ASD, the rims were often soft or not intact and the large ASO weight was also a negative factor for the ASO fixing, so the selected ASO size was much larger than the ASD diameter.

The success of shunt closure was based on the results of TEE with color Doppler imaging. Patients were considered to have undergone successful ASD closure if they had *(a)* no left-to-right flow across the atrial septum, *(b)* trivial flow (jet width, <1 mm), or *(c)* a small (jet width, ≥1 but <2 mm) residual shunt at color Doppler TEE.

The position, shape and diameter of ASD are key factors for deciding whether an ASD can be occluded through a catheter, what ASO size is required and the possibility of success. The method used for evaluating whether an ASD is suitable for interventional therapy should be convenient and accurate. The generally used methods include TTE and TEE. TTE examination is relatively easy and convenient. A pediatric cardiologist with extensive experience in echocardiography and interventional therapy in congenital heart disease, can in general, through a thorough, multi-view examination with TTE, obtain sufficient information about the ASD diameter, shape, position and rims. The image quality demonstrated by TTE is usually influenced by factors such as age, obesity, intercostal space, thoracic deformity and lung diseases. The Amplatzer septal occluder has a central waist for closure of the defect and two disks for fixation. Accurate assessment of the location and size of the ASD and surrounding rims is essential for determining whether implantation of an Amplatzer septal occluder or surgical repair is appropriate (4–17). TTE is a useful screening tool for the detection of ASD. However, signal dropout at TTE due to inability to evaluate the atrial septum perpendicularly may lead to a false-positive diagnosis of ASD (8, 9). In TTE, technical difficulty in locating the optimal perpendicular axis of the ASD to various anatomic landmarks may result in inadequate assessment of the surrounding rims (8–12). ASD with anterior superior rim deficiency is common, the reported incidence ranging from 28% to 54% (4, 5, 13, 16, 17). The "true" diameter of the ASD is somewhat enigmatic, especially since the ASD might be oval (from its origin as the fossa ovalis). It is likely that the standard method for sizing—using the stretched diameter—may stretch the ASD, necessitating the use of a larger device or even excluding the patient from transcatheter ASD closure. However, if the ASD is >10 mm by TTE, it is likely that TTE will underestimate the size of the defect, necessitating further testing with TEE, which can be performed in the cardiac catheterization laboratory before the catheterization procedure. Huang et al. (17) reported that Amplatzer septal occluder closure is effective for ASD with a deficient anterior superior rim. Nevertheless, some anatomic conditions have been described as being troublesome for Amplatzer septal occluder closure. These conditions include small atrial capacity, which can limit full expansion of the disks of the Amplatzer occluder; floppy septum, which can cause prolapse of the occluder; thin septum, which can easily be torn after deployment of the occluder, and large ASD, which can be underestimated with resultant dislodgment of the occluder (15–17).

Study limitations

Not all ASDs can be treated by percutaneous techniques. In fact, more than 50% children age ≤5 years who were assessed for ASD repair underwent percutaneous closure due technical reasons.

Clinical implications

The demonstration of the safety and efficacy of ASD percutaneous closure in young and very young children has some clinical im-

plications. The first important advantage of percutaneous techniques is related to their lesser psychological impact. In fact, the absence of skin scars, the shorter hospitalization, and the avoidance of admission to an intensive care unit are widely appreciated by patients and parents. There may also be some advantages during the follow up. First, the absence of a scar on atrial myocardium may reduce the incidence of incisional arrhythmias. Second, bypass surgery is complicated by a late decline in cognitive function, as shown by Newman et al. (27) in patients undergoing coronary artery bypass graft. Even in pediatric patients, there is some evidence that bypass surgery may be related to a slightly poorer neuropsychological outcome at follow-up (28). Appropriate handling of a noninvasive tool capable of facilitating accurate measurement of ASD and detailing anatomic information would be beneficial for treatment planning and avoiding a large proportion of intraprocedural failures.

Conclusion

TTE, used to measure ASD diameter, can accurately direct the selection of the ASO needed for successfully closure of ASD, especially for relative small ASDs. The larger the ASD, the much larger the ASO needed and the more difficult the interventional procedure. TTE is effective and safe as an imaging guide for ASD transcatheter closure. The obtained formulas represents "our" default size of the SBD, based on measurements of TTE and TEE: M-SBD (TTE)=6.02+0.86×TTE and M-SBD(TEE)=3.93+ 0.86×TEE.

Authors' contributions: Conception and design: MH, SMD and RG; Acquisition, analysis and interpretation of data: MH and RG; Drafting the article: MH, SMD; Revising it critically for important intellectual content: SMD, ZB, AK.

Conflict of interest: The authors declare that they have no conflict of interest. This article was not sponsored by any external organization.

References

1. Dickenson DE, Arnold R, Wilkinson JL. Congenital heart disease among 160 480 liveborn in Liverpool 1960-1969. Implication for surgical treatment. Br Heart J. 1981;46:55-62.
2. Kirklin JW, Barratt-Boyces BG. Cardiac surgery. 2nd ed. New York: Churchill Livingstone; 1993. p. 609-44.
3. Galal MO, Wobst A, Halees Z, Hatle L, Schmaltz AA, Khougeer F, et al. Perioperative complications following surgical closure of atrial septal defect type II in 232 patients: a baseline study. Eur Heart J. 1994;15:1381-4.
4. Du ZD, Hijazi ZM, Kleinman CS, Silverman NH, Larntz K. Comparison between transcatheter and surgical closure of secundum atrial septal defect in children and adults: results of a multicenter nonrandomized trial. J Am Coll Cardiol. 2002;39:1836-44.
5. Masura J, Gavora P, Podnar T. Long-term outcome of transcatheter secundum-type atrial septal defect closure using Amplatzer septal occluders. J Am Coll Cardiol. 2005;45:505-7.
6. Yew G, Wilson NJ. Transcatheter atrial septal defect closure with the Amplatzer septal occluder: five-year follow-up. Catheter Cardiovasc Interv. 2005;64:193-6.
7. Faletra F, Scarpini S, Moreo A, Ciliberto GR, Austoni P, Donatelli F, et al. Color Doppler echocardiographic assessment of atrial septal defect size: correlation with surgical measurement. J Am Soc Echocardiogr. 1991;4:429-34.
8. Marx GR, Sherwood MC, Fleishman C, Van Praagh R. Three-dimensional echocardiography of the atrial septum. Echocardiography. 2001;18:433-43.
9. Lu JH, Hsu TL, Hwang B, Weng ZC. Visualization of secundum atrial septal defect using transthoracic three-dimensional echocardiography in children: implications for transcatheter closure. Echocardiography. 1998;15:651-66.
10. Acar P, Roux D, Dulac Y, Rouge P, Aggoun Y. Transthoracic three-dimensional echocardiography prior to closure of atrial septal defects in children. Cardiol Young. 2003;13:58-63.
11. Durongpisitkul K, Tang NL, Soongswang J, Laohaprasitiporn D, Nana A, Kangkagate C. Cardiac magnetic resonance imaging of atrial septal defect for transcatheter closure. J Med Assoc Thai. 2002;85(Suppl 2):S658-66.
12. Durongpisitkul K, Tang NL, Soongswang J, Laohaprasitiporn D, Nanal A. Predictors of successful transcatheter closure of atrial septal defect by

cardiac magnetic resonance imaging. Pediatr Cardiol. 2004;25:124-30.

13. Mazic U, Gavora P, Masura J. The role of transesophageal echocardiography in transcatheter closure of secundum atrial septal defects by the Amplatzer septal occluder. Am Heart J. 2001;142:482-8.
14. Zhu W, Cao QL, Rhodes J, Hijazi ZM. Measurement of atrial septal defect size: a comparative study between three-dimensional transesophageal echocardiography and the standard balloon sizing methods. Pediatr Cardiol. 2000;21:465-9.
15. Carcagni A, Presbitero P. New echocardiographic diameter for Amplatzer sizing in adult patients with secundum atrial septal defect: preliminary results. Catheter Cardiovasc Interv. 2004;62:409-14.
16. Mathewson JW, Bichell D, Rothman A, Ing FF. Absent posteroinferior and anterosuperior atrial septal defect rims: factors affecting nonsurgical closure of large secundum defects using the Amplatzer occluder. J Am Soc Echocardiogr. 2004;17:62-9.
17. Huang CF, Fang CY, Ko SF, Chien SJ, Lin IC, Lin YJ, et al. Transcatheter closure of atrial septal defects with superior–anterior rim deficiency using Amplatzer septal occluder. J Formos Med Assoc. 2007;106:986-91.
18. Lin SM, Tsai SK, Wang JK, Han YY, Jean WH, Yeh YC. Supplementing transesophageal echocardiography with transthoracic echocardiography for monitoring transcatheter closure of atrial septal defects with attenuated anterior rim: a case series. Anesth Analg. 2003;96(6):1584-8.
19. Helgason H, Jonsdottir G. Spontaneous closure of atrial septal defects. Pediatr Cardiol. 1999;20:195-9.
20. Radzik D, Davignon A, Van Doesburg N, Fournier A, Marchand T, Ducharme G. Predictive factors for spontaneous closure of atrial septal defects diagnosed in the first 3 months of life. J Am Coll Cardiol. 1993;22:851-3.
21. Rastegari M, Redington A, Sullivan ID. Influence of the introduction of Amplatzer device on the interventional closure of defects within the oval fossa in children. Cardiol Young. 2001;11:521-5.
22. Rao PS, Langhough R, Beekman RH, Lloyd TR, Sideris EB. Echocardiographic estimation of balloon-stretched diameter of secundum atrial septal defect for transcatheter occlusion. Am Heart J. 1992;124:172-5.
23. Jan SL, Hwang B, Lee PC, Fu YC, Chiu PS, Chi CS. Intracardiac ultrasound assessment of atrial septal defect: comparison with transthoracic echocardiographic, angiocardiographic, and balloon-sizing measurements. Cardiovasc Intervent Radiol. 2001;24:84-9.
24. Zhang J. The echo diagnosis and intervention of atrial septal defect. In: Zhang YS, Zhu XY, Zhang J, editors. Congenital heart disease intervention and echo diagnosis progress. Xi'an: World Book Publishing Co. Ltd; 2005. p. 108-14.
25. Demkow M, Ruzyllo W, Konka M, Kepka C, Kowalski M, Wilczynski J, et al. Transvenous closure of moderate and large secundum atrial septal defects in adults using the Amplatzer septal occluder. Catheter Cardiovasc Interv. 2001;52:188-93.
26. Durongpisitkul K, Soongswang J, Laohaprasitiporn D, Nana A. Intermediate term follow-up on transcatheter closure of atrial septal defects by Amplatzer septal occluder. J Med Assoc Thai. 2000;83:1045-53.
27. Newman MF, Kirchner JL, Phillips-Bute B, Gaver V, Grocott H, Jones RH, et al. Longitudinal assessment of neuro- cognitive after coronary by-pass surgery. N Engl J Med. 2001;344:395-402.
28. Visconti KJ, Bichell DP, Jonas RA, Newburger JW, Bellinger DC. Developmental outcome after surgical versus interventional closure of secundum atrial septal defect in children. Circulation. 1999;100(19 Suppl):II145-50.

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Suicide among childhood cancer survivors in Slovenia

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Objective. Suicide is one of the causes of late mortality among childhood cancer survivors. The aim of our study was to analyse the risk of suicide among childhood cancer survivors compared with that of the general population of Slovenia. **Patients and methods**. This retrospective study included patients with childhood cancer registered at the Cancer Registry of Slovenia between 1978-2008, with an observation period of 1978-2010. Childhood cancer patients and control subjects from the general population of Slovenia were matched by sex, year and age at the beginning of follow-up and time of follow-up in years. Data on the general population of Slovenia were obtained from the Statistical Office of the Republic of Slovenia. **Results**. A total of 1647 patients were recorded in the Cancer Registry as having cancer during childhood, with 3 patients committing suicide. All three were male. Their age at diagnosis of cancer was 12, 13 and 2 years old; their age at suicide was 19, 32 and 28 years old. The mechanism of death was asphyxiation in all three deaths. The calculation of the expected number of suicides in the group of individuals with childhood cancer from the general Slovene population revealed the number of 3.16 persons. **Conclusion**. The comparison of the observed and expected probability showed that there was no statistically significant difference in the suicide rate between childhood cancer survivors and the general population of Slovenia.

Key words: Childhood cancer, Late mortality, Slovene population, Suicide risk.

Introduction

Suicide is the 13th leading cause of death in the world (1). In Slovenia, with a population close to 2 million, suicide accounts for 2.4% of all deaths in the general population (from the 10-14 years age group to the 80 years and above age group)(2). Slovenia is ranked fifth among European countries in terms of suicides (3).

One of the causes of late mortality in childhood cancer survivors is suicide.An increased risk of suicide has been reported among childhood cancer survivors (4, 5). It has been suggested that when viewed in the context of population–based studies of

suicide, adult survivors of childhood cancer have an elevated risk for suicidality (4, 5) while others have reported no correlation between childhood cancer associated with a risk of suicide (6, 7). The variation in the frequency of suicides among different countries (8) might be correlated to several cultural and behavioral factors, which also reflect suicidal behavior among childhood cancer survivors.

In an analysis of 228 childhood cancer survivors aged 18 to 61 years (114 women and 114 men), treated and followed up at the Oncological Institute in Slovenia, patients were matched by sex and age with a control group of individuals, who did not experience any chronical disease during childhood (9). However, the study showed there were higher rates of depression among childhood cancer survivors than among controls. Suicidal thoughts were, however, present in childhood cancer survivors in equal frequency as in the controls, and a plan for suicide was present in both groups in 8.7% (9). It has been recommended that depressive disorder should alert for risk assesment of suicidal adolescents (10).

The aim of this paper was to compare suicide frequency among children treated for cancer with the suicide frequency of the general population in Slovenia.

Materials and methods

Subjects

The study population was comprised of individuals with childhood cancer, registered with the Cancer Registry of Slovenia between 1978-2008 and treated at the Department of Oncology and Haematology of the University Children's Hospital in Ljubljana. The observation period was 1978–2010.

Inclusion criteria: diagnosis of childhood cancer, patient age at the start of observation ≥5 years old, first diagnosis made between 1978–2008, or year of first diagnosis before 1978, but patient still alive in 1978.

Exclusion criteria: nonresidents were excluded.

All children with cancer in Slovenia are treated at the Department of Oncology and Haematology of the University Children's Hospital in Ljubljana. After treatment, all are followed up by the same center for at least five years or until they reach the age of 18 years old. Since 1986, all childhood cancer survivors have been followed up regularly, at least once every year, at the outpatient Clinic for Late Effects at the Institute of Oncology, Ljubljana (11).

Demographic data (age, sex) and medical information on diagnosis, date of diagnosis and treatment were obtained from medical records, while information on patient status (alive/suicide/other cause of death) was obtained from the Cancer Registry of Slovenia or from the Clinic for Late Effects at the Institute of Oncology.

For the purpose of this study, subjects from the general population of Slovenia were used as a comparison group. Childhood cancer patients and control subjects from the general population were matched by the following characteristics: sex, year of the beginning of follow-up, age at the beginning of follow-up, and time of follow-up in years.

The year of the beginning of follow-up was either:

(a) the year of diagnosis between 1978–2008 if at diagnosis the patient was aged 5 years or more, or

(b) the year of patient's age of 5 years if the diagnosis was set between 1978–2008 and at diagnosis the patient was younger than 5 years, or

(c) the year of patient's age of 5 years if the diagnosis was set before 1978 and in 1978 the patient was younger than 5 years, or

(d) the year when observation period started (i.e. the year 1978) if the diagnosis was set before 1978 and in 1978 the patient was still alive and aged 5 years or more.

The time of follow-up was defined as the time from diagnosis or first year of the beginning of follow-up to the event, which was defined as suicide, death to other causes, or year of last follow-up (i.e. 2010 was taken as the end of the observation period, or some earlier year if the patient was lost from follow-up).

Data on the general population of Slovenia were obtained from the Statistical Office of the Republic of Slovenia, SORS (12). As the data on death to suicides in Slovenia were recorded at SORS separately by sex as late as 1978, we had to adjust our observation period to 1978 – 2010, although the first records in the Cancer Registry of Slovenia dated from 1950.

Statistical analysis

Basic demographic and clinical characteristics were presented using descriptive statistics. The SORS reports data on the general population and deaths in 5-years age groups, our data is reported in a similiar manner. Descriptive analysis was carried out using SPSS 20.0 statistical software (SPSS Inc., Chicago; IL, USA), while the R language (13) was used for inferential analysis.

To compare the observed number of suicides from the patient population and the expected number of suicides, if we assume that the suicide rate among cancer patients is smiliar to that of the general population, the binomial test was applied. The expected number of suicides in the patient population was calculated from the general Slovene population, comparable to the patient population in terms of 4 characteristics, noted in the Subjects section. To locate the comparable general population group and for calculating the expected number of suicides, a special programme in R language was used, prepared at the Institute for Biostatistics and Medical Informatics in Ljubljana specifically for this purpose.

The expected number of suicides was calculated as follows: in the general population, for each patient a comparable individual, in terms of sex, year and age at the beginning of follow-up, was looked for. For this individual, on the basis of data of the number of suicides and the number of residents the probability of suicide was calculated for each year of follow-up. Finally, the probabilities of suicides for all years of follow-up were summed up, which gave us the expected number of suicides in the study population according to the general population for this specific patient. To gain the overall expected number of suicides, all probabilities summed up per patient were summed up for all patients.

Results

A total of 1647 individuals with cancer during childhood were included in the analysis. Demographic and clinical information is presented in Table 1. Patients were followed up from 1 to 33 years. At the end of the follow-up period, the individuals' ages ranged from 5 to 66 years old, with the majority (43%) of them 20 to 39 years old. The most frequent diagnosis was leukemia (26%) and tumors of the central nervous system (19%). The majority of patients were treated with both irradiation and chemotherapy (29%),

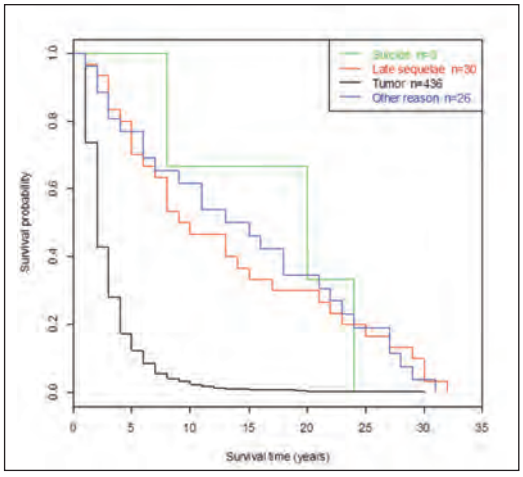

Figure 1 Estimated survivorship functions for subjects in respect to cause of death.

Table 1 Demographic and clinical characteristics of individuals with childhood cancer

Characteristic	Subjects (n=1647)
Age at cancer diagnosis, years	8.2 (4.9), [0-18]
Age at the beginning of the follow-up, years	9.4 (4.4), [5-34]
Age at the end of the follow-up, years	22.3 (11.9), [5-66]
Age at the end of the follow-up, n (%)	
<10 years	261 (16)
10-19 years	507 (31)
20-39 years	708 (43)
≥40 years	171 (10)
Time of observation, years	13.9 (10.7), [1-33]
Sex, n (%)	
Female	713 (43)
Male	934 (57)
Cancer diagnosis, n (%)	
Leukemia	430 (26)
Central nervous system	317 (19)
Hodgkin's disease	150 (9)
Non-Hodgkin's lymphoma	140 (9)
Renal tumors	94 (6)
Neuroblastoma	59 (4)
Rhabdomyosarcoma	62 (4)
Malignant bone tumors / Ewing's and PNET	111 (7)
Soft-tissue sarcomas	54 (3)
Gonads	55 (3)
Carcinomas	109 (7)
Other neoplasms	51 (3)
Unspecified malignant neoplasms	15 (1)
Cancer treatment*, n (%)	
SR=Surgery	232 (14)
RT=Radiation therapy	41 (2)
ChT=Chemotherapy	235 (14)
SR + RT	169 (10)
RT + ChT	478 (29)
SR + ChT	230 (14)
SR + RT + ChT	243 (15)

Values are mean (standard deviation) and [min, max], unless otherwise specified. *Missing data in six patients; percentages calculated per 1641 subjects. PNET=Primitive neuroectodermal tumor.

while 8 (0.5%) patients received no therapy because they had died before therapy started, and 5 (0.3%) had no treatment (four with IV S neuroblastoma and one with brain tumor).

At the end of the follow-up, 1152 (70%) individuals were still alive, while 495 (30%) had died. According to the cause of death, estimated survivorship functions are shown in Figure 1. The longest median survival time was recorded for deaths from suicide (20 years), a shorter median survival time was recorded for deaths due to other reasons

Table 2 Comparison of observed and expected probability of suicide, calculated by binomial test

	Observed	Expected	P value
Number of suicides	3	3.1555	-
Probability of suicides	3/1647=0.0018214	3.1555/1647=0.0019159	1

Table 3 Demographic and clinical characteristics of individuals who committed suicide

Characteristic	Person 1	Person 2	Person 3
Sex	Male	Male	Male
Diagnosis	Osteogenic sarcoma	Brain tumor	Soft tissue sarcoma
Treatment	SR+ChT	SR+RT	SR+ChT
Year of birth	1966	1974	1981
Age at diagnosis (yrs)	12	13	2
Date of suicide	May 1985	July 2006	June 2009
Time of follow-up (yrs)	7	19	23
Age at suicide (yrs)	19	32	28
Method of suicide	Strangulation	Strangulation	Strangulation

ChT=chemotherapy; RT=radiation therapy; SR=surgery.

(injury, traffic accident, asphyxiation) and due to late sequelae (13 and 9 years, respectively), while the shortest median survival time was observed for deaths because of the primary malignant disease (2 years).

Out of 1647 individuals, only 3 suicides were recorded during the observation period. The calculation of the expected number of suicides in the group of individuals with childhood cancer from the general Slovene population was 3.1555 persons. The probability of suicide, calculated from among the study population, was 0.0018214, (95% confidence interval: 0.0003758-0.0053139). The comparison of observed and expected probability showed that there was no statistically significant difference between the two groups (p=1) (Table 2). The characteristics of the three individuals that committed suicide are shown in Table 3.

Discussion

Suicide is a significant public health issue in Slovenia and around the world. Any attempt to ascertain the reason for committing suicide may prevent future acts. In this current study, we identified 3 suicides among patients treated for childhood cancer. All the victims were males aged 19, 32 and 28 years old. This study provided some insight into the possible reasons that triggered suicide. One patient (Patient #1) was dissatisfied due to physical distress following exarticulation of the femur, while another patient (Patient #2) was distressed following treatment by cranial radiation. These two patients did not have regular follow-up. Some reports have cited that dissatisfaction with physical appearance, poor physical health and treatment with cranial radiation were associated with psychological distress (14). Cranial radiotherapy, causing a specific pattern of cognitive and educational sequelae, is associated with suicidal ideation (15). The third patient, who was regularly followed at the outpatient clinic for late effects, was in perfect health with no complaints 19 months prior to death. The reason for the suicide was not determined, although it was reported that he consumed huge amounts of alcohol.

None of these three patients were included in the group of childhood cancer survivors analysed for depression conducted in 2006 (9). In that study 146 childhood cancer survivors, all under 18 years old at diagnosis, were followed up at the Oncological Institute in Ljubljana (9). About 40% of them reported that they thought they would rather be dead, among them twice as many women, 29% had active suicide thoughts, while 8.7% had a suicide plan. Seven (7/146, 5%) survivors actually tried to commit suicide, in comparison with one (1/87, 1%) in the control group. This was the only statistically significant ($p<0.001$) difference comparing the depressive symptoms of the study group and the control group. There were no successful suicides among the childhood cancer survivors. It would seem that subjects with the experience of childhood cancer appreciate life in a way which protects them from committing suicide.

Also among those survivors who had been attending group meetings (once per month), only a half of them reported depressive symptoms (9).

In a study by Recklitis (14), childhood cancer survivors who completed screening with the Symptom Checklist 90 Revised (SCL-90), reported little (15%) or no (84%) distress, but had a positive screen on the Mental Scale, indicating significant psychological distress. Suicidal symptoms were reported in 14% of the children. Though it noted important differences, risk factors for suicide attempts and completion are well established (16), it still seems hard to predict suicide in a certain individual.

Regarding reports of the high risk for suicide among childhood cancer survivors a question arose: Have any of those survivors in the study, whose responses led the investigators to conclude that they were at risk for suicide actually committed suicide after the completion of data collection? Zebrack proposed an analysis that would have made a comparison to see if the same respondents who indicated suicidality in their survey also were assesed and indentified by clinicians as demonstrating indicators of suicide risk ideation (17).

In our study, the observed suicide frequency among children treated for cancer did not differ from the expected frequency in to the general Slovene population (3 versus 3.16, $p=1$). Slovenia is a small country, with only 2 million inhabitants, so it is hard to make conclusions in this respect. However, our results were similar to previously published large-scale studies which suggest the risk of suicides in survivors does not exceed the risk observed in the appropriately matched general populations (18-23). However, a recent review reports evidence that the prevalence of completed suicides is greater in a cancer population than in the general population (16).

In countries where suicide research is strong, suicide rates have been declining due to universal preventive interventions. As pointed out by Nordentoft (24), suicide is a major public health problem and it should be given high priority with regard to prevention and research.

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References

1. Krug EG. World Report on Violence and Health. World Health Organization. Geneva; 2002.
2. Statistical Yearbook 2010 (database on the Internet]. SORS [cited 2012 Aug 20]. Available from: http://www.stat.si/letopis/LetopisVsebina.aspx?poglavje=4&lang=si&leto=2010
3. Death due to suicide [database on the Internet]. EUROSTAT [updated 2012 Apr 02; cited 2012

Aug 20]. Available from: http://epp.eurostat.ec.europa.eu/tgm/table.do?tab=table&init=1&plugin=0&language=en&pcode=tps00122

4. Recklitis CJ, Diller LR, Li X, Najita J, Robinson LL, Zeltzer L. Suicide ideation in adult survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. J Clin Oncol. 2010;28(4):655-61.
5. Howard RA, Inskip PD, Travis LB. Suicide after childhood cancer. J Clin Oncol. 2007;25(6):731.
6. Mertens AC. Cause of mortality in 5-year survivors of childhood cancer. Pediatr Blood Cancer. 2007;48(7):723-6.
7. Teta MJ, Del Po MC, Kasl SV, Meigs JW, Myers MH, Mulvihill JJ. Psychosocial consequences of childhood and adolescent cancer survival. J Chronic Dis. 1986;39(9):751-9.
8. Centers of Disease Control and Prevention (CDC). Rates of homicide, suicide, and firearm–related death among children – 26 industrialized countries. MMWR Morb Mortal Wkly Rep. 1997;46(5):101-5.
9. Svetičič J, Marušič A, Jereb B. Ali je med preživelimi raka v otroštvu več depresivnosti in samomorilnega vedenja? Onkologija. 2006;10(2):75-80.
10. Nrugham I, LarssonB, Sund AM. Specific depressive symptoms and disorders as associates and predictors of suicidal acts across adolescence. J Affect Disord. 2008;111(1):83-93.
11. Jereb B. Model for long-term follow–up of survivors of childhood cancer. Med Pediatr Oncol. 2000;34(4):256-8.
12. Statistical Office of the Republic of Slovenia [homepage on the Internet]. Ljubljana: The Statistical Office [cited Aug 22]. Available from: http://www.stat.si/eng/index.asp
13. R Core Team [homepage on the Internet]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna (Austria); ISBN: 3-900051-07-0; c2012 [cited Aug 22]. Available at: http://www.R-project.org
14. Recklitis CJ, Lockwood RA, Rothwell MA, Diller LR. Suicidal ideation and attempts in adult survivors of childhood cancer. J Clin Oncol. 2006;24(24):3852-7.
15. Recklitis C, O'Leary T, Diller L. Utility of routine psychological screening in the childhood cancer survivor clinic. J Clin Oncol. 2003;21(5):787-92.
16. Robson A, Scrutton F, Wilkinson L, MacLeod F. The risk of suicide in cancer patients: a review of the literature. Psychooncology. 2010;19(12):1250-8.
17. Zebrack BJ, Ell K, Smith WB. Suicide Risk in Childhood Cancer Survivors. J Clin Oncol. 2007;25(6):732-3.
18. Mertens AC, Yasui Y, Neglia JP, Potter JD, Nesbit ME Jr, Ruccione K, et al. Late mortality experience in five-year survivors of childhood and adolescent cancer: the Childhood Cancer Survivor Study. J Clin Oncol. 2001;19(13):3163-72.
19. Möller TR, Garwicz S, Barlow L, Falck Winther J, Glattre E, Olafsdottir G, et al. Decreasing late mortality among five-year survivors of cancer in childhood and adolescence: A population-based study in the Nordic countries. J Clin Oncol. 2001;19(13):3173-81.
20. Robertson CM, Hawkins MM, Kingston JE. Late deaths and survival after childhood cancer: implications for cure. BMJ. 1994;309(6948):162-6.
21. Hawkins MM, Kingston JE, Kinnier Wilson LM. Late deaths after treatment for childhood cancer. Arch Dis Child. 1990;65(12):1356-63.
22. Hudson MM, Jones D, Boyett J, Sharp GB, Pui CH. Late mortality of long-term survivors of childhood cancer. J Clin Oncol. 1997;15(6):2205-13.
23. Cardous-Ubbink MC, Heinen RC, Langeveld NE, Bakker PJ, Voûte PA, Caron HN, et al. Long-term cause-specific mortality among five-year survivors of childhood cancer. Pediatr Blood Cancer. 2004;42(7):563-73.
24. Nordentoft M. Prevention of suicide and attempted suicide in Denmark. Epidemiological studies of suicide and intervention studies in selected risk groups. Dan Med Bull. 2007;54(4):306-69.

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Diagnosing and treating hemophagocytic lymphohistiocytosis in the tropics: Systematic review from the indian subcontinent

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Background. Hemophagocytic lymphohistiocytosis (HLH) is a catastrophic syndrome of unrestrained immune activation. Evaluation and management of HLH in the tropics is challenging. **Objectives.** To examine the reported etiologies and management of HLH reported from the sub-continent. **Methods.** Systematic review of all published cases from the Indian sub-continent. **Results.** We found only 156 published cases of HLH from the sub-continent. HLH was reported from the immediate perinatal period to 46 years of age. Infection-associated HLH (IAHS) constituted 46.8% of all cases of HLH (44% and 51% in children and adults respectively). In adults, tropical infections triggered 51% of these cases of IAHS. Steroids were used in 47% of children and 10% of adults. Etoposide and/or cyclosporine were used in 8% children and 8% of adults only. Intravenous immunoglobulin was used in another 30% of children and 4% of the adults. HLH-related mortality occurred in 31.8% and 28% of children and adults respectively. **Conclusions.** HLH is under-reported in the sub-continent and has high mortality. Cyclosporine and etoposide are seldom administered early despite diagnosis of HLH. Larger cohorts with IAHS triggered by tropical infections are urgently needed to understand its natural history and implications of this differing prescription pattern on mortality.

Key words: Hemophagocytic lymphohistiocytosis, Hemophagocytic syndrome, India, Indian sub-continent.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a potentially fatal syndrome secondary to immune hyperactivation (1). HLH may be genetic in origin or arise secondary to infectious, rheumatologic, malignant or metabolic disorders (Table 1).

Irrespective of cause, the unrestrained immune hyperactivation characteristic of this syndrome leads to host tissue damage (2). The diagnosis of this syndrome is cur-

Table 1 Etiology of Hemophagocytic lymphohistiocytosis

Category	Specific causes	Defect
Genetic HLH		
FHLH1	Unknown	Unknown
FHLH2	PRF1	Vesicle content
FHLH3	Munc13.4	Vesicle priming
FHLH4	STX11	Vesicle docking and fusion
FHLH5	STXBP2	Vesicle docking and fusion
Associated with other syndromes		
Chediak-Higashi I	Autosomal recessive, oculocutaneous albinism, easy bruising and frequent pyogenic infections, due to decreased chemotaxis and bactericidal activity; large neutrophil granules & abnormalities in LYST gene	
Griscelli II	Autosomal recessive syndrome; hypomelanosis with immunologic abnormalities with or without neurologic impairment, caused by mutation in the RAB27A gene; normal leukocyte pigmentation	
Hermansky-Pudlak II	Homozygous mutations of β3A subunit of the AP3 complex (AP3β1) on chromosome 5q14.1. Partial oculocutaneous albinism, platelets lacking dense bodies and storage of ceroid-like material in tissues. May include interstitial lung disease, renal abnormalities, cardiomyopathy	
XLPI (X-linked)	Mutation in the SH2D1A gene encoding SLAM-associated protein (SAP). Phenotype has severe or fatal mononucleosis, acquired hypogammaglobulinema, HLH and lymphoma. May include aplastic anemia, red cell aplasia, and lymphomatoid granulomatosis	
XLPII (X-linked)	Similar phenotype; mutation in X-linked inhibitor of apoptosis	
Metabolic syndromes		
Congenital lysinuric protein intolerance, Di- George's, Omenn's and Wiskott-Aldrich syndrome		
Infections		
Viral	EBV (most common); CMV, HIV, Avian influenza, Parvovirus B19, dengue shock syndrome, HHV-6,8, Varicella-Zoster, Herpes simplex	
Bacterial	Salmonella spp, leptospirosis, Rickettsia	
Protozoal	Plasmodium, leishmania	
Mycobacterial	*Mycobacterium tuberculosis* (disseminated tuberculosis)	
Fungal	Invasive aspergillosis, Penicillium marneffi infection	
Malignancy		
	T-cell lymphoblastic leukemia	
Rheumatologic disorders		
	Adult-onset still disease, juvenile-onset rheumatoid arthritis, systemic lupus erythematosus	

Abbreviations: FHL-Familial hemophagocytic lymphohistiocytosis, HLH- hemophagocytic lymphohistiocytosis, XLP-X-linked lymphoproliferative syndrome (Duncan's syndrome), EBV-Epstein Barr virus, CMV-cytomegalovirus, HHV-Human herpes virus, PRF1- pore forming protein 1, STX11 syntaxin 11, STXBP2-syntaxin binding protein 2, Munc-mammalian uncoordinated protein gene, LYST-lysosomal trafficking regulator gene, RAB27A- Ras-related protein Rab-27A gene.

rently based on meeting the HLH-2004 criteria (Table 2).

In the tropics, especially in adults, infections are common triggers of HLH (3) and the prevalence of perforin mutations is unknown. The clinical features of tropical infectious triggers can overlap with that of HLH and the diagnosis of the inciting etiology can be challenging (Table 3) (4).

Table 2 Revised diagnsotic criteria for hemophagocytic lymphohistiocytosis (1)

The diagnosis of hemophagocytic lymphohistiocytosis can be established if one of either one or two is fulfilled
1. A molecular diagnosis consistent with hemophagocytic lymphohistiocytosis OR
2. Diagnostic criteria for HLH fulfilled (At least 5 out of the 8 below to be fulfilled)
1. Fever
2. Splenomegaly
3. Cytopenias (affecting ≥2 of 3 lineages in the peripheral blood with Hemoglobin <10 g/dl, platelets <100 x 109/l and neutrophils <1.0x109/l)
4. Hypertriglyceredemia and/or hypofibrogenemia (fasting triglycerides > 265 mg/dl, fibrinogen ≤1.5 g/dl)
5. Hemophagocytosis in bone marrow or spleen or lymph nodes[1]
6. Low or absent NK-cell activity (according to local laboratory)[2]
7. Ferritin ≥500/ µg/dl
8. Soluble CD25 (Soluble Interleukin-2 receptor) ≥2400 U/ml[3]

[1] Hemophaogcytosis may be absent at initial evaluation; repeated marrow and/or tissue aspirates and biopsies may be needed;
[2] Sustained low NK cell activity suggests perforin and/or granzyme pathyway abnormality and screening for CD107a (LAMP-1 cell surface expression by cytometry) expression is recommended; NK-cell activity may be transiently depressed in IAHS. Persistent NK-cell activity depression even in the face of clinical resolution and absent known mutations should trigger referral for bone marrow transplantation.
[3] This is the most sensitive biochemical test and levels co-relate with response and prognosis; levels are age-dependent and local data needs to be ascertained.

Table 3 Unique practice concerns during evaluation and treatment of HLH in the tropics (compared to the western world)

TROPICS	WESTERN WORLD
Age of onset is bimodal with a larger adolescent-young adult population	Most cases are infants and young children; smaller adolescent and adult group
Frequency of familial HLH not characterized in any major ethnic group in the tropics; familial HLH reported but mutation NOT characterized	
Screening studies of immunologic dysfunction1 not widely available	Defined mutations (5 in females and 7 in males part of the initial genetic evaluation2)
Confirmatory genetic tests for HLH not available and frequency undefined	
Extensive search needs to be focused on ruling out tuberculosis, leishmaniasis & malaria as causes of HLH	Similar considerations; however tropical infectious causes are extremely rare as triggers of HLH
HLH secondary to tropical infections may have a better prognosis	Viral (EBV)-related HLH similar prognosis and treatment; increased mortality if etoposide delayed
Case series indicate tuberculosis, leishmaniasis, malaria, rickettsia & dengue-related HLH recover with pathogen-specific treatment ± steroids alone	EBV-related HLH treated with similar (HLH-2004) regimen; relapses may occur. Antivirals also in conjunction
HLH recognition may be delayed, especially in adults with acute presentation because symptoms & signs mimic leishmaniasis, disseminated tuberculosis, malaria or sepsis syndrome	HLH is less common in this age group and these infections are uncommon except in immigrants
IVIG is used along with steroids as the main immunomodulatory therapy	Cyclosporine, etoposide and dexamethasone; methotrexate intrathecally in select cases
No large series or published experience as part of a protocol; case reports and small single-centre series only	Enrollment in HLH-2004 trials; established HLH-2004 or ATG-steroid-BMT protocol

[1] Includes perforin and granzyme assays and/or CD107a expression in all and SAP protein and XIAP protein expression in males only;
[2] Includes PRF1, MUNC13-14, STXBP2, STX11, RAB27A; (SH2D1A, BIRC4 in males only).
Abbreviations: HLH=Hemophagocytic lymphohistiocytosis, ATG=anti-thymocyte globulin, HCT=hematopoietic stem cell transplantation, EBV=Epstein Barr virus, ATG=Anti-thymocyte globulin, IVIG-Intravenous immunoglobulin, BMT=Bone marrow transplantation.

Intense immunosuppression administered for HLH without appropriate antimicrobial therapy can have disastrous consequences and the course of tropical infections associated with HLH may be different from other causes of HLH (5). We performed a systematic review to identify the common etiologic triggers of HLH and suggest an appropriate initial etiologic evaluation and management strategy for HLH in the sub-continent.

Methods

Literature search

Two of the authors (Dr. R.S and Dr. N.S) conducted a systematic search of English literature independently using the terms "hemophagocytosis", "hemophagocytic lymphohistiocytosis" "Macrophage activation" *and* "Asia" *or* "India" in the MEDLINE, OVID and CINAHL databases. This was further supplemented by search of IndMED, the internet search engine GOOGLE and a hand search of the references and our personal databases for published cases of hemophagocytic lymphohistiocytosis from the Indian sub-continent including India, Pakistan, Sri Lanka, Bangladesh, Nepal and Bhutan.

Only those articles were included for analysis which were reported in English literature and included patients with diagnosed HLH fulfilling the 2004 criteria of the Histiocyte society as evidenced by 1) genetic diagnosis of HLH *or* 2) At least 5 out of the 8 criteria for clinical diagnosis of HLH (Table 1).

Data extraction

Both the abstracts and full text articles, where available, were reviewed. Where neither was available, the authors were mailed for data on their published cases. Data was extracted in a pre-designed data extraction form regarding the age, sex, etiology of HLH, treatment of HLH administered and outcomes. Data was extracted and expressed in a descriptive fashion (Mean, SD; Table 4)

Results

Our search yielded 682 references. This included 156 cases of HLH (Table 4 A&B) in 56 published reports, including 63 adults (≥8 years of age) and 93 children.

Full text or abstracts were available for all 156 cases in the articles reviewed (4-59). An additional 24 reports were excluded because they reported cases from outside the subcontinent, they did not meet current criteria for HLH (60-64) or did not report any new case of HLH (65). Cases reported in duplicate were not included for the analysis (26).

HLH was reported from the immediate perinatal period (55) to 46 years of age (36). Adults formed 40.4% (N=63/156) of the total number of cases reported (Table 4 A&B). The male to female ratio was 2:1 in adults (N=15) and children (N=49). Most published cases were single case reports. Clinical series reported a confirmed infection as a triggering factor for HLH in 42-43% (5, 9, 56) of their cohorts.

In children, definite (known mutations) or possible familial HLH (FHLH), as suggested by family history, multiple relapsing courses or prominent neurologic disease at onset was reported as the cause in 24.7% (13/93) of the pediatric cohort. No series reported the results of genetic testing for HLH. Another 2.1% (2/93) were due to inherited causes such as Griscelli syndrome (17) and Chediak-Higaski syndrome (51). Infectious triggers were seen in 44% (41/93) overall; Viruses (56%, 23/41) and tropical infectious agents (32%, 13/41) were the agents recognized. Viruses that were found on evaluation included unknown agents 39% (9/23), Epstein-Barr virus (EBV) 17.3% (4/23), Dengue 26% (6/23), Cytomegalovirus and Parvovirus B19 (2/23, 8.7% each). Connective tissue disease, especially Still's disease, triggered another 18.3% (17/93) of cases of HLH.

Table 4 A Systematic search of all published reports of HLH in adult patients published from South Asian subcontinent

Author	Age	No. of cases	Etiology of HLH	Treatment	Outcome	Comments
Kumar et al. [8]	NA	2	Histoplasmosis	Empiric ATT; No antifungals -Died at 48 hours		HLH: Node (1), spleen (1)
Bhutani et al. [10]	28/M	1	VL	AmB	Alive	VL diagnosis-serology alone
Pahwa et al. [12]	Bone marrow review (n=14)		VL (9), P. vivax (2), P. falciparum (3)		Died (3/14; all VL-HLH bleeding, SSG-related myocarditis)	
Saluja et al. [15]	43/M	1	Histoplasmosis	Itraconazole orally x 6 months		Alive
Karthik et al. [18]	50/M	1	? Tuberculosis	Empiric ATT; no immunomodulation-Alive		Clinical response to ATT alone
Pinto et al. [20]	Chart review; 8/13 adults-mean age 45.75 years			CTD (4); Nephrotic (1); CLD (1); Malignancy (2)		5/13 died; Rx details NA
Rajagopala et al. [4]	23/M	1	VL	Amphotericin B	Alive	rk-39 ELISA; LD negative
Singh et al. [27]	2/14 patients with Still's disease; mean 29.8 years			CSA, steroids, sulfasalazine		Died (1), alive (1)
Patel et al. [31]	NA	1	VL	NA		HIV co-infection
Prasad et al. [33]	NA	2/3 cases	VL	Amphotericin	Alive	LD positive; rk-39+
Premaratna et al. [34]	NA	2	Rickettsia	Antibiotics	Alive	Serology diagnosis
Aggarwal et al. [36]	46/F	1	B-cell lymphoma	Supportive; died < 48 hours after admission		BMA ante-mortem
Gopal et al. [39]	3 patients over a year		Scrub typhus	Antibiotics alone	Alive	Weil-Felix, ELISA+
Koul et al. [40]	40/M	1	Salmonella typhi	Antibiotics alone	Alive	Blood cultures positive; BMA HLH (Ferritin data missing for 3 patients)
	18/F	1				
	25/M	1				
	25/F	1	?VAHS	Methylprednisolone	Alive	No recurrence
	45/M	1	? FLH	HLH-94	Died; recurrent disease	Recurrence @ 4 months
Valsalan et al. [45]	22/M	1	Scrub typhus	Antibiotics alone	Alive	Weil-Felix, ELISA+
Ray et al. [50]	24/F	1	Dengue fever	Steroids; slow taper	Alive @ 6 months	NS1 antigen detection
Chandra et al. [53]	38/F	1	Histoplasmosis	Antifungals	NA	HIV co-infection; BMA+
John et al. [54]	28/M	1	? IAHS Acinetobacter super-infection	Steroids, CSA, antibiotics	Alive; etiologic evaluation poor	
Nayan et al. [57]	19/M	1	?IAHS	Steroids, etoposide	Died @ 96 hours	Shock, ARDS
Mishra et al. [56]	Retrospective review 14 cases 43% (6/14) IAHS; EBV (3/6) Parvovirus B19 (2/6) and CMV (1/6)					3/14 died

Table 4B Systematic search of all published reports of HLH in pediatric patients published from South Asian subcontinent

Author	Age	No. of cases	Etiology of HLH	Treatment	Outcome	Comments
Joseph et al. [6]	7/M	1	? Viral HLH	None	Died; S. aureus sepsis	Post-mortem Bx
Biswal et al. [7]	2 month/M	1	?FLH	None	Died @ 10 days	Evaluation limited
Mathew et al. [9]	N=7, Median 1 year	M (4); F(3)	IAHS (? 4); FLH (3)	Supportive	IAHS 2/4 died; FLH (3/3) died	Bone marrow (5/7); liver Bx HLH (2/7)
Kakkar et al. [11]	Autopsy series; antenatal diagnosis (1/4) All FLH with advanced HLH on autopsy; supportive Rx only					
Bakhshi et al. [13]	9/M	1	EBV	Alive; Supportive only. No antivirals or etoposide		
Dutta et al. [14]	13/F	1	Parvovirus B19	Antibiotics; antifungals	Died	IPA also at autopsy
Agarwal et al. [16]	6/M	1	VL	SSG	Alive	BMA-amastigotes, HLH
Malhotra et al. [17]	4 months		Griscelli syndrome	Supportive	Died	Superadded infection
Karthik et al. [18]	17/M	1	Salmonella	Ceftriaxone x 14 days	Alive	Resolution by 7th day
Mathur et al. [19]	4/M	1	VL	SSB; Later AmB-died DIC		LD bodies; rk-39+
Pinto et al. [20]	Chart review; 5/13 children-mean age 13.7 years			CTD (4); VL (1)		5/13 died; Rx details NA
Das et al. [21]	2/F	1	?Viral HLH	AmB for Candidemia; Alive		Marrow C/S-Candida
Jain et al. [22]	14/F	1	Dengue	Supportive care alone; Alive @ 1 month		IgM Dengue ELISA+
Medhi et al. [23]	11/M	1	T-cell lymphoma	Chemotherapy	Alive	Panniculitis
Rajam et al. [24]	13/F	1	Juvenile-onset R.A	Methylprednisolone	Alive; ARDS, shock and liver dysfunction	
	14/F	1	SLE	Methylprednisolone	Alive; cardiac tamponade	
Raka et al. [25]	1.5 mo/F	1	FLH	Supportive	Died	
Balasubramaniam [26]	52 days/M	1	Tuberculosis	ATT and IVIG	Died; ARDS, gastric aspirate C/S-M tuberculosis	
Gosh et al. [28]	3/M	1	?VAHS	Supportive (antibiotics)	Alive; Acinetobacter super-infection	
Gupta et al. [29]	17/M	1	Tuberculosis	ATT, steroids	Alive; Repeated negative Bx of nodes for AFB	
Juneja et al. [30]	12/F	1	soJRA	Steroids, CSA	Died	Long delay to Rx
Pramanik et al. [32]	8/M	1	? Viral	Steroids alone	Alive	Etiology unclear
	10/M	1	? FLH ?Viral	Steroids alone	Alive at 10 months	Seizures
	12/F	1	? Viral	Steroids, IVIG	Alive	Rx 8 weeks
	6/M	1	Salmonella	Antibiotics alone	Alive	Rx 4 weeks
	3/M	1	? FLH	Steroids alone	Died	Jaundice, ascites

Continuation of Table 4B Systematic search of all published reports of HLH in pediatric patients published from South Asian subcontinent

Author	Age	No. of cases	Etiology of HLH	Treatment	Outcome	Comments
Puliyel et al. [35]	12/M	1	FLH	HLH-2004	Remission; relapse 8 months and died	
Dass et al. [37]	16/M	1	Falciparum malaria	Methylprednisolone	ARDS, Shock; steroids taper over 14 days	
Deshpande et al. [38]	2 month/M	1	Miliary tuberculosis	ATT and steroids	Alive; AFB+, granulomas BMA	
Kumar et al. [41]	6/M	1	soJRA	Steroids	Alive	
Mondal et al. [42]	2.5 month/M	1	Brucellosis	Antibiotics alone	Bone marrow HLH; C/S-Brucella	
Ramesh et al. [43]	9/M	1	soJRA	Antibiotics, CSA, steroid	Alive; urinary tract infection	
	10/M	1	Hodgkin's lymphoma	Chemotherapy	Better; malignancy on 2..nd BMA only	
Suresh et al. [44]	3/F	1	Kawasaki's disease	Aspirin, IVIG, steroids	Better; BMA negative; no response to IVIG	
Ali et al. [46]	NA	1	FLH	HLH protocol and BMT	Alive; report from Pakistan	
Gupta et al. [47]	2 month/M	1	CMV	Ganciclovir alone	Better; IgM, PCR+ BMA-negative, FNA Node+	
Jayakrishnan et al. [48]	5/F	1	Scrub typhus	Antibiotics alone	NA	
Kumar et al. [49]	6/F	1	SoJRA	Steroids, cyclosporine	Alive; PRES during steroid therapy	
Ramachandran et al. [5]	N=32*; Age mean 46 months; 33/43 over 2 years fulfilled HLH criteria		Dengue (5), EBV (3), CMV, leptospira and bacterial (5)	Steroids (67%), IVIG (64%), CSA (33%), Etoposide (15%)	Died (8, 26%); BMA-86% HLH; 2/33 only treated with HLH-2004 protocol x 8 weeks	
Roy et al. [51]	1/5 of CHS with HLH 8months/F			Supportive	Died <48 hours	
Vinoth et al. [52]	11 month/M	1	Falciparum malaria	Artesunate alone	Better; peripheral smears negative; BMA parasites	
Maheshwari et al. [55]	Perinatal	1	Tuberculosis	NA		
Sood et al. [59]	16/M	1	Parvovirus B19	Antibiotics alone	Klebsiella bacteremia;	
Singh et al. [58]	6 patients (5 M; 1 F)-13 years		soJRA	Steroids; IVIG (2/6)	1/6 died; BMA (4); Nodes (1)	

Abbreviations: Male (M), Female (F); NA- not available; + present; - absent; Bx – Biopsy; ATT- anti-tuberculosis therapy; HLH - Histo-pathologic evidence of hemophagocytic lymphohistiocytosis; VL - Visceral leishmaniasis; AmB - Amphotericin B desoxycholate: P. vivax - Plasmodium vivax; SSG - Sodium stibogluconate; CTD - connective tissue disease: CLD - Chronic liver disease; Rx – Treatment; LD - Leishman-Donovan bodies; CSA - cyclosporin A; HIV - Human immunodeficiency virus; BMA-Bone marrow aspiration; ELISA-Enzyme - linked immunosorbent assay; FLH - Familial HLH; IAHS - Infection associated HLH; ARDS - Acute respiratory distress syndrome; EBV - Epstein Barr virus; CMV – Cytomegalovirus; C/S - culture & sensitivity; RA - Rheumatoid arthritis; soJRA - systemic onset Juvenile rheumatoid arthritis (Still's disease); IVIG – immunoglobulin; BMT - Bone marrow transplantation; PRES - Posterior reversible encephalopathy syndrome. *Duplicate reporting of one case of tuberculosis removed.

In adults, no confirmed or probable case of FLH has been reported from the sub-continent. Infections were reported as the most common triggers for HLH. In adults, tropical infectious diseases were reported to have triggered 51% (32/63) of the cases of HLH [Visceral leishmaniasis (VL) 40.6% (13/32), Rickettsia 18.8% (6/32), Malaria 15.6% (5/32), Histoplasmosis 12.5% (4/32), Enteric fever 9.4% (3/32), Tuberculosis 1/32]. Viral agents were reported as possible triggers in another 30% (19/63) cases of HLH, but the etiologic agent was unrecognized in 68.5% of cases (13/18). Where an etiologic agent was reported, EBV 16.7% (3/18) and Parvovirus B19 (11.1%) were the triggers most often. Connective-tissue disease and malignancy were other important recognized triggers in adults (9.5%, 6/63 and 4.8%, 3/63 respectively).

Data on the use of immunomodulatory treatments for HLH were available in 93.5% (87/93) and 79.4% (50/63) of adults. Steroids were the most common immunomodulatory agents used in 47% (41/87) of children and 10% (5/50) of adults. Etoposide and/or cyclosporine were used in 8% (7/87) children and 4/50 (8%) of adults only. Intravenous immunoglobulin (IVIG) was used in another 30% of children (26/87) and 2/63 (4%) of the adult cases reviewed. HLH-related mortality occurred in 31.8% (29/91) and 28% (17/61) of children and adults respectively.

Discussion

Hemophagocytic lymphohistiocytosis (HLH) is an entity which presents major diagnostic and therapeutic challenges (1). HLH is a clinical syndrome secondary to hyper-cytokinemia and organ infiltration by phagocytizing histiocytes (Figure 1) resulting from defects in critical regulatory pathways responsible for the termination of inflammatory responses (1, 2).

This entity is often under-recognized, especially in adults, and specific therapy is not considered early in the disease course. Our systematic review found less than 160 cases in a population of 1.2 billion, indicating under-recognition of this entity. Most cases were single reports and most of these reports clustered around the same centers across the sub-continent (Table 4 A&B). Given the prevalence of the tropical triggers of HLH in the sub-continent, the possibility of under-diagnosis remains highly likely, especially in adults. One of the largest series and a seminal report of HLH from India was not included as it did not fulfill the current requirements for diagnosis of HLH (60).

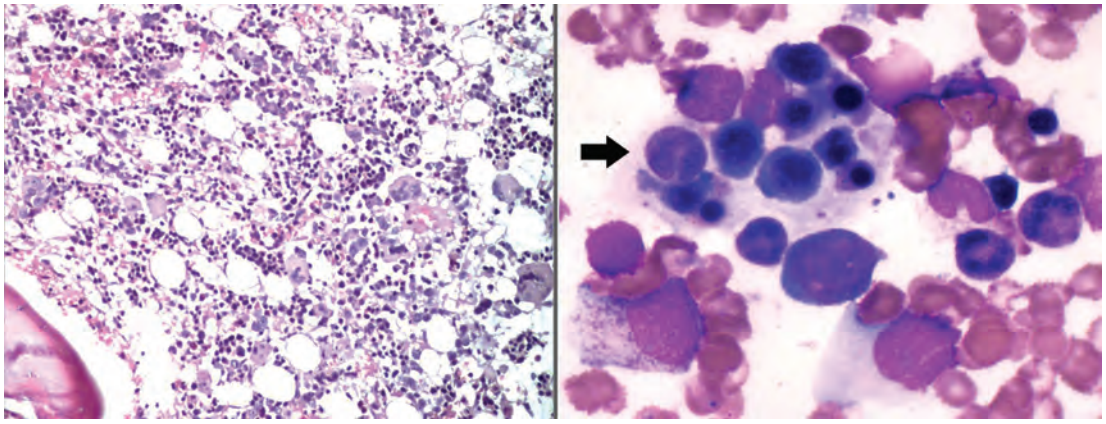

Figure 1 Composite figure showing low-power photomicrograph (x 100 hematoxylin stain) of bone marrow in a patient with HLH. The oil immersion image (right x 400) of the same section shows histiocytes with hemophagocytosis and leukophagocytosis.

Genetic HLH may be: familial (FHL), autosomal recessive], sporadic (sHLH) or complicate certain well characterized syndromes involving defects in the cytolytic T-cell pathway (Table 1). FHL has an estimated incidence of around 1:50,000 and is invariably fatal if untreated, with a median survival of two months. The presentation is often in infancy, though onset in adolescence and adults is well known. The family history may be non-contributory, given the recessive nature of inheritance and the absence of large families (66); furthermore, FHL may often be triggered by infections. Infections, notably Epstein-Barr virus, are another leading cause of HLH (67). Salmonella, tuberculosis, malaria and leishmaniasis are tropical infections that are well-recognized triggers of infection-associated hemophagocytosis (IAHS) (Table 1). In the tropics, infections are overwhelmingly the most common triggers of HLH. In our systematic review, age was not a predictor of etiology of HLH. Tuberculosis has been reported in the neonatal period (55) when FLH is usually prevalent in the Western world. Caution is however required in interpreting our results. The authors did not use a common etiologic evaluation panel, evaluation was often limited, unknown triggers constitute a large sub-group and genetic tests for FLH were seldom done, even in children.

The clinical features of HLH, secondary to such unrestrained immune activation, are not specific and mimic tropical infections (VL, disseminated tuberculosis, and severe malaria), hematological malignancy and auto-immune disease in adults. The diagnosis of HLH is made by fulfilling the revised HLH criteria (1) (Table 2) which were primarily designed to select enrollment into clinical trials. The sensitivity of these criteria for early HLH is unknown given the lack of a gold standard test. Importantly, the clinical picture might be aggressive and the diagnostic criteria might not be fulfilled at onset, making management extremely challenging. In particular, the finding of bone marrow hemophagocytosis is not sensitive for the diagnosis of HLH or the underlying trigger (4, 43). Also, the finding of isolated marrow hemophagocytosis in the absence of the clinical syndrome does not qualify for the diagnosis of HLH (Table 2). In the West and South-East Asia, IAHS is usually viral (EBV)-triggered and a distinction between FHL and IAHS at onset is *not* made; indeed, delay in administration of etoposide to cases of EBV-related HLH is associated with increased mortality. In contrast, tropical HLH may be triggered by *tuberculosis, VL, Salmonella, Plasmodium, dengue or Parvovirus B19*. Several case reports and small series suggest that the natural history of IAHS may be different from EBV-triggered HLH (3, 5, 18).

The HLH-2004 protocol uses upfront cyclosporine [with etoposide, dexamethasone] and intrathecal methotrexate for patients with neurological signs, persistent active CNS disease and CNS reactivation of HLH. All children with familial disease, known mutations, severe and persistent non-familial disease and relapsed HLH are treated with continuation phase etoposide, dexamethasone, and CSA. Stem-cell transplantation is performed as early as possible, when an acceptable donor is available. Therapy is discontinued otherwise at remission (8 weeks) as the completed regimen for patients with possible sHLH and viral-triggered HLH. Patients with refractory disease are treated with ATG, rituximab or alemtuzumab for remission induction. Our systematic review shows that steroids and IVIG (in children) are the common regimens reported; the use of CSA and/or etoposide was very low. The reasons for this may include fulminant presentation, late recognition, the inability to rule out tropical-triggers of HLH, physician perception on the differing profiles of non-viral infection-triggered HLH and severe cytopenia (4). Data from systematic reviews

suggest that HLH secondary to VL, tuberculosis, malaria and dengue may recover with *early* anti-microbial therapy and steroids alone. Indeed, the major correlate with mortality is the time to diagnosis (and treatment) of the offending pathogen. Further, the co-existing organ dysfunction due to HLH may also complicate drug administration [e.g. anti-tubercular drugs and HLH-related liver dysfunction, cytopenia and etoposide dose]. Our adult series also highlights these difficulties; infections were the most common triggers (80%; 10% unknown), short presentation was (median 11 days, IQR 9.25-30) and HLH criteria not being fulfilled at ICU admission median of 4 (IQR 2-4.25) (3).

A uniform protocol for rapid early evaluation of suspected HLH and initiation of therapy (Table 5, Figure 2) is important.

Such a protocol, especially in adults, should balance the exhaustive search for tropical triggers and early initiation of HLH-2004 (including etoposide) in patients with viral-triggered HLH and patients with tropical infection-triggered HLH not responding to steroids alone (Figure 2).

Table 5 Summary of suggested evaluation of a suspected patient with hemophagocytic lymphohistiocytosis in the Indian sub-continent

1. Tests to confirm the diagnosis of hemophagocytic lymphohistiocytosis (HLH)
Complete blood counts, peripheral smear, reticulocyte counts
Liver function tests
Serum creatinine, bicarbonate
Prothrombin time, activated partial thromboplastin time, fibrinogen, D-dimers
CSF (if symptomatic): Total counts, protein levels and cytology
Serum triglycerides, serum ferritin levels
Bone marrow aspirate or tissue aspirates (where involved)
Soluble CD25 levels
NK-cell activity (if possible)
2. Etiological work-up for proven HLH
Review of marrow for *Leishman-donovan* bodies, granulomas, *Histoplasma* inclusions, malignancy, *Plasmodium* inclusions or normoblasts; *Candida* and CMV in neonates; Request mycobacterial and bacterial cultures
Peripheral smears for malaria
Blood cultures, Widal test where applicable
Anti-EBV VCA IgM (PCR if available)
CMV PP65; Qualitative PCR in neonates, immunosuppressed and neutropenia
Human immunodeficiency virus ELISA
rk-39 ELISA for leishmanisis
IgM Parvovirus ELISA
IgM Dengue ELISA or Macro agglutination assay for dengue and leptospirosis
IgM ELISA or Macro agglutination assay for leptospirosis
Weil-Felix test, IgM immunochromotographic test for scrub typhus
Anti-nuclear antibody ELISA or Immunofluorescence for anti-nuclear antibodies
Lymph node biopsy (If prominent lymphadenopathy and sub-acute course suggests lymphoma)
Assay for perforin expression by flow cytometry and/or CD107a expression (*if FLH suspected and all assays for IAHS negative*)
3. Search for complications
Echocardiography (for pericardial effusions), ejection fraction
Contrast-enhanced CT-Head or MRI-Brain (if neurological symptoms)
4. Research in proven FLH in the tropics
Perforin mutations (in association with research centers or western centers)

Abbreviations: FHL - Familial hemophagocytic lymphohistiocytosis; HLH - hemophagocytic lymphohistiocytosis; CD - Cluster of differentiation; EBV - Epstein Barr Virus; CMV - Cytomegalovirus; C/S - culture & sensitivity; ELISA - Enzyme-linked Immunosorbent assay; CT - computed tomography; MRI - Magnetic resonance imaging; FLH - Familial HLH; IAHS - Infection associated HLH; CSF - Cerebrospinal fluid analysis; PCR - Polymerase chain reaction.

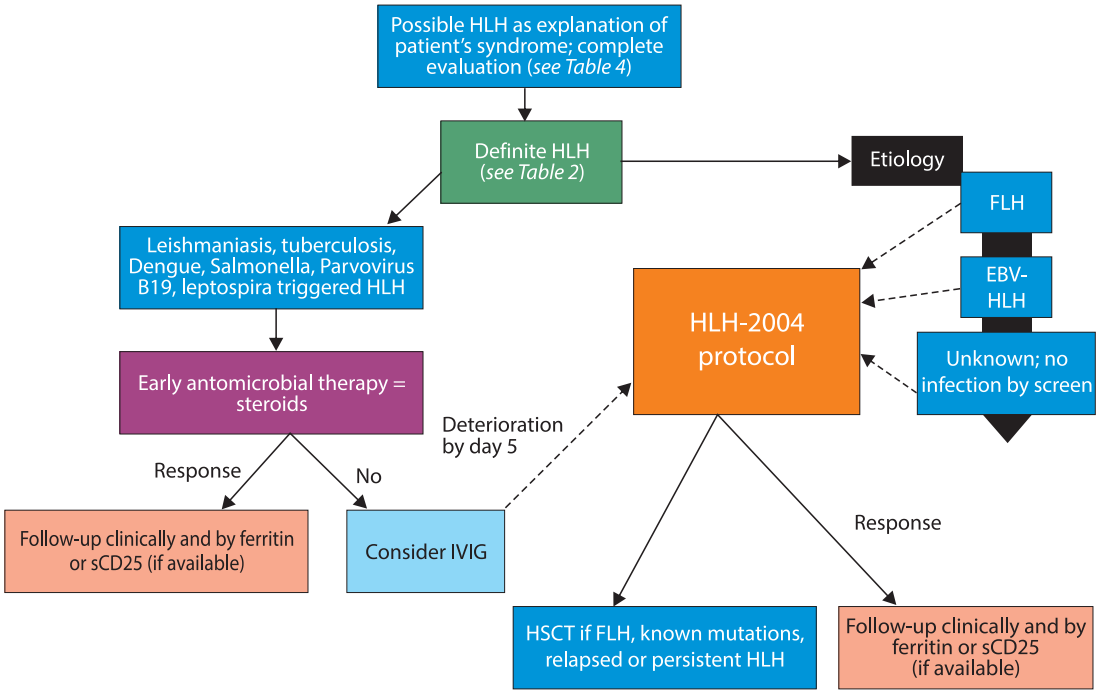

Figure 2 Suggested flow-chart for evaluation of an adult patient with suspected HLH in the sub-continent based on our systematic review.

Lastly, multi-centric prospective data from the sub-continent with such a common evaluation protocol and longitudinal outcomes in cohorts of patients with IAHS will clarify the etiology, management and outcomes of HLH in the sub-continent and whether the prescription patterns in the sub-continent merit reconsideration.

Conclusions

In conclusion, HLH is a catastrophic and fulminant clinical syndrome of immune activation. Heightened clinical recognition of this entity and early evaluation with rapid initiation of treatment may help in better outcomes. More data and multi-centric prospective studies from the tropics are urgently required.

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References

1. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124-31.
2. Creput C, Galicier L, Buyse S, Azoulay E. Understanding organ dysfunction in hemophagocytic lymphohistiocytosis. Intensive Care Med. 2008;34(7):1177-87.
3. Rajagopala S, Singh N, Agarwal R, Gupta D, Dass R. Severe hemophagocytic lymphohistiocytosis in adults-experience from an intensive care unit from North India. Indian J Critical Care Medicine. 2012. in press.
4. Rajagopala S, Dutta U, Chandra KS, Bhatia P, Varma N, Kochhar R. Visceral leishmaniasis as-

sociated hemophagocytic lymphohistiocytosis--case report and systematic review. J Infect. 2008;56(5):381-8.
5. Ramachandran B, Balasubramanian S, Abhishek N, Ravikumar KG, Ramanan AV. Profile of hemophagocytic lymphohistiocytosis in children in a tertiary care hospital in India. Indian Pediatr. 2011;48(1):31-5.
6. Joseph VJ, Sunny AO, Pandit N, Yeshwanth M. Hemophagocytic syndrome. Indian Pediatr. 1992;29(7):897-900.
7. Biswal N, Shareef S, Nalini P, Sreenisasan S, Basu D. Hemophagocytic lymphohistiocytosis. Ind J Pediatrics. 1999;66:632-5.
8. Kumar N, Jain S, Singh ZN. Disseminated histoplasmosis with reactive hemophagocytosis: aspiration cytology findings in two cases. Diagn Cytopathol. 2000;23(6):422-4.
9. Mathew LG, Cherian T, Sudarshanam A, Korah I, Kumar NKS, Raghupathy P. Hemophagocytic Lymphohistiocytosis: A Case Series. Indian Pediatrics. 2000;37:526-31.
10. Bhutani V, Dutta U, Das R, Singh K. Hemophagocytic syndrome as the presenting manifestation of visceral leishmaniasis. J Assoc Physicians India. 2002;50:838-9.
11. Kakkar N, Vasishta RK, Banerjee AK, Marwaha RK, Thapa BR. Familial hemophagocytic lymphohistiocytosis: an autopsy study. Pediatr Pathol Mol Med. 2003;22(3):229-42.
12. Pahwa R, Singh T, Khurana N. Hemophagocytic syndrome in malaria and kala-azar. Indian J Pathol Microbiol. 2004;47(3):348-50.
13. Bakhshi S, Pautu JL. EBV-associated Hemophagocytic Lymphohistiocytosis with Spontaneous Regression Indian Pediatrics. 2005;42:1253-5.
14. Dutta U, Mittal S, Ratho RK, Das A. Acute liver failure and severe hemophagocytosis secondary to parvovirus B19 infection. Indian J Gastroenterol. 2005;24(3):118-9.
15. Saluja S, Sunita, S Bhasin, D K Gupta, B Gupta, S P Kataria, et al. Disseminated Histoplasmosis with Reactive Haemophagocytosis Presenting as PUO in an Immunocompetent Host. J Assoc Physicians India. 2005;53:906-8.
16. Agarwal S, Narayan S, Sharma S, Kahkashan E, Patwari AK. Hemophagocytic syndrome associated with visceral leishmaniasis. Indian J Pediatr. 2006;73(5):445-6.
17. Malhotra AK, Bhaskar G, Nanda M, Kabra M, Singh MK, Ramam M. Griscelli syndrome. J Am Acad Dermatol. 2006;55(2):337-40.
18. Karthik R. Infectious Causes of Macrophage Activation Syndrome. J Assoc Physicians India. 2007;55:877.
19. Mathur P, Samantaray JC, Samanta P. Fatal haemophagocytic syndrome and hepatitis associated with visceral leishmaniasis. Indian J Med Microbiol. 2007;25:416-8.
20. Pinto L, Kagalwala F, Singh S, Balakrishnan C, Prabhu S, Khodaiji S. Macrophage Activation Syndrome: Experience from a Tertiary Referral Centre. J Assoc Physicians India. 2007;55:185-7.
21. Das S, Kalyani R. Hemophagocytic syndrome. Ind J Pathol Microbiol. 2008;51(1):125-6.
22. Jain D, Singh T. Dengue virus related hemophagocytosis: a rare case report. Hematology. 2008;13(5):286-8.
23. Medhi K, Kumar R, Rishi A, Kumar L, Bakhshi S. Subcutaneous panniculitis like T-cell lymphoma with hemophagocytosis: complete remission with BFM-90 protocol. J Pediatr Hematol Oncol. 2008;30(7):558-61.
24. Rajam L, Prasad V, Yatheesha BL. Reactive hemophagocytic syndrome. Indian J Pediatr. 2008;75(12):1261-3.
25. Raka S, Nayar P, Godbole R, Manchanda R. Familial hemophagocytic lymphohistiocytosis. Indian J Hematol Blood Transfus. 2008;25(2):78-80.
26. Balasubramanian S, Kaarthigeyan K, Aparna V, Srinivas S. Tuberculosis associated hemophagocytic syndrome in infancy. Indian Pediatr. 2008;45:593-5.
27. Singh S, Samant R, Joshi VR. Adult onset Still's disease: a study of 14 cases. Clin Rheumatol. 2008;27(1):35-9.
28. Gosh JB, Roy M, Bala A. Infection Associated with Hemophagocytic Lymphohisticytosis triggered by nosocomial Infection. OMJ. 2009;24:223-5.
29. Gupta AP, Parate SN, Bobhate SK, Anupriya. Hemophagocytic syndrome: A cause for fatal outcome in tuberculosis. Ind J Pathol Microbiol. 2009;52(2):260-2.
30. Juneja M, Jain R, Mishra D. Macrophage activation syndrome in an inadequately treated patient with systemic onset juvenile idiopathic arthritis. Kathmandu Univ Med J (KUMJ). 2009;7(28):411-3.
31. Patel KK, Patel AK, Sarda P, Shah BA, Ranjan R. Immune reconstitution visceral leishmaniasis presented as hemophagocytic syndrome in a patient with AIDS from a nonendemic area: a case report. J Int Assoc Physicians AIDS Care (Chic). 2009;8(4):217-20.
32. Pramanik S, Pal P, Das PK, Chakrabarty S, Bhattacharya A, Banerjee S. Reactive Haemophagocytic Lymphohistiocytosis. Indian Journal of Pediatrics. 2009;76:643-5.

33. Prasad R, Muthusami S, Pandey N, Tilak V, Shukla J, Mishra OP. Unusual presentations of Visceral leishmaniasis. Indian J Pediatr. 2009;76(8):843-5.
34. Premaratna R, Williams HS, Chandrasena TG, Rajapakse RP, Kularatna SA, de Silva HJ. Unusual pancytopenia secondary to haemophagocytosis syndrome in rickettsioses. Trans R Soc Trop Med Hyg. 2009;103(9):961-3.
35. Puliyel MM, Rose W, Kumar S, Moses PD, Gibikote S. Prolonged neurologic course of familial hemophagocytic lymphohistiocytosis. Pediatr Neurol. 2009;41(3):207-10.
36. Aggarwal D, Gupta R, Singh S, Gupta K, Kudesia M. Hemophagocytic Lymphohistiocytosis in B-Cell Lymphoproliferative Disorder: Report of a Rare Association. Indian Journal of Hematology and Blood Transfusion. 2010;26(2):74-6.
37. Dass R, Barman H, Duwarah SG, Choudhury V, Jain P, Deka NM, et al. Macrophage activation syndrome in malaria. Rheumatol Int. 2010;30(8):1099-101.
38. 38. Deshpande A, Nayar PS, Pradhan AM, Manchanda RV. Miliary tuberculosis with hemophagocytosis in a two months old infant. Indian J Hematol Blood Transfus. 2010;26(3):115-7.
39. Gopal GK, Anugrah C, Boorugu H. Scrub typhus associated macrophage activation syndrome. Trop Doct. 2010;40(4):249-50.
40. Koul PA, Khan U, Shah S, Jan MR, Wani DA, Abdul D, et al. Hemophagocytic Lymphohistiocytosis: A 25-Year Experience at a Tertiary Care Hospital. WebmedCentral INFECTIOUS DISEASES [serial on the Internet]. 2010;1(9):Available from: http://www.webmedcentral.com/wmcpdf/Article_WMC00674.pdf.
41. Kumar S, Vaidyanathan B, Gayathri S, Rajam L. Systemic onset juvenile idiopathic arthritis with macrophage activation syndrome misdiagnosed as Kawasaki disease: case report and literature review. Rheumatol Int. 2010. Available from: https://springerlink3.metapress.com/content/84213931j616105v/resource-secured/?target=fulltext.pdf&sid=ceyydymehif4ssuffc14q4o3&sh=www.springerlink.com
42. Mondal N, Suresh R, Acharya NS, Praharaj I, Harish BN, Mahadevan S. Hemophagocytic syndrome in a child with brucellosis. Indian J Pediatr. 2010;77(12):1434-6.
43. Ramesh M, Singh V, Ghuliani R, Kapur BN, Singh J, Shankar S. Histiocytosis Syndromes of Childhood: A report of four cases. J Nepal Paediatr Soc. 2010;30(3):171-4.
44. Suresh N, Sankar J. Macrophage activation syndrome: a rare complication of incomplete Kawasaki disease. Ann Trop Paediatr. 2010;30(1):61-4.
45. Valsalan R, Kosaraju K, Sohanlal T, Kumar PSP. Hemophagocytosis in scrub typhus. J Postgrad Med. 2010;56:301-2.
46. Ali N, Fadoo Z, Masood N, Adil SN. Successful Engraftment and Survival Following Allogeneic Hematopoietic Stem Cell Transplant in a Child with Familial Hemophagocytic Lymphohistiocytosis. Indian J Pediatr. 2011. Available from: http://www.springerlink.com/content/9272744w8016562t/
47. Gupta A, Sen R, Batra C, Banerjee D, Jain M. Hemophagocytic syndrome secondary to cytomegalovirus infection in an infant. J Cytol. 2011;28(1):36-8.
48. Jayakrishnan MP, Veny J, Feroze M. Rickettsial infection with hemophagocytosis. Trop Doct. 2011;41(2):111-2.
49. Kumar S, Rajam L. Posterior reversible encephalopathy syndrome (PRES/RPLS) during pulse steroid therapy in macrophage activation syndrome. Indian J Pediatr. 2011;78(8):1002-4.
50. Ray S, Kundu S, Saha M, Chakrabarti P. Hemophagocytic Syndrome in Classic Dengue Fever. J Glob Infect Dis. 2011;3(4):399-401.
51. Roy A, Kar R, Basu D, Srivani S, Badhe BA. Clinico-hematological profile of Chediak-Higashi syndrome: Experience from a tertiary care center in south India. Ind J Pathol Microbiol. 2011;54(3):547-9.
52. Vinoth PN, Thomas KA, Selvan SM, Suman DF, Scott JX. Hemophagocytic syndrome associated with Plasmodium falciparum infection. Indian J Pathol Microbiol. 2011;54(3):594-6.
53. Chandra H, Chandra S, Sharma A. Histoplasmosis on bone marrow aspirate cytological examination associated with hemophagocytosis and pancytopenia in an AIDS patient. Korean J Hematol. 2012;47(1):77-9.
54. John TM, Jacob CN, Ittycheria CC, George AM, Jacob AG, Subramaniyam S, et al. Macrophage activation syndrome following Acinetobacter baumannii sepsis. Int J Infect Dis. 2012;16(3):e223-4.
55. Maheshwari P, Chhabra R, Yadav P. Perinatal Tuberculosis associated Hemophagocytic Lymphohistiocytosis. Indian J Pediatr. 2012:;79(9):1228-9.
56. Mishra B, Varma N, Appannanavar S, Malhotra P, Sharma M, Bhatnagar A, et al. Viral markers in patients with hemophagocytosis: A prospective study in a tertiary care hospital. Indian J Pathol Microbiol. 2012;55:215-7.
57. Nayan D, Bhagyalakshmi S, Nitin K, Farah J, Chandrakala S. Hemophagocytic Lymphohistiocytosis in a 19 Year Old Critically Ill Patient. Indian Journal of Hematology and Blood Transfusion. 2012;28(2):117-20.

58. Singh S, Chandrakasan S, Ahluwalia J, Suri D, Rawat A, Ahmed N, et al. Macrophage activation syndrome in children with systemic onset juvenile idiopathic arthritis: clinical experience from northwest India. Rheumatol Int. 2012;32(4):881-6.
59. Sood N, Yadav P. Hemophagocytic syndrome associated with concomitant Klebsiella and Parvovirus B-19 infection. Indian J Pathol Microbiol 2012;55:124-5.
60. Currimbhoy Z. An outbreak of an infection associated with circulating activated monocytes and hemophagocytes in children in Bombay, India. Am J Pediatr Hematol Oncol. 1991;13(3):274-9.
61. Vijayalakshmi AM, Ganesh VRR. Hemophagocytic Syndrome Associated with Dengue Hemorrhagic Fever. Indian Pediatrics. 2009;46:545.
62. Sharma M, Dass J, Tyagi S. ATRA Induced Reactive Hemophagocytosis: a Case Report. Mediterr J Hematol Infect Dis. 2011;3(1):e2011034.
63. Gupta A, Modi CJ, Gujral S. Hemophagocytosis by leukemic cells in biphenotypic acute leukaemia: a rare case. Indian J Pathol Microbiol. 2010;53(2):370-1.
64. Avasthi R, Mohanty D, Chaudhary S, Mishra K. Disseminated Tuberculosis: Interesting Hematological Observations. J Assoc Physicians India. 2010;58:243-4.
65. Bhattacharyya M, Ghosh MK. Hemophagoctic lymphohistiocytosis--recent concept. J Assoc Physicians India. 2008;56:453-7.
66. Henter JI. Biology and treatment of familial hemophagocytic lymphohistiocytosis: importance of perforin in lymphocyte-mediated cytotoxicity and triggering of apoptosis. Med Pediatr Oncol. 2002;38(5):305-9.
67. Imashuku S, Tabata Y, Teramura T, Hibi S. Treatment strategies for Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH). Leuk Lymphoma. 2000;39(1-2):37-49.

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Trace elements and cell-mediated immunity in gestational and pre-gestational diabetes mellitus at third trimester of pregnancy

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Objective. The aim of the study: To evaluate the correlations between Zn2+, Cu2+, Mg2+, Se2+ and Cr3+ and alteration in T cell subsets during diabetic and normal pregnancy. **Methods**. The study involved 63 women with gestational diabetes mellitus (GD) and 16 pregnant women with Type 2 diabetes and 48 healthy, non-pregnant women were included as controls. Ten ml of whole venous blood from each participant was analyzed for electrolytes by atomic absorption; total antioxidant activity, individual enzymatic antioxidants by spectrophotometry; and lymphocyte sub-populations by flow cytometry. **Results**. There were significant changes in lymphocyte sub-populations: Naïve T cells were decreased and memory T-cells and activated T cells (CD4+HLA-DR+, CD4+CD29+) were increased in diabetes in pregnancy. Zn2+ and Cr3+ deficiency were observed in Type 2 diabetics with an increase in Cu2+ in all pregnant cohorts. In healthy pregnant subjects, CD4+-HLA-DR+ was increased in direct proportion to serum Mg2+ ($p<0.05$) and Se2+ ($p<0.01$). In insulin-treated GD patients, CD4+CD29+ cells were increased proportionally to serum Zn2+ ($p<0.05$) while in diet controlled GD cohort CD45RO+/ CD45RA+ T cells correlated directly with serum Mg ($p<0.05$) and Zn2+ ($p<0.01$) while it correlated inversely with serum Cu2+ ($p<0.01$). **Conclusions**. The results of the present study show a correlation between trace element deficiency and increased lipid peroxidation in diabetes in pregnancy and lymphocyte activation. Dietary manipulation may, therefore, point to improvement in existing approaches to management of diabetes mellitus in pregnancy.

Key words: Gestational diabetes, Lymphocyte activation, Trace elements.

Introduction

In approximately 3% of pregnancies, there is diminished insulin secretion, with varying degrees of insulin resistance and diabetes in pregnancy (1-4). Gestational diabetes mellitus (GDM) is carbohydrate intolerance that begins or is first recognized during pregnancy (5). This could be newly diagnosed type 1, type 2 Diabetes Mellitus or secondary to metabolic changes related to pregnancy (6). In GDM, there is enhanced ability of glu-

cose to cross the placenta, with resultant fetal hyperglycaemia, hyperinsulinaemia and macrosomia. This may lead to a variety of fetal pathologies postpartum and pregnancy-associated morbidity, such as preeclampsia (7-9) and susceptibility to development of GD in subsequent pregnancies. Up to 90% of GDM-afflicted women develop type 2 diabetes (10). GDM may therefore, serve to unmask women who are predisposed and destined to develop type 2 diabetes later in life (11).

The pathogenic effect of high glucose in concert with fatty acids is mediated via increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These free radicals directly induce tissue damage by activating a number of cellular stress sensitive pathways, such as nuclear factor kB (NF-kB), p38 mitogen-activated protein kinase and NH_2-terminal jun kinase/stress-activated protein kinase (12). There is abundant evidence linking oxidative stress to insulin resistance (13). Oxidative stress has long been associated with the etiology of late complications of diabetes mellitus (14). Both insulin resistance and decreased insulin secretion are major features of the pathophysiology of Type 2 Diabetes mellitus (15) and GDM. Early diagnosis of gestational diabetes minimizes the exposure of the developing fetus to suboptimal conditions and prevents perinatal complications and their sequelae (15).

There are increased percentages of HLA-DR+ and CD56+ activated T-cells during the first trimester (16), increased soluble interleukin-2 receptor (sIL-2R) and CD8+ T cell levels (17) and maternal serum factors which down regulate B cell activity (18). Decreased absolute numbers of circulating T helper and NK cells late in pregnancy have also been suggested to play a role in maintenance of healthy gestation (19).

Iron, zinc, selenium and copper are necessary for the activity of the enzymes that neutralize free radicals, in the form of superoxide dismutase, catalase and glutathione peroxidase (20). Zinc deficiency is associated with increased plasma glucocorticord levels, which induces apoptotic loss of precursor and immature G-cells in the bone marrow and pre- T cells in the thymus (21, 22) and an imbalance in T helper cytokine secretion by PBMC (10, 23). A significant decrease in Zn++ and Se++ levels in whole blood has been reported in diabetic pregnancy (24, 25, 26), compared to normoglycemic pregnancy. The potential beneficial effects of Zinc and Chromium have been reported in patients with type 2 diabetes mellitus (27-29).

The objective of the present study is to evaluate the relationship of concentrations of the trace elements and glycemic control with insulin and diet in GDM, and establish any correlation between the trace elements and lymphocyte sub-population in diabetic pregnancies. The Hypothesis of the study includes: (1) Correlations between use of diet or insulin for glycemic control women with GDM or Type 2 diabetes and serum levels of electrolytes, including Zn++, Cu++, Se++, Mg++ and Cr+++, would suggest a role for these elements in the management of diabetes in pregnancy; (2) Correlation between the elements and T lymphocyte subset status, suggests an immunoregulatory role for the trace elements. This would open the gate to future innovative therapeutic intervention.

Patients and methods

Subjects

The study included 63 women with gestational diabetes (43 women controlled with diet and 20 women controlled with insulin) and 16 pregnant women with Type 2 diabetes (5 women controlled with diet and 15 women controlled with insulin), and, as the control, 44 normal pregnant women and 48 healthy non-pregnant women were included. The diet-treated versus insulin-treated groups were included to assess the severity

of the diabetes and at the same time to afford the opportunity to assess the effect of treatment on the level of trace elements and the T lymphocyte subsets. In order to evaluate the effect of normal pregnancy on the level of the trace elements and the T lymphocytes subset status, a control group of 48 healthy, non-pregnant women and 44 normal pregnant women were included. They were matched for age and parity the diabetic study patients, none of whom had a history of diabetes, preeclampsia, hypertension or renal disease. All the patients had clinical evaluation confirmation of gestational diabetes, Type 2 diabetes and the allocation of patients, to dietary control or insulin, was according to the practice guidelines of the Hospital. About 10-15 ml of blood was drawn from each patient without the use of tourniquet, in the third trimester of pregnancy, for estimation of the trace elements and electrolytes and lymphocyte sub-populations. Gestational diabetes was diagnosed for the first time during pregnancy with a 75g glucose tolerance test (GTT). Type 2 diabetics were those women who had pregestational diabetes that had started a few years before and had been on treatment before the onset of the current pregnancy. The diet-controlled groups managed their diabetic condition solely through a diabetic diet. All women had a blood glucose profile to determine the insulin requirement. The insulin regimen included a combination of short-acting (Actrapid® and Monotard®) morning and evening doses. Participation in this study was voluntary and conducted with the informed consent of all participants. Approval for the study was obtained from the Institutional Ethics for Safety of Human Subjects for Research.

Lymphocyte analysis

Five ml of peripheral venous blood was collected from each individual in EDTA tubes and analyzed for the percentage of immune activation-associated lymphocyte subsets within 4-6 hours following phlebotomy. Lymphocyte subpopulations analyzed for included CD4+ T helper cells expressing CD25, HLADR, CD54, CD45RA (naïve T cells), CD45RO (memory T cells), CD29 (Helper/inducer T cells); CD8+ cytotoxic T cells expressing CD25, HLADR or CD38; CD19+ B cells bearing the autoimmune-associated antigen CD5; and T cells expressing natural killer cell antigens: CD3+CD16+CD56+. Fifty µl of blood was incubated for 30 min at room temperature including death *in utero*, delayed organ maturation, high birth weight and many other abnormalities (10- 12). The disorder is transitory, and insulin utilization and blood sugar levels may return to normal with 5 µl of flourescein-isothiocyanate (FITC) or phycoerythrin (RD1) conjugated monoclonal antibodies (mAb), to surface markers of interest. The cells were then treated with Q-prep (Coulter Corporation, Hialeah, FL, USA) for hemolysis, stabilization and amplification of the antigen-antibody reaction and fixation, with paraformaldehyde. A two-color fluorescence analysis using an automated flow cytometer (Coulter, Altra cell sorter, San Diego 92121, California, USA*)* was performed. Positive analysis regions for cells expressing specific surface antigens were set against isotypic controls and specific binding of fluorophore-conjugated monoclonal antibodies was analyzed by cytoflurograph according to standard methods recommended by the manufacturer. Monoclonal antibodies specific for human T-lymphocytes and subpopulations (CD3, CD4, CD8), B-lymphocytes (CD19) and lymphocyte activation (CD25, CD29, CD38, CD45RA, CD45RO, CD54, CD62, TCR-vB6, HLA-DR) were purchased from Dakopatts, A/S, Copenhagen, Denmark and from Immunotech, Coulter Corporation, Hialeah, FL, USA.

Trace element analysis

Ten ml of venous blood was collected from each patient in metal-free tubes (Becton-Dickinson, New Jersey, USA), without any anticoagulant. Serum was separated and analyzed for trace metal content using graphite furnace atomic absorption. Briefly, Cu++, Zn++, Se++ and Cr+++ in the serum samples were measured with the use of an atomic absorption spectrophotometer (model 2380, Perkin-Elmer, Oak Brook, IL, USA) with a graphite furnace HGA 300 and analytical wavelengths of 324.8 nm for Cu++, 213.8 nm for Zn++, 196.0 nm for Se++ and 357.9 nm for Cr+++. Serum Mg++ levels were measured in the same spectrophotometer, using an air-acetylene flame and one-slot burner equipped with a deuterium lamp and an analytical wavelength of 258.2 nm. Duplicates of each measurement were made, with the use of both aqueous and serum quality controls. Results are reported as μg/L of whole blood for Zn++, Cu++, Se++ and Mg++; and in ng/dl for Cr+++.

Measurement of malondialdehyde

Malondialdehyde (MDA) is a natural product formed in all mammalian cells as a product of lipid peroxidation. MDA is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation, it is a marker of oxidative stress, which is associated with hyperglycemia and insulin dysfunction. MDA readily combines with several functional groups on molecules including proteins, lipoproteins, and DNA. In the present study malondialdehyde was measured by a spectrophotometric method as previously (30- 32).

Statistical analysis

All statistical analysis was performed using the SPSS for Windows statistical package (Norusis/SPSS, Inc.) version 17. Data on trace elements, electrolytes and malondialdehyde are presented as mean±SD, while values of T cell lymphocytes subsets were presented as mean %±SEM. Statistical analysis between two groups was performed using a two-tailed student t-test, comparisons involving multiple groups were performed with regression analysis. Pearson correlation was used to measure the association between variables. A value of $p < 0.05$ was considered statistically significant.

Results

Lymphocyte sub-populations

The differences in profiles of activated cellular phenotypes between non-pregnant subjects, healthy pregnant women, gestational diabetics treated with dieting and gestational diabetics treated with insulin are shown in Table 1. The following lymphocyte subsets were higher in healthy pregnant women compared to their non-pregnant counterparts; they included activated T-helper cells (CD+CD25) ($p<0.01$), CD4+ HLA-DR+ ($p<0.01$), and CD8+HLA-DR+ ($p<0.01$). There were significant increases in T cells with NK markers, activated T-helpers cells CD4+CD25+ ($p<0.01$) and CD4+HLA-DR+ ($p<0.01$), memory cells CD4+CD45RO+ and activated T-cytotoxic cells CD8+HLA-DR ($p<0.01$) in the insulin controlled GDM compared to the diet treated GDM and healthy pregnant women. Generally, there were much less activated cellular phenotypes in diet treated gestational diabetics compared to insulin treated GDM. However, while the frequency of CD4+HLA-DR+ and CD45RO+CD45RA+ cells was significantly elevated in the diet-treated Type 2 diabetes cohort ($p<0.05$), while CD4+ CD25+ were significantly elevated in the insulin treated cohort ($p<0.05$) relative to the non-pregnant cohort, no changes were observed compared to the normal pregnancy cohort.

Table 1 Trace electrolyte concentrations in peripheral blood of non-pregnant control subjects, healthy pregnant women, and women with gestational diabetes being treated with dietary therapy alone or with insulin at 3rd trimester of pregnancy

Serum trace element	Non-pregnant subjects (n = 40)	Healthy pregnant subjects (n = 33)	Gestational diabetes Dieting (n = 26)	Gestational diabetes Insulin-treated (n = 10)	Pregnant-Type 2 diabetes Dieting (n = 5)	Pregnant-Type 2 diabetes Insulin-treated (n = 10)
Zn (µg/l)	788.4 ± 30.2	645.9 ± 23.9**	671.6 ± 37.8*	664.5 ± 46.5	437.0 ± 22.7**¶	650.6 ± 91.4
Cu (µg/l)	1552.4 ± 86.2	2840 ± 102.6***	2672.2 ± 98.1***	3094.0 ± 112.0***	3067.0 ± 67.5***	2442.0 ± 157.5***
Se (µg/l)	75.2 ± 2.5	63.0 ± 3.3**	72.2 ± 3.5	78.1 ± 5.7	93.2 ± 4.7¶	71.0 ± 6.7¶
Mg (µg/l)	15917.5 ± 266.2	13139.0 ± 57.9**	15987.5 ± 288.0	14400.0 ± 50	16000.0 ± 110.7¶¶	16540.0 ± 423.9
Cr (ng/dl)	19.9 ± 6.3	24.2 ± 6.9	14.9 ± 5.9	6.0 ± 6.0*¶	29.0 ± 5.6	6.6 ± 32.4¶

* p<0.05, ** p<0.01 and *** p<0.001 relative to non-pregnant subjects, ¶ p < 0.05 as compared to healthy pregnant subjects at 3ed trimester, ψ p < 0.05 as compared to healthy pregnant subjects at 2nd trimester.

Table 2 Malondialdehyde levels in peripheral blood of non-pregnant control subjects, gestational diabetes patients and pregnant type 2 diabetes women at third trimester of pregnancy

Malondialdehyde levels	Non-pregnant subjects (n = 30)	Healthy pregnant subjects (n = 31)	Gestational diabetes Dieting (n = 26)	Gestational diabetes Insulin-treated (n = 10)	Women with Type 2 diabetes Dieting (n = 5)	Pregnant women with Type 2 diabetes Insulin-treated (n =10)
Malondialdehyde (µmol/L)	0.38 ± 0.05	0.535 ± 0.18	0.411 ± 0.13	0.385 ± 0.27	1.10 ± 0.01**¶	0.72 ± 0.24*

Trace elements and diabetic pregnancy

As shown in Table 2, serum Zn++ levels were significantly lower in normal pregnancy as compared to non-pregnant women (p<0.01). No change in Zn++ levels was observed in GDM cohorts. However, a significantly lower level was observed in the diet controlled Type 2 diabetes group compared to the healthy pregnant cohort (p<0.05). Conversely, Cu++ levels were elevated in all the diabetic pregnant cohorts compared to non-pregnant women (p<0.001) with no significant difference between the diabetic cohorts. Apart from the lower levels of Se++ and Mg++ in normal pregnancy compared to non-pregnant women (p<0.01) there were no other changes in the GD cohorts. Compared to healthy pregnancy, in Type 2 cohorts both diet-controlled and insulin-treated showed a significant increase in Se++ (p<0.05) while only the insulin-treated showed a significant increase in Mg++. A significant decrease in Cr+++ was observed in both the gestational diabetes and the Type 2 diabetes cohorts controlled with diet, compared to normal pregnancy (p<0.05).

Correlations between immunophenotype of lymphocyte subpopulations and serum trace element concentration

As shown in Table 3, in normal pregnancy, Cu++ had a significant correlation with CD4+CD25+; r=0.388 (p<0.05) and CD3+CD16+CD56+; r=0.435 (p<0.01) but an inverse or negative correlation with naïve T cells (CD4+CD45RA+); r=-0.372 (p<0.05). There was a highly significant correlation between CD4+HLA-DR+ and Se++; r=0.480 (p<0.01) and with Mg++; r=0.836 (p<0.01). Among the diet controlled GDM, Cu++ had a highly negative correlation with CD45RO+/CD45RA+; r=-748 (p<0.05). Conversely,

Zn++ and Mg++ had a strong correlation with CD45RO+/CD45RA+; r=0.625 ($p<0.01$) with Zn++ and r=0.550 ($p<0.05$) with Mg++. In the insulin treated GDM group, Zn++ had a positive correlation with CD4+CD29+; r= 0.997 ($p<0.05$). There were changes in HLA-DR+ cells that correlated with serum trace element concentrations, with CD4+-HLA-DR+, but not CD8+HLA-DR+ increasing in direct proportion to serum Mg++ ($p<0.05$) and Se++ ($p<0.01$) in healthy pregnant and with Mg++ in insulin-treated GD patients ($p<0.01$). This may be a reflection of the role of these elements in regulation of lymphocyte activation.

The CD4+CD29+ cells (as a percentage of the total CD4+ population), were observed to decrease significantly from the second to third trimester in Type 2 diabetes treated with insulin ($p<0.01$). Interestingly, these cells exhibited an increase in percentage in association with serum Mg++ in healthy, pregnant subjects; and to serum Zn++ and serum Se++ in insulin-controlled GDM patients. Another significant finding was the association between CD4+ CD25+ *and trace elements*. In Type 2 diabetic women treated with insulin, Cr+++ had a strong correlation with CD4+CD25+; r= 0.906 ($p<0.05$).

Analysis of the association between memory/naïve and trace elements

There was an inverse correlation between percentage expression of CD45RO+/ CD45RA+ T cells (reflecting immune activation) and serum Cu++ within the GDM diet and GD insulin cohorts, which was not observed in the healthy cohort. On the other hand, Zn++ correlated directly with this cell population in the diet controlled GDM cohort ($p<0.01$). A positive correlation between Mg++ and CD45RO+/ CD45RA+ T cells was also observed in healthy pregnancy, diet- and insulin -controlled GDM cohorts ($p<0.05$).

Table 3 Frequencies of peripheral blood lymphocyte subpopulations in non-pregnant control subjects, healthy pregnant subjects, and women with gestational diabetes being treated with diet therapy or insulin

Lymphocyte subpopulation	Non-pregnant subjects (n = 48)	Healthy pregnant subjects (n = 44)	Gestational diabetes dieting (n = 43)	Gestational diabetes insulin-treated (n = 20)	Pregnant Type 2 diabetes dieting (n = 5)	Pregnant Type 2 diabetes insulin-treated (n = 11)
CD3+CD16+CD56+*a*	5.9±1.0	6.4±1.5	4.8±2.0	10.3±2.8	5.4±2.0	8.7±2.8
CD4+CD25+*b*	3.4±0.5	6.5± 0.9**	10.6±2.1**	13.5±3.9**¶	3.7±2.1	14.4±6.3*
CD4+HLA-DR+*b*	4.0±0.4	5.8±0.8*	5.5±0.8*	9.3±1.5**¶Ψ	8.6±2.8*	11.5±4.6
CD4+CD45RO+*b*	49.8±1.9	48.9±1.8	54.4±1.6*¶	56.2±3.1*¶	42.2±8.6	53.7±3.6
CD4+CD45RA+*b*	41.1±1.6	46.9±2.4*	36.8±2.1*¶	38.3±3.3*¶	44.8±4.1	43.3±5.7
CD4+CD29+*b*	40.4±8.9	44.5±3.4	59.5±1.8***¶¶¶	62.4±5.1***¶¶	46.7±7.4	44.9±6.5
CD8+CD25+*d*	0.96±0.3	1.8±0.6	3.8± 1.1**¶	2.8±1.2*	3.8±1.7	4.7±2.3
CD8+HLA-DR+*d*	7.9±1.3	17.4±3.1**	10.7±1.8*¶	12.7±2.5*	10.7±2.8	16.2±5.5
CD45RO+/CD45RA+	4.5±1.2	7.1±3.8	8.2±1.8*	4.6±1.3	11.2±3.2*	6.5±2.0

Values (mean %±SEM) are given as percentage of lymphocytes in each major lymphocyte population (CD3, CD4, and CD8). [a]inCD3+, [b]in CD4+, [c]in CD8+. Pregnant women compared to non-pregnant controls, *p<0.05, **p<0.01, ***p<0.001. Pregnant women with diabetes compared to healthy pregnant subjects, ¶p<0.05, ¶¶p <0.01, ¶¶¶p < 0.001. Diet-treated GD patients compared to insulin treated GD Ψp<0.05.

Table 4 Correlations between immunophenotype of lymphocyte subpopulations and serum trace element concentrations in peripheral blood of healthy pregnant women, gestational diabetes patients and pregnant Type 2 diabetes women at third trimester of pregnancy

Trace element	Immunopheno-type	Correlation coefficient
NP		
Cu	CD4+CD25+	0.388*
Cu	CD4+CD45RA+	-0.372*
Cu	CD3+CD16+CD56+	0.435**
Se	CD4+-HLA-DR+	0.480**
Mg	CD4+-HLA-DR+	0.836**
GD (Diet-controlled)		
Cu	CD45RO+/ CD45RA+	-0.748**
Zn	CD45RO+/ CD45RA+	0.625**
Mg	CD8+CD25+	0.632*
Mg	CD45RO+/ CD45RA+	0.550*
GD (Insulin-treated)		
Zn	CD4+CD29+	0.997*
Pregnant with Type 2 diabetes (Insulin-treated)		
Cr	CD4+CD25+	0.906*

Immunophenotypic values are listed as percentage of total peripheral blood lymphocyte population (mean percent ± SEM). Mean values for trace element s± SEM are listed in ug/l for Zn, Cu, se and Mg, and in ng/l for Cr. Pearson correlation was used to measure the association between with significance of each correlation assessed using Student's 2-tailed t-test. * Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01.

Oxidative stress and immune regulation

An inverse correlation of MDA with CD4+CD25+ cells was observed in the present study. Conversely, MDA activity was observed to increase in direct proportion to CD45RO+ memory T cells in the blood of women with diet-controlled GDM ($p<0.05$), and in inverse proportion to memory cells in diet-treated women afflicted with Type 2 diabetes ($p<0.05$), a possible role of MDA in immune regulation needs further investigation. Malondialdehyde activity showed an inverse correlation with CD4+-HLA-DR+ ($p<0.01$) cells in insulin-treated GDM patients but not CD8+HLA-DR+. CD8+CD25+ cells frequency varied directly with MDA in healthy pregnant women ($p<0.05$) as shown in Table 4. An inverse correlation was observed between MDA concentration and CD8+CD25+ in insulin controlled Type 2 diabetes- in the third trimester.

Discussion

Lymphocyte activation

The percentage of CD4+CD25+ (presumed suppressor-inducer cells) was higher in blood of GDM versus healthy pregnant subjects, which was not observed in blood of Type 2 diabetes-afflicted cohorts (Table 1). A subset of T-helper (CD4+) cells expressing IL-2R have been shown to constitute a population of "professional" suppressor T cells (T-reg cells) that prevent induction of organ-specific autoimmune disease (33). Our finding that these cells are elevated in GDM versus healthy pregnant subjects may be an

indication of the occurrence of a protective mechanism mounted by the host immune system in response to some fundamental element of GDM pathogenesis. This study also revealed the expansion of CD4+HLA-DR+ and CD8+HLA-DR+ activated T cells in diabetic pregnancy which exceeds those in healthy controls. Our observation that naïve T cells, CD4+CD45RA+ (which may act as suppressors of immune activation (34) were present at lower frequencies in GDM blood with a concomitant increase in the percentage of memory T cells, CD4+CD45RO+ (which increase in immune activation) than in healthy pregnant women (Table 1) was expected. An increase in naïve T cells during late pregnancy suggests a protective role. The lack of increase in this population in the blood of GDM- or Type 2 diabetes-afflicted women indicates an increase in immune activation versus immunosuppression during late pregnancy in diabetic patients and may in turn correlate with a greater risk to the pregnancy.

A higher number of CD4+CD29+ (presumed helper/inducer cells) activated T cell subpopulations were also observed in GDM patients than in the blood of women experiencing a normal pregnancy, and a previous investigation showed this population to be down-regulated in normal pregnancy (35). These observations suggest that maternal immunosuppression, which is normally increased during pregnancy to compensate for the immunostimulatory activity of paternal antigens, may not be as effective in GDM patients as in healthy women. However this subpopulation was not observed to vary significantly from healthy control values in the peripheral blood of Type 2 diabetic women (Table 1). These cells are a T-helper/inducer subset, observed to be increased in the peripheral blood of patients with T cell-mediated diseases, such as Guillain-Barre syndrome (36). Their activity may or may not contribute to the pathogenesis of GDM, however, their increased frequency in patients may indicate a decreased capacity to control pregnancy-induced immune activation. The fact that changes in activated lymphocyte sub-population were not observed in Type 2-diabetic women is an indicator of differences in the immunopathogenesis of GDM versus Type 2 diabetes.

Mechanisms of immune modulation in type 2 and gestational diabetes mellitus: Trace elements and oxidative stress in immune regulation

The effect of diabetes in pregnancy may arise through two related mechanisms, namely, the direct effect of trace elements and oxidative stress on immune regulation. A significant decrease in Zn++ was shown in the diet–treated diabetic group relative to healthy pregnancy ($p<0.05$) (Table 2), which supports the hypothesis that Zn++ and Cu++ may play a role in the mechanisms regulating the immune response (30, 31). A significant increase in Cu++ was observed in all pregnant cohorts relative to non-pregnant women $p<0.01$), but not compared with the diabetic groups. In previous work we demonstrated a strong positive correlation between serum Cu++ and the size of T cell (CD3+) populations expressing the NK markers CD16 and/ or CD56 in Kuwaiti women with normal pregnancy (31). Also in the present study we found that serum Cu++ increased in direct proportion to the percentage of CD4+CD25+ T cells (Treg cells) in healthy pregnant subjects ($p<0.05$) and in GDM patients treated with diet ($p<0.01$) (Table 3). Hence, increase of serum Cu++ may be a normal response in late pregnancy. These findings support the hypothesis that Zn++ and Cu++ may play a role in the mechanisms regulating the immune response (23). Mg++ was significantly higher in the Type 2 diet treated cohort relative to healthy women ($p<0.01$) (Table 2). It is of interest to note that CD4+CD29+ cells exhibited an in-

crease in percentage proportional to serum Mg++ in healthy, pregnant subjects; and to serum Zn++ and serum Se++ in insulin-treated GDM patients (Table 3). Similarly, CD4+-HLA-DR+, but not CD8+HLA-DR+, was increased in direct proportion to serum Mg++ ($p<0.05$) and Se++ ($p<0.01$) in healthy pregnant and with Mg++ in insulin-treated GDM patients ($p<0.01$) (Table 3). Mg++ ions have been reported to specifically inhibit the antigen-presenting capacity of human epidermal Langerhans cells, which was associated with a reduced expression of HLA-DR by these cells (37). Another study found that deficiency of Mg++ is associated with immunosuppression in athletes, suggesting that Mg++ has a role in immunoregulation (38, 39), which is consistent with our findings in this study. Furthermore, an inverse correlation was noted between percentage expression of CD45RO+/ CD45RA+ T cells (reflecting immune activation) and serum Cu++ within the GDMM diet and GD insulin cohorts, which was not observed in the healthy cohort (Table 3). On the other hand, Zn++ correlated directly with this cell population in the GDM diet cohort ($p<0.01$). These results reflect the role of these elements in regulation of lymphocyte activation.

Relative to subjects with normal pregnancy, Cr+++ levels were observed in this study to be significantly decreased in GD and Type 2 women treated with insulin (Table 2). These results suggest that this element also contributes at some level to the pathogenesis of GD and pregnancy in diabetes. This is consistent with the role of this metal as a regulator of carbohydrate metabolism in pregnancy (40). Serum Cr+++ was observed to vary in direct proportion to CD4+CD25+ T cells in the blood of insulin-treated Type 2 diabetes patients ($p<0.05$) (Table 3) suggesting that Cr+++ may also be involved in immune regulatory mechanisms.

Oxidative stress is an immunological hallmark of type 1 diabetes, therefore the inverse correlation of MDA with CD4+CD25+ cells observed in the present study, may suggest the involvement of some immunoregulatory mechanisms. This suggests that increased levels of MDA in some subjects of this group may be associated with apoptotic deletion of activated T cells expressing HLA-DR, but the same was not observed in healthy pregnant subjects. Although no statistically significant difference was observed in serum Se++ between GDM women and the healthy pregnant cohort, in type 2 diabetes, a significant increase in serum Se++ relative to healthy pregnancy was observed ($p<0.05$). Se++ is an active oxygen species scavenger (41) as well as being an integral part of the antioxidant enzyme glutathione peroxidase. Normal gestation induces an increase of lipid peroxidation products, and this process is substantially increased in diabetes. An inverse correlation was observed between MDA concentration and this population in Type 2 diabetic women treated with insulin in the third trimester however, which suggests a possible apoptotic deletion of CD8+CD25+ activated population in some of the Type 2 diabetes women treated with insulin, which may be induced by reactive oxygen species, as was described previously (42, 43).

Conclusion

In conclusion, this study also revealed the expansion of memory T cells CD4+CD45RO, CD4+HLA-DR+ and CD8+HLA-DR+ activated T cells in diabetic pregnancy and lower frequencies of naïve T cells, CD4+CD45RA+, which act as suppressors of immune activation. An increase in naïve T cells during late pregnancy suggests a protective role. The present study has suggested two mechanisms through which diabetes in pregnancy causes adverse immunoregulation of T. lymphocytes. The results of the present study reflect the role of deficiencies

of trace elements Zn++, Se++, Cu++ in regulation of lymphocyte activation. Secondly, in this study, increased lipid peroxidation products, measured as malondialdehyde concentration, were increased in diabetes in pregnancy. Oxidative stress may cause apoptotic deletion of CD8+CD25+ activated population in diabetic women in pregnancy. Dietary manipulation of antioxidant status with Zn++, Se++, Cu++, Mg++ and Cr++ may therefore allow substantial improvements to be made in existing approaches to management of diabetes in pregnancy.

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References

1. Damm P. Gestational diabetes mellitus and subsequent development of overt diabetes mellitus. Dan Med Bull. 1998;45(5):495-509.
2. Buchanan TA, Xiang AH. Gestational diabetes mellitus. J Clin Invest. 2005;115:485-91.
3. Chu SY, Callaghan WM, Kim SY, Schmid CH, Lau J, England LJ, et al. Maternal Obesity and risk of Gestational Diabetes Mellitus. Diabetes Care. 2007;30:2070-6.
4. Zhang C, Ning Y. Effect of dietary and lifestyle factors on the risk of gestational diabetes: review of epidemiologic evidence. Am J Clin Nutr. 2011;94:S1975-9.
5. Ben-Haroush A, Yogev Y, Hod M. Epidemiology of Gestational Diabetes Mellitus and its association with Type 2 Diabetes. Diabet Med. 2004;21:103-13
6. Yogev Y, Visser G. Obesity, gestational diabetes and pregnancy outcome. Seminars in Fetal and Neonatal Medicine. 2009;14(2):77-84.
7. Correa A, Gilboa SM, Besser LM, Botto LD, Moore CA, Hobbs CA, et al. Diabetes mellitus and birth defects. Am J Obst Gynecol. 2008;199:2371-8.
8. Solomon CG, Seely EW. Brief review: hypertension in pregnancy: a manifestation of the insulin resistance syndrome? Hypertension. 2001;37(2):232-9.
9. Tamas G, Kerenyi Z. Gestational diabetes: current aspects on athogenesis and treatment. Exp Clin Endocrinol Diabetes. 2001;109(Suppl 2):S400-11.
10. Lupo VR, Stys SJ. Recurrence of gestational diabetes in subsequent pregnancies. In: Weiss PM, Coustan DR, editors. Gestational Diabetes. Vienna, Austria: Springer-Verlag; 1988. p. 123-6.
11. Rice GE, Illanes SE, Mitchell MD. Gestational diabetes mellitus: a positive predictor of type 2 diabetes? Int J Endocrinol. 2012;2012:721653.
12. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are Oxidative Stress-Activated Signaling Pathways Mediators of Insulin Resistance and ß-Cell Dysfunction? Diabetes. 2003;52:1-8.
13. Paolisso G, Giugliano D. Oxidative stress and Insulin action. Is there a relationship? Diabetologia. 1996;39:357-63.
14. West IC. Radicals and Oxidative stress in diabetes. Diabet Med. 2000;17:171-80.
15. Georgiou HM, Lappas M, Georgiou GM, Marita A, Bryant VJ, , Hiscock R, et al. Screening for biomarkers predictive of gestational diabetes mellitus. Acta Diabetol. 2008;45:157–65.
16. Kuhnertm M, Strohmeier R, Stegm Yller M, et al. Changes in lymphocyte subsets during normal pregnancy. Eur J Obstet Gynecol Reprod Biol. 1998;76:147-51.
17. Liu CL, Xu HY, Liu Y. Serum level of soluble interleukin-2 receptor and T lymphocyte subpopulations in normal pregnancy. Chung Hua Fu Chan Ko Tsa Chih. 1994;29:518-20.
18. Vanderbeeken YE, Duchateau J, Gregoire M, Vandermeersch B, Collet H, Lucas A. Modulation of B cell stimulation by maternal serum. Immunol Invest. 1991;20(3):287-304.
19. Watanabe M, Iwatani Y, Kaneda T, Hidaka Y, Mitsuda N, Morimoto Y, et al. Changes in T, B, and NK lymphocyte subsets during and after normal pregnancy. Am J Reprod Immunol. 1997;37:368-77.
20. Chandra RK. Nutrition and Immunology: From the clinic to cellular biology and back again. Proc Nutr Soc. 1999;58:681-3.
21. Xiang AH, Peters RK, Trigo E. Multiple metabolic defects during late pregnancy in women at high risk for type 2 diabetes. Diabetes. 1999;48:848-54.
22. Fraker PJ, King LE. Programming. Annu Rev Nutr. 2004;24:277-98.
23. Beck FW, Prasad AS, Kaplan J, Fitzgerald JT, Brewer GT. Changes in cytokines production and T cell sub-population in experimentally induced zinc deficient human. Am J Physiol. 1997;272:E1002-7.
24. Wang Y, Tan M, Huang Z, Sheng L, Ge Y. Elemental Contents In Serum Of Pregnant Women With

Gestational Diabetes Mellitus. Biological Trace Element Research. 2002;88:113-8.

25. Borella P, Szilagyi A, Than G, Csaba I, Giardino A, Facchinetti F. Maternal plasma concentration of magnesium, calcium, zinc and copper in normal and pathological pregnancies. Science of the Total Environment. 1990;99(1-2):67-76.
26. Huijgen A, Sanders G. Intracellular and extracellular, ionized and total Mg++ in pre-eclampsia and uncomplicated pregnancy. Clinical Chemistry and Laboratory Medicine. 1999;37:55-9.
27. Anderson RA, Roussel AM, Zouari N, Mahjoub S, Matheau JM, Kerkeni A. Potential Antioxidant Effects of Zinc and Chromium Supplementation in People with Type 2 Diabetes Mellitus. J Am Coll Nutr. 2001;20:212-18.
28. Roussel AM, Kerkeni A, Zouari N, Mahjoub S, Matheau JM, Anderson RA. Antioxidant Effects of Zinc Supplementation in Tunisian s with Type 2 Diabetes Mellitus. J Am Coll Nutr. 2003;22:316-21.
29. Ryan GJ, Wanko NS, Redman AR, Cook CB. Chromium as Adjunctive Treatment for Type 2 Diabetes. Ann Pharmacother. 2003;37:876-85.
30. Abul HT, Mahmoud FF, El-Rayes SK, Haines DD, Omu A. Potential aetiological involvement of Zn++, Cu++, Se++ and Mg++ in pre-eclamptic and hypertensive parturient women in Kuwait. Trace Elements and Electrolytes. 2001;18(1):20-5.
31. Abul H, Mahmoud F, Haines D, Mannazhath N. Pregnancy-Associated Relationships Between Serum Content of Cu2+, Mg2+, Zn2+, Se2+ and Peripheral Blood Lymphocyte Sub-populations in Kuwaiti Women. Trace Elements and Electrolytes. 2004;21(3):168-73.
32. Jentzsch AM, Bachmann H, Furts P, Biesalski HK. Improved analysis of malondialdehyde in human body fluids. Free Radical Biology and Medicine. 1996;20(2):251-6.
33. Thornton A, Shevach E. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. J Immunol. 2000;164:183-90.
34. Clement LT. Isoforms of the CD45 common leukocyte antigen family: markers for human T-cell differentiation. J Clin Immunol. 1992;12:1-10.
35. Matthiesen L, Berg G, Ernerudh J, Hakansson L. Lymphocyte subsets and mitogen stimulation of blood lymphocytes in normal pregnancy. Am J Reprod Immunol. 1996;35:70-9.
36. Sindern E, Oreja-Guevara C, Raulf-Heimsoth M, Baur X, Malin JP. A longitudinal study of circulating lymphocyte subsets in the peripheral blood during the acute stage of Guillain-Barre syndrome. J Neurol Sci. 1997;151:29–34.
37. Schempp CM, Dittmar HC, Hummler D, Simon-Haarhaus B, Schulte-Mönting J, Schöpf E, et al. Magnesium ions inhibit the antigen-presenting function of human epidermal Langerhans cells in vivo and in vitro. Involvement of ATPase, HLA-DR, B7 molecules, and cytokines. J Invest Dermatol. 2000;115(4):680-6.
38. Nielsen FH, Lukaski HC. Update on the relationship between magnesium and exercise. Magnes Res. 2006 Sep;19(3):180-9.
39. König D, Weinstock C, Keul J, Northoff H, Berg A. Zinc, iron, and magnesium status in athletes--influence on the regulation of exercise-induced stress and immune function. Exerc Immunol Rev. 1998;4:2-21.
40. Sharma S, Agrawal RP, Choudhary M, Jain S, Goyal S, Agarwal V. Beneficial effect of chromium supplementation on glucose, HbA1C and lipid variables in individuals with newly onset type-2 diabetes. J Trace Elem Med Biol. 2011;25(3):149-53.
41. Xu H, Liu Q, Zhou J, Zuo P, Wang J. The mechanism for the effect of Se++ supplementation on immunity. Biol Trace Elem Res. 1995;48(3):231-8.
42. Agostini M, Di Marco B, Nocentini G, Delfino DV. Oxidative stress and apoptosis in immune diseases. Int J Immunopathol Pharmacol. 2002;15(3):157-64.
43. Hildeman DA, Mitchell T, Teague TK, Henson P, Day BJ, Kappler J, et al. Reactive oxygen species regulate activation-induced T cell apoptosis. Immunity. 1999;10(6):735-44.

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Care providers' needs and perspectives on suffering and care in Bosnia and Herzegovina and Cambodia

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This exploratory study aimed to obtain insight into field-level care providers' views on suffering and healing as well as existing obstacles and needs related to providing care to their clients. This research provides a "snapshot" for a better understanding of existing care systems in two post-conflict settings. By identifying existing approaches to care and the needs of the care provider community, this research might be useful in guiding psychosocial assistance programming in post-conflict settings. Utilizing a semi-structured questionnaire, 45 care providers were interviewed, including local health care practitioners, traditional/spiritual healers, and humanitarian relief workers, in Bosnia and Herzegovina and Cambodia. This study found that the majority of care providers in both settings perceived poverty and violence as significant causes and consequences of human suffering and, at the same time, felt ill-equipped in addressing these issues and related problems. Other issues that hindered these healers in providing care included: limited government/institutional support; lack of training; material resources and funding. Study findings point to a new framework for developing effective interventions and the need for further emphasis on supporting care providers in their work, and most specifically, in identifying and responding to poverty and violence.

Key words: Traditional healing, Mental health, Psychosocial assistance, Poverty, Violence.

Introduction

Growing research reveals serious psychological distress resulting from mass violence, with the prevalence of depression in some cases being three times greater than that found among non-traumatized populations (1-3). The toll of conflict evidenced in "invisible wounds" is expected to escalate in the future (4). Currently, the devastating mental health sequelae of conflict and effective approaches to treatment are factored into the public health response in conflict-affected settings (5, 6).

Conflict's toll, in terms of its destruction of social and economic systems and related serious mental health outcomes of conflict,

demands an effective response from the healing community, including local, national and international actors. The response among international organizations and non-governmental organizations to address these "invisible wounds" has been embodied in the provision of *psychosocial assistance*.

This area of assistance is characterized by various activities, efforts and approaches ranging from those interventions that use a trauma and individual-focused 'psychological' approach or treatment, to those that focus on strengthening community mobilization and cooperation, skills development and capacity-building (7-12). The wide variation of approaches is likely attributable to the term that describes the field itself. The term *psychosocial* describes and acknowledges the "dynamic relationship between psychological and social effects" that are, "continually influencing each other" (13). The overarching objectives of psychosocial interventions are to "[to both] promote existing psychosocial protective factors and minimize psychosocial stressor factors." (14).

This study aimed to obtain field-level perspectives from the care provider community, including health care providers, traditional and spiritual healers, and humanitarian relief organization (HRO) staff working in Bosnia and Herzegovina (BIH) and Cambodia. Specifically, this exploratory study aimed to obtain further insight into these care providers' views on: 1) suffering and healing and 2) existing obstacles and needs related to providing assistance. Through the lens of these providers on-the-ground, this research can provide a snapshot for a better understanding of existing care systems in two post-conflict settings. This information may be useful in guiding psychosocial assistance and approaches.

Participants and methods

The study was undertaken in Siem Reap Province, Cambodia and Travnik and Sarajevo Cantons, BIH. These settings were selected because they are both post-conflict environments and have received high levels of humanitarian assistance. Additionally, interviewees were readily accessible as a result of the involvement of the Harvard Program in Refugee Trauma (HPRT) in each of these settings for many years.

During the Khmer Rouge genocide in Cambodia from 1975 to 1979, though estimates vary, an estimated 1.5 to 3 million people were killed (between 20% and 40% of the Cambodian population) (15, 16). Hundreds of thousands of people became refugees, with over 300,000 Khmer displaced persons living in Thailand more than a decade later (17). Under the Khmer Rouge, Siem Reap suffered one of the largest single attacks by the Khmer Rouge and mass graves have been found in the province (18-20). Fighting continued in this area up until 1997 with elements of the Khmer Rouge, with much of it occurring in the region around Siem Reap.

In 1992, violent conflict in an ethnically diverse BIH began after the referendum vote for BIH independence from the former Yugoslavia. The Dayton Peace Accords were signed three years later, in 1995. The total number of war deaths has been estimated at 103,000 and a total of 1.3 million were forcibly displaced, accounting for one-quarter of the country's pre-war population (21). During the war, there was heavy inter-ethnic fighting in the Travnik Canton (22). At the time of this study, BIH was widely considered a post-conflict country with significant difficulties related to the return of displaced populations to their pre-war homes.

Given the exploratory nature of this project, a semi-structured questionnaire was developed for use with study participants, to include: (1) HRO staff (local and international, working for international organizations (IOs) or non-governmental organizations (NGOs)); (2) health care providers (this classification refers to, for example, medical

doctors, nurses, psychologists, etc.); and (3) traditional and spiritual healers.[1] Consisting of 75 questions, the 90 minute interview elicited participants' views on causes and consequences of human suffering; material, psychological and social needs of clients; helping activities; and barriers to care, including training and supervision resources, job stress and burnout, and cross-sector collaboration. The interview contained both open-ended questions and Likert-scale ratings. Interviews were conducted by SDK, ST, LM and MH, with translation provided by ST and MH. Language-specific written and verbal informed consent and study procedures were approved by the Massachusetts General Hospital Institutional Review Board Human Research Committee. HPRT had several years of experience and local presence in these countries. Local HPRT staff recruited 45 participants (22, 23) from a list of organizations and practitioners representing each of the provider types.[2] The majority of them were interviewed separately; in BIH, two groups of two individuals from the same organization were interviewed together.

In order to analyze study data, first, we conducted a content analysis (23) of open-ended interview responses. Coding and analysis relied on a two-pronged content analytic approach (24) of constant comparison (25, 26) and grounded theory mapping (27). Open-ended responses were systematically coded into four *a priori* categories of human functioning: social, physical, mental, and spiritual. *Post hoc* analyses were conducted to uncover any emergent themes, which led to the development of two additional themes of poverty and war. These were developed due to the overwhelming number of responses referring to one or both of these. Two of the authors (SDK and LM) separately coded the responses with any disagreements resolved through discussion. The resulting data are reported in terms of proportions of provider responses within each theme and specific quotes are provided to highlight relevant issues. For Likert-scale questions, answers are presented quantitatively using descriptive statistics (e.g., mean and standard deviation). Group differences between types of providers were conducted with t-tests or ANOVA's as appropriate.

[1] Traditional healers included those who practice traditional medicine and spiritual healers comprised those individuals who are authorized to conduct worship ceremonies and perform other duties associated with their role in a religious organization. Some examples of spiritual healers include, among others, hafiz, nuns, and monks.

[2] Of the potential participants approached in Cambodia, two individuals refused to participate, giving reasons related to lack of time and in one case because of concerns about confidentiality based on prior experience with international research projects. In Bosnia, one individual refused to participate due to lack of time and the need to contact a parent organization for permission to participate.

Results

Provider training

In both cohorts, health care providers and HRO staff typically reported acquiring technical or clinical skills through prior related experience, formal education and degree-granting institutions, and specific training programs offered by their respective governments or other HROs. For example, a nurse in the Cambodian sample had received a nursing degree and continued training in emergency health care from an international NGO. While such training was typically supported by both institutions and governments, this participant valued her practical experience during the war more highly:

Sometimes there were not enough doctors and at the same time we had fighting. [I had to] help the patients…[There were] a lot of wounded, I had to do surgery to get bullets, sometimes amputate…I learned from my experience a lot more.

Table 1 Background characteristics by country

Characteristics	Cambodia (n = 22)	Bosnia and Herzegovina (n=23)
	Mean (SD) or %	
Male gender (n)	15	10
Age in years	46.9 (8.9) (27 – 60)	39.0 (11.7) (25 – 65)
Direct care provision (face to face with clients)	72%	91%
Supervisor /Administrative responsibilities	72%	39%
Number of years in position	6.6 (5.3) (1 – 22)	5.6 (8.4) (0.5 – 38)
Number of years in this or related field	14.8 (8.7) (3 – 30)	11.0 (9.3) (0.5 – 38)
Education level	Varied by provider type	Majority post-graduate +
Mental health trained/ provision of services	18%	61%

For traditional and spiritual healers, preparation often consisted of mentorship by elders or spiritual guides, which typically continued for years, often decades. For some traditional healers in Cambodia, the healing method consisted of the "guiding spirit" or *proling santhita* taking possession of the care provider's body; and, therefore, it was reported that no preparation was necessary for this healing method. In BIH, several providers had received formal religious education and training.

Causes of human suffering

Participants' views on the causes and consequences of human suffering were categorized into four *a priori* themes of human functioning: social, physical, mental, and spiritual. As described above, upon data review, two additional themes of poverty and war were developed due to the overwhelming number of responses referring to one or both of these. Across providers in both cohorts, war- and poverty-related issues were identified as the bedrock for human suffering, most typically linked to specific social ills. Poverty, war, and social themes combined accounted for the overwhelming majority of responses by country (95.7% BIH, 90.9% Cambodia, see Table 2). As stated by an HRO staff member in BIH when asked for her views on the cause(s) of human suffering:

Poverty, definitely poverty. A lot of different reasons for it [human suffering], but everything is based on poverty. [Also] what happened during the war… [but] everything is based on poverty.

Health care providers predominantly reported poverty as a cause of human suffering, exacerbating physical health problems linked to social causes, such as a lack of education or limited access to transportation and financial resources needed to ensure timely access to health care. A health care provider in BIH stated that the causes of suffering are:

Poverty and war. People can't get the right treatment. For example, because of the road a patient can't get to the hospital and can't pay [when they get there].

HRO staff typically reported societal causes related to poverty and war, such as government corruption, lack of social safety or social norms, and disparity in access to education and health care. A Cambodian HRO staff commented:

…Poverty is first and human resources second. [People] are poor with no money or education…Human resources [means] people lost their trust and confidence…Before we helped each other…before the conflict, the community would help. Now it's different.

Table 2 Causes of human suffering categorized by type of provider and theme†

Type of provider	Themes (%)					
	Poverty	War	Social	Physical	Mental	Spiritual
Health care providers	84.2	31.6	57.9	63.2	15.8	5.3
Humanitarian relief organization (NGO or IO)	46.2	46.2	76.9	53.8	38.5	7.7
Traditional or spiritual healer	38.5	53.8	38.5	46.2	7.7	38.5

†Percentages may total more or less than 100% for several reasons (e.g., missing data, responses contain more than one theme).

Table 3 Consequences of human suffering by type of provider and theme†

Type of provider	Themes (%)			
	Social	Physical	Mental	Spiritual
Health care providers	26.3	63.2	57.9	0
Humanitarian relief organization (NGO or IO)	61.5	30.8	46.2	0
Traditional or spiritual healer	23.1	61.5	53.8	0

†Percentages may total more or less than 100% for several reasons (e.g., missing data, responses contain more than one theme).

People have lost trust and confidence. Now when we talk to [people] they say "I don't believe you".

Although human suffering related to spiritual causes was infrequently reported by other providers, traditional and spiritual healers often mentioned spiritual or religious issues in relation to physical and social causes. A BIH spiritual healer commented on the lack of faith playing a causal role in societal suffering, "a communist country…going towards disaster [and] supported something negative. A society without God had no moral values." Other traditional and spiritual healers mentioned the impact of spirits or fate as the cause of human suffering.

Consequences of human suffering

As Table 3 reveals, health care providers predominantly mentioned the physical and mental consequences of human suffering. These typically included disease, illness, and specific psychological symptoms (e.g. worry, depression, feeling unhappy, and hopelessness). The majority of HRO staff reported social and mental consequences. As an HRO staff member in BIH noted, due to societal breakdown, "communities were destroyed…[and] displacement [of people to other countries or outside their home communities] caused the breakdown of families." While traditional and spiritual healers perceived causes of human suffering as spiritual in nature, they primarily reported physical and mental consequences.

Client/Patient needs

Participants' perceptions of the clients' most significant needs and problems were grouped into four categories: (1) economic concerns (e.g., poverty, unemployment, basic needs); (2) health concerns (e.g., health problems, need for medical treatment); (3) psychological concerns (e.g., psychological symptoms and psychosomatic problems); and (4) family concerns (e.g., family support needs and family violence).

Across providers in both cohorts, client needs were congruent with the larger societal ills, poverty and war, as described above. The most frequent concerns cited were (1) economic, often linked to concerns about

Table 4 Help for human suffering by type of provider†

Type of provider	Helping themes (%)			
	Supportive communication	Medical treatment	Material assistance	Spiritual/Relief guidance
Health care providers	70.6	66.7	35.3	0.0
Humanitarian relief organization (NGO or IO)	75.0	8.3	25.0	8.3
Traditional or spiritual healer	45.5	9.1	9.1	54.5

†Percentages may total more or less than 100% for several reasons (e.g., missing data, responses contain more than one theme).

the future or psychological, health, and family problems. In ranking needs and problems, health care providers' responses were distributed roughly equally across categories (economic: 36.8%, health: 36.8%, psychological: 31.6%; and family: 36.8%).

All HRO staff mentioned clients' economic concerns (1), with fewer citing the other concerns (health: 25%; post-conflict adaptation; psychological and family: 16.7%). An HRO staff member in BIH stated that, "Housing, financial problems lead to emotional problems… people [who are] unemployed don't have the resources or opportunity to restore their identities." Almost one-half (42.8%) of the traditional and spiritual healers[1] reported their clients' economic concerns, while the majority (71.4%) cited health concerns; with other categories carrying less weight, including psychological concerns (28.6%); post-conflict adaptation (28.6%); and family-related concerns (0%).

Assistance

Participants' responses when asked about their beliefs about what helps people who are suffering were grouped into four themes (Table 4).

The majority of participants reported that they thought supportive communication helped people who are suffering. Generally, mental health providers (e.g., classified in this study as health care providers) mentioned more formalized communication, such as counseling. Others reported more informal communication through talking, listening, or providing education (e.g., on appropriate medical care). A care provider in BIH noted the importance of listening in helping someone feel "supported and [able] to talk openly." An HRO staff in Cambodia stated that it is important to "find out why the patient thinks they are suffering, to provide options."

Secondary to supportive communication, helping themes were associated with provider type. Health care providers were more likely to mention medical treatment, such as medical care or referral. The majority of traditional and spiritual healers reported spiritual and religious guidance, such as preaching and using specific spiritual practices. HRO staff primarily reported material assistance as a helping theme.

Barriers to care

Information provided by participants on existing barriers to effectiveness was categorized into themes: lack of (1) institutional/governmental support, (2) physical support (e.g. resources, tools, and infrastructure); (3) knowledge, training, and (in some cases) insufficient supervision; (4) community stigma; and (5) the need for continued donor funding. Self-care and burnout issues

[1]Responses for five of the 13 traditional healers and spiritual/religious leaders could not be coded due to missing data or unclear statements.

(6) were also revealed. Across providers in both countries, 65% of responses pertained to the themes (1) (2) and (3).

Many health care providers in BIH reported that the lack of institutional support (1) contributed to employee frustration and negatively impacted their ability to provide clinical care. Several health care providers in Cambodia noted the lack of physical support (2), mentioning the lack of resources for free medical care and the need for updated medical equipment and supplies.

Lack of knowledge and training, including both education prior to taking a position and ongoing training, were also frequently cited as there were some limitations in supervision (3). The majority of participants, in both cohorts, mentioned the general need for continuing position-related training relevant to their positions. A lack of resources (including institutional awareness and financial) for training was often reported.

Clinical skill deficits were mentioned by the majority of health care providers and some traditional healers, focusing on the need for updated training and materials. All international HRO staff (N=3) reported prior training in cultural issues with a need for more intensive language training. Equal numbers of traditional healers and health care providers commented on the need for basic training in the identification and treatment of mental health disorders.

One health care provider stated:

I don't know how to help patients [with mental health needs]. It's very good to learn about psychiatric diagnoses. [For example] patients with malaria have the same symptoms as depression. Some patients…get cured [with malaria treatment] but some have symptoms still there that need to be diagnosed, but I don't know how.

Regular supervision was reported for all health care providers and HRO staff in Cambodia and in BIH, supervision was reported for 86% of HRO staff and 50% of health care providers. Supervision was reported to a lesser extent among traditional and spiritual healers. Several health care providers noted that supervision had occurred previously, and frequently associated this with the work of outside/international HROs. Many reported that formal supervision had ceased to exist when the involvement of International HROs ended. A BIH HRO staff member expressed that it "would be useful to have a supervisor but we can't afford it."

The impact of community stigma (4) on program effectiveness was mentioned by 13% of participants. Such stigma was typically associated with mental health and some physical health problems (e.g., HIV infection). A number of care providers implied that there was stigma surrounding their use. Donor funding issues, including adequacy and the need for continued support (5), were mentioned by 10% of HRO staff. An HRO staff person in Cambodia stated that, "equipment will deteriorate because foreign staff can access outside funding…the knowledge [from training] will stay."

This study's findings with regards to job stress and burnout (6) were limited, with several participants reporting no job stress or personal problems. Findings may have been influenced by cultural context (e.g., in Cambodia, for example, it is not generally acceptable to discuss one's own problems openly). Participants did, however, report several job-related stressors, including feeling overwhelmed by client needs or the amount of work. An HRO staff member in Cambodia noted often feeling "frustrated because he couldn't do more due to limited time and resources." While in BIH, an HRO staff member reported "being close to burnout…I got sick, tired, irritable, [it was] hard to concentrate, I couldn't listen." Job stress was reported to impact work performance, with providers reporting changes in attitude towards work and anger towards other staff. Activities that helped with job stress includ-

Table 5 Collaboration by type of provider

Sector	Type of provider (Respondent to survey)†			
	Health	HRO	TH/Spir	
	Mean (SD)	Mean (SD)	Mean (SD)	F
Other HRO	2.9 (1.3)	4.3 (0.9)	2.0 (1.5)	8.39**
Health care	3.6 (1.5)	4.0 (1.0)	2.4 (1.6)	3.49*
Traditional healers	1.3 (0.8)	1.6 (0.8)	2.0 (1.6)	1.38
Spir./Rel. leaders	1.7 (1.1)	2.1 (1.0)	2.3 (1.8)	0.79
Community leaders	2.4 (1.2)	4.1 (0.9)	1.8 (1.4)	11.27***
Schools/Teachers	3.6 (1.6)	4.0 (1.0)	1.8 (1.6)	6.71**

†Health = Health care providers; HRO = Humanitarian relief organization (NGO or IO); TH/Spir = Traditional or Spiritual healer). *p<0.05; **p<0.01; ***p<0.001. There were 2 degrees of freedom for all between groups ANOVAs.

ed talking with others (co-workers, family, and friends), taking time to rest and relax, engaging in social and spiritual activities, and exercise.

Collaboration

HRO staff reported a higher level of collaboration across sectors than either health care providers or traditional and spiritual healers. *Post hoc* analyses were conducted using the Bonferroni correction for multiple tests. According to their report, HRO staff collaborated more with other HRO staff than did health care providers (mean difference = 1.3, $p<0.05$) and traditional and spiritual healers (mean difference = 2.3, $p<0.001$). The results were similar for collaboration with community leaders, with higher rates for HRO staff than health care providers (mean difference = 1.7, $p<0.01$) and traditional and spiritual healers (mean difference = 2.3, $p<0.001$). There were no significant differences in collaboration with either sector for health care providers and traditional and spiritual healers.

Ratings of collaboration with the health care sector were high for health care providers and HRO staff, with HRO staff indicating significantly more collaboration than traditional and spiritual healers (mean difference = 1.6, $p<0.5$). It is interesting to note that this could be interpreted as health care providers perceiving less collaboration with HRO than the HRO themselves. There was no difference in collaboration between health care providers and traditional and spiritual healers. Per report, traditional and spiritual healers collaborated less with schools and teachers than did health care providers (mean difference = 1.85, $p<0.05$) and HRO staff (mean difference = 2.3, $p<0.01$). No group collaborated significantly with traditional and spiritual healers.

Collaboration with traditional and spiritual healers is limited, and this group has the lowest rates of collaboration across sectors. A spiritual leader in Cambodia indicated role constraint impacting collaboration, stating "people come to me for help; as a monk, I can't do anything more than that." However, a traditional healer in Cambodia noted the value of indigenous healers, stating that, "traditional healers are [most] community oriented, [fitting within] the cultural structure." A health care provider in BIH reported no direct collaboration with traditional healers, but that, "we know the client goes and will support them in going."

Several providers in BIH commented on organizational, personal, or cultural issues that impacted collaboration. A health care provider reported some cooperation with other sectors, but stated that there, "should be better cooperation, but it's not our fault

or theirs…just a problem in organization." A spiritual healer noted some collaboration with HRO in the past, but that "humanitarian aid provided all the time might make [clients] lazy." Speaking about collaboration with the health care sector, an HRO staff stated:

They are increasingly turning to [us]. At first they thought we were interfering foreigners. Now they realize we are on their side, that we have qualifications and something to say.

Discussion

This study provides a microcosm of healing communities in two different post-conflict settings from the viewpoint of field-level care providers. Both the study approach and its findings provide some useful guidance for psychosocial assistance approaches and activities.

Listening to the voices of care provider community

This research demonstrates the value of eliciting the input of the care provider community to better understand the context, current approaches, and needs of care providers and the larger community in post-conflict settings. We believe that this type of exploratory research might be a useful approach/tool in guiding and negotiating a response between affected communities and outside agencies that aim to provide psychosocial assistance. While psychosocial assistance activities aim to acknowledge the experience, knowledge, capacity and contribution of local providers in the post-conflict setting and avoid depleting mainstream services, this provides a concrete framework for eliciting and analyzing community input to guide program decisions and negotiations between the war-affected community and outside agencies. This type of exploratory research might be included as a central component in the planning and design phases for psychosocial assistance programs in post-conflict settings.

This study highlights the centrality of poverty and violence as areas responsible for suffering where support is needed, and we would expect these social ills to be highlighted in other post-conflict settings (which are also often characterized by high levels of poverty and violence). However, the support the care providers need in addressing these issues might be different and/or other social issues might also emerge through this exploratory research. This research and rich data obtained from care providers can provide the basis of an action plan for the care provider and social service community. Not only does such an approach reflect sensitivity to local capacities, approaches, and needs, but it also gives local communities their rightful role as key players in post-war recovery and rehabilitation (28).

Poverty and violence in BIH and Cambodia

Study findings also provide some evidence that additional support in addressing issues related to poverty and violence might be useful in post-conflict settings, to promote and improve the psychosocial care of war-affected populations. Given its broad mandate, addressing both psychological and social issues, the field of psychosocial assistance is well-placed to respond to this identified need for support. Concrete guidance in addressing these specific issues through psychosocial assistance is needed. While additional social issues might be identified in other settings, we believe that specific support in these two areas should be further considered in psychosocial assistance programming. This viewpoint is based not only the prevalence of poverty and violence in these settings, but also on a growing body of research which shows interlinkages be-

tween poverty, violence and mental health outcomes (29-31).

Integrative and collaborative care systems are needed

The multi-dimensional experience and impact of poverty and violence warrants further consideration in psychosocial assistance, and highlights the need for activities which support an integrated healing system. Some manifestations of poverty and violence include, for example, child abuse, domestic violence, substance dependence and crime, other high risk activities and poor health (32-35). Therefore, it might be useful in post-conflict settings to strengthen linkages among local care providers themselves and between local care providers and the larger social service system. For example, a network could be established linking care providers to services such as child care, training in parenting and self-protection, substance use prevention activities, skills training, supported employment, and other efforts to support socio-economic integration, etc. While various approaches to healing, such as supportive communication, are viewed as helpful, their effectiveness is further strengthened with the provision of concrete social assistance. One important issue raised by care providers in BIH was a lack of adequate housing, yet neither health care providers nor HROs reported assisting with housing needs. Though they may not be *responsible* for providing housing, they should be able to facilitate and guide clients towards accessing assistance to meet this need.

An integrated approach would be particularly useful in settings where, for example, mental health care is highly stigmatized. It can also play an important role in reducing duplication of services. Within a collaborative and integrated care system, an individual's various needs can be met regardless of where they enter the care system. Any effective system must also consider a community's reliance on and the role of spiritual and/or traditional healers in healing activities and care (36-37) and ensure their linkages to the larger care system.

Resources and capacity-building

This analysis provided evidence that training for local care providers in BIH and Cambodia in addressing poverty and violence might be useful. This is not surprising as, for example, research has found that mental health issues often go unnoticed in the primary care setting. Research shows that training programs have been effective in increasing the capacity of health care workers to identify and address issues related to mental health, and other outcomes associated with poverty and violence (38-44).

In this setting, it is not surprising that limited access to resources and support was cited as a barrier to providing effective care as was training, more generally. Care providers indicated that they needed training in supervision, languages, and in writing. Further, training in specific areas of care might be warranted. For example, most care providers did not have confidence in addressing substance abuse/dependence issues. In addition, awareness-raising and training self-care might be useful in improving the care provider community's capacity to provide adequate care to their clients while protecting their own health and well-being.

Emphasizing evidence-based practice

Since this research was undertaken, significant strides have been made in developing the evidence base for psychosocial assistance activities and approaches. Further, a number of resources have been collaboratively developed, including, for example, the Inter-Agency Standing Committee on Mental Health and *Psychosocial Support in*

Emergency Settings (45) to guide efforts and approaches in psychosocial care. The field should continue to focus on building the research base, documenting promising practices and continue to promote and set guidelines related to standards of care. This is not just adding to the 'toolbox' of psychosocial interventions, but calls for thinking 'outside the box' in terms of identifying and designing interventions and measuring their impact. One example of this might be providing people with opportunities to help others, as there is evidence that giving people the opportunity to practice altruistic behavior is associated with better mental health outcomes (46). Over time, this is an important way that resources for such assistance can be secured – a particular concern, as found in this study, and more generally today in the context of limited and competing resources in post-conflict settings. Further, while the community can provide important guidance and insight into areas where assistance would be useful, practitioners in this field can also provide important useful information and guidance on the basis of evidence-based approaches that have been effective in other settings.

Limitations of study

This study provided important insight into psychosocial practices and field-level realities in two post-conflict environments from the prospective of the care provider community. There are some limitations, including the study's small sample size and the absence of interviews with all types of relief providers (e.g., development organizations) and clients. Information from clients, and other important providers (e.g., human rights, advocacy and protection sector actors), would provide important insights into this study's findings, as well as into the utility and feasibility of its recommendations. Finally, these data were collected in 2003 and, therefore, may not perfectly reflect practice today. However, it is our view that the central tenets of the paper and study findings remain useful and valid today.

Conclusion

Effective approaches to healing and capacity for providing such care are already present in post-conflict settings, though they may have been damaged during conflict. A systematic effort to listen to the field-level care providers' "voices" can provide important insight into their capacities and needs, in turn guiding psychosocial programming. This approach reflects the central tenets of relief and development assistance, to ensure that activities to assist communities are informed and guided by the communities themselves. On this basis, the psychosocial field is well placed to ensure effective care is provided to address both psychological and social needs.

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References

1. Robins L, Reigier D, editors. Psychiatric disorders in America: The Epidemiologic Catchment Area Study. New York: The Free Press; 1991.
2. Norris FH, Byrne CM, Diaz E, Kaniasty K. The range, magnitude, and duration of effects of natural and human-caused disasters: a review of the empirical literature. National Center for Post-

Traumatic Stress Disorder, Department of Veterans Affairs; 2001.

3. Roberts B, Damundu EY, Lomoro O, Sondorp, E. Post-conflict mental health needs: a cross-sectional survey of trauma, depression and associated factors in Juba, Southern Sudan. BMC Psychiatry. 2009;4(9):7.
4. Mollica RF. Invisible Wounds. Sci Am. 2000;282(6):54-7.
5. Waldman R, Martone G. Public Health and Complex Emergencies: New Issues, New Conditions. Am J of Public Health. 1999;89(10):1483-5.
6. Mollica RF, Lopes Cardozo B, Osofsky HJ, Raphael B, Salama P. Mental health in complex emergencies. The Lancet. 2004;364(9450):2058-67.
7. Ager A. Tensions in the psychosocial discourse: Implications for the planning of interventions with war-affected populations. Dev Pract. 1997;7(4):402-7.
8. Summerfield D. Assisting survivors of war and atrocity: Notes on 'psychosocial' issues for NGO workers. Dev Pract. 1995;5(4):352-60.
9. Summerfield D. A critique of seven assumptions behind psychological trauma programmes in war-affected areas. Soc Sci Med. 1999;48:1449-62.
10. Bracken P, Giller JE, Summerfield D. Rethinking Mental Health Work with Survivors of Wartime Violence and Refugees. Journal of Refugee Studies. 1997;10:4431-42.
11. Population Council. Psychosocial benefits of a mentoring program for youth-headed households in Rwanda. 2007. Found November 13, 2012 from: http://www.popcouncil.org/pdfs/horizons/RwandaPsychOVCImpactSum.pdf
12. McDonald L. Psychosocial Rehabilitation of Civilians in Conflict-Affected Settings (Chapter 10). In Martz E, editor. Trauma Rehabilitation after War and Conflict. New York: Springer; 2010.
13. Policy guidelines on the psychosocial care and protection of children in armed conflict: Recommendations. Nairobi, Kenya. UNICEF; 1997.
14. Arts PGH. Draft guidelines for programmes: Psychosocial and mental health care assistance in (post) disaster and conflict areas. Netherlands Institute for Care and Welfare; 2001.
15. Kiljunen K. Power politics and the tragedy of Kampuchea during the seventies. Bull Concern Asian Sch. 1985:1749-64.
16. Hannum H. International law and Cambodian genocide: the sounds of silence. Hum Rights Q. 1980;11:82-138.
17. Mollica RF, Jalbert RR. Community of confinement: The mental health crisis in Site Two (Displaced persons camps on the Thai-Kampuchean border). Report for the Committee on Refugees and Migrants, World Federation for Mental Health; 1989.
18. Duffy T. Toward a Culture of Human Rights in Cambodia. Human Rights Quarterly. 1994:16(1);82-104
19. Reuters. Mass grave found in Cambodian village. August 7, 2012. Found on November 13, 2012 at: http://www.reuters.com/video/2012/08/07/mass-grave-found-in-cambodian-village?videoId=236903252
20. Yale University. Cambodian Genocide Program. Provincial Killing Fields: Director for province links. (Map) Found November 13, 2012 at http://www.yale.edu/cgp/maps/directory.html
21. Cain J, Duran A, Fortis A, Jakubowski E. In Cain J, Jakubowski E, editors. Heath care systems in transition: Bosnia and Herzegovina. Copenhagen: European Observatory on Health Care Systems; 2002.
22. CESPI (Centro Studi di Politica Internazionale). Local Democratic Governance in Travnik Municipality. First Report. SeeNet Programme; 2010.
23. Bazeley P. Computerized data analysis for mixed methods research. In Tashakkori A, Teddlie C, editors. Handbook of mixed methods in social and behavioral research. Thousand Oaks, CA: Sage; 2003.
24. Holsti OR. Content analysis for the social sciences and humanities. Reading, MA: Addison-Wesley; 1969.
25. Glasser B, Strauss A. Discovery of grounded theory: Strategies for qualitative research. Chicago: Adeline; 1967.
26. Charmaz K. Reconstructing grounded theory. In Alasuutari P, Bickman L, Brannen J, editors. The SAGE handbook of social research methods. Los Angeles: Sage; 2008. p. 461-78.
27. Clarke AE. Situational analyses: Grounded theory mapping after the postmodern turn. Symbolic Interaction. 2003;26:553-76.
28. Mollica RF, McDonald L. Refugees and mental health: Old stereotypes, new realities. UN Chronicle. 2002;39(2):29-30.
29. Patel V, Araya R, de Lima M, Ludermir A, Todd C. Women poverty and common mental disorders in four restructuring societies. Soc Sci Med. 1999;49:1461-71.
30. Costello EJ, Compton SN, Keeler G, Angold A. Relationships between poverty and psychopathology: a natural experiment. JAMA. 2003;290;2023-9.
31. Roberts GL, Williams GM, Lawrence JM, Raphael B. How does domestic violence affect women's mental health? Wom Health. 1998;28(1):117-29.

32. Djeddah C, Facchin P, Ranzato C, Romer C. Child abuse: Current problems and key public health challenges. Soc Sci Med. 2000;51(6):905-15.
33. Oyemade A. Child abuse and neglect: A global phenomenon. African Journal of Medicine and Medical Sciences. 1991;20(1):5-9.
34. Huch MH. Violence against women: a worldwide problem. Nur Sci Q. 2000;13(4):339-40.
35. Armstrong TD, Costello EJ. Community studies on adolescent substance use, abuse, or dependence and psychiatric comorbidity. Journal of Consulting and Clinical Psychology. 2002;70(6):1224-39.
36. Kayombo EJ, Mbwambo ZH, Massila M. Role of traditional healers in psychosocial support in caring for the orphans: A case of Dar-es Salaam City, Tanzania. Journal of Ethnobiology and Ethnomedicine. 2005;1-3.
37. Ngoma MC, Prince M, Mann A. Common Mental disorders among those attending primary health clinics and traditional healers in urban Tanzania. Br J Psychiatry. 2003;183:349-55.
38. World Health Organisation (WHO). World Report on Violence and Health. Geneva: WHO; 2002.
39. Domestic Violence: A Resource Manual for Health Care Professionals. Chapter 5: Education and Training. Cardiff: The National Assembly for Wales; 2001.
40. World Health Organisation (WHO). The World Health Report 2001: Mental Health: New Understanding, New Hope. Geneva: WHO; 2001.
41. World Health Organisation (WHO). Diagnosis and Management of Common Mental health disorders in primary health care. Geneva: WHO; 1998.
42. Day A, Thurlow K, Woolliscroft J. Working with childhood sexual abuse: a survey of mental health professionals. Child Abuse Negl. 2003;27:191-8.
43. Henderson, DC, Mollica RF, Tor S, Lavelle J, Hayden, D. Building primary care practitioners' confidence in mental health skills in a post-conflict society: a Cambodian example. J Nerv Ment Dis. 2005;193(8):551-9.
44. Bower P, Garralda E, Kramer T, Harrington R, Sibbald B. The Treatment of child and adolescent mental health problems in primary care: a systematic review. Fam Pract. 2001;18:373-82.
45. Inter-agency Standing Committee. IASC Guidelines on mental health and psychosocial support in emergency settings. Geneva: IASC. 2007. Found November 12, 2012 at: http://www.who.int/hac/network/interagency/news/iasc_guidelines_mental_health_psychososial.pdf
46. Mollica, RF, Cui X, McInnes K, Massagli MP. Science-based policy for psychosocial interventions in refugee camps: a Cambodian example. J Nerv Ment Dis. 2002;190:158-66.

Review article

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Radiological imaging of rectal cancer

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This article discusses the possibilities of diagnosing abdominal imaging in patients with rectal cancer, detecting lesions and assessing the stage of the lesions, in order to select the appropriate therapy. Before the introduction of imaging technologies, the diagnosis of colorectal pathology was based on conventional methods of inspecting intestines with a barium enema, with either a single or double contrast barium enema. Following the development of endoscopic methods and the wide use of colonoscopy, colonoscopy became the method of choice for diagnosing colorectal diseases. The improvement of Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI), gave us new possibilities for diagnosing colorectal cancer. For rectal cancer, trans-rectal US (TRUS) or endo-anal US (EAUS) have a significant role. For staging rectal cancer, the Multi Slice Computed Tomography (MSCT) is not the method of choice, but Magnetic Resonance Imaging (MRI) is preferred when it comes to monitoring the rectum. The role of the MRI in the T staging of rectal cancer is crucial in preoperative assessment of: thickness – the width of the tumor, the extramural invasion, the circumference of resection margin (CRM), and the assessment of the inclusion of mesorectal fascia. For successful execution of surgical techniques, good diagnostic imaging of the cancer is necessary in order to have a low level of recurrence. According to medical studies, the sensitivity of FDG-PET in diagnosing metastatic nodals is low, but for now it is not recommended in routine diagnosis of metastatic colorectal carcinoma.

Key words: Colorectal cancer, TRUS, MSCT, MRI, Staging.

Introduction

Colorectal cancer is a malignant disease on the rise. Annually, about 300,000 new cases of colorectal cancer are diagnosed per 500 million EU citizens. Colorectal cancer is the third most common cancer among men (only after lung and prostate cancer), and the second most common cancer among women (after breast cancer) (1). It represents the second cause of death in both sexes, in men after lung cancer and breast cancer in women. Colorectal cancer is the second most common cancer in general and the most common of the gastrointestinal tract cancers. It

is equally common in both genders, mostly in those over 50 years of age. In about 50% of cases the cancer occurs in the rectum and colon recto-sigmoid, the other 25% occurs in the sigma, while the remaining 25% is found in the rest of the colon. Rectal cancer is a major problem precisely because of its high incidence. For many years the primary radiological diagnostic method in diagnosing abnormalities of the colon and rectum was barium enema. Double contrast technique is used in detecting small lesions (<1 cm), documenting inflammatory diseases and detecting rectal pathology. Before the introduction of colonoscopy, barium enema was the method of choice for screening colorectal cancer. The development of colonoscopy is changing the algorithm because, unlike colonoscopy, barium enema is a method of low sensitivity when it comes to detecting polyps and cancer (2) and it is performed only when colonoscopy is not possible or unsuccessful. With the use of Computerized Tomography (CT) imaging, Multi Slice Computed Tomography (MSCT), with the advantages of computerized colonography (CT colonography) and Magnetic Resonance Imaging (MRI), diagnosis of colorectal pathology, particularly the detection of the colon and rectal cancer, has been enhanced (3-6). These methods provide insight into intramural and extra-colic pathological changes in the colorectal region. Both of these methods have their limitations, particularly with older generation CT and MRI scans, in assessing the stage of the tumor (TU) in the area of the bowel wall and the invasion to regional lymph nodes. MSCT colongraphy remains the method of choice after the proven insufficiency of colonoscopy (7-11). In 2004, a 64-slice CT scan was introduced in the diagnostic process. Compared to the older CT generation, 64-slice CT scan has a shorter scanning time (7 sec for 40 cm), with improved spatial and temporal resolution images. However, even in devices with the option of "low dose", there is still a high limit of radiation dose for colorectal screening (12).

Transrectal US (TRUS) is an accurate diagnostic method for rectal cancer compared to older CT and MRI technologies. MRI, with high resolution T2 weighted sequences, clearly depicts the details of the rectal wall and perirectal anatomy. The rectal wall can be recognized as three different layers: a thin inner line of low signal intensity represents the mucosal layer, a middle layer of high signal intensity represents the submucosa, and an outer layer of low signal intensity represents the muscularis propria (13, 14). The mesorectal fascia, which forms the boundary of the surgical excision plane in total mesorectal excision, is identified as a thin, low – signal intensity structure on the MRI, that envelops the rectum and the surrounding perirectal fat (14).

Radiological imaging of rectal cancer

Determining an optimal treatment plan for an individual patient with rectal cancer is a complex process. In addition to facing decisions regarding the intent of rectal cancer surgery, consideration must also be given to the validity of the treatment results, including the probability of maintaining or restoring normal bowel function/anal continence and preserving genitourinary functions. Careful patient selection with respect to particular treatment options and the use of sequenced multimodality therapy for selected patients, which combines chemo – radiation with operative treatment as part of the treatment regimen, is recommended. The main objective in diagnostically imaging patients with rectal cancer is to detect lesions and assess the extent of those lesions, in order to better assist the selection of appropriate therapy. Patients with rectal cancer appropriate for resection require complete staging evaluation, including full colonoscopy to evaluate the synchronous lesions, rigid

proctoscopy to determine the location of the cancer, baseline computed tomography scans of the chest, abdomen and pelvis, and a complete physical examination.

The accessibility of evaluating rectal cancer by certain imaging modalities, such as endo-anal US (EAUS) and MRI scan, makes pre-operative assessments of the depth of tumor penetration and the presence of local lymph nodal metastases possible. Additional information regarding the extent of the disease and the occurrence of distant metastases can be determined preoperatively through CT scans. Thus, endorectal ultrasound, or endorectal or pelvic MRI, CT scans of the chest, abdomen and pelvis, are recommended for the preoperative staging of rectal cancer. A positron emission tomography scan is not routinely indicated at baseline in the absence of evidence of synchronous metastasis disease.

The most common method for determining the stage of colorectal cancer is the AJCC/TNM system CC VII (15). General CT and MSCT are now the most widely used methods in determining the stage of colorectal cancer (Figure 1 and Figure 2) (9, 10).

In contrast to some limitations of CT and MRI scans when it comes to predicting the stage of rectal cancer, high resolution MRI of the rectum has recently been proven to be highly accurate and reproducible in assessing the absolute extramural depth of tumor invasion in rectal cancer (16, 17). Phased – array surface coil is preferred for high resolution MRI scanning of the rectum, because the endorectal coil MRI offers a limited field of vision, despite its superior spatial resolution: a complete assessment of perirectal structures is difficult with the endorectal coil because portions of the mesorectal fascia, mesorectal fat, and lymph nodes lie outside the field of view (16).

In the preoperative evaluation based on MRI scans, attention is given to an accurate assessment of the depth of tumor invasion, extra-mural invasion of the circumference of resection margin (CRM), and the mesorectal fascia, which is equal to the circumference border of the surgical excision (17–24). The standard surgical procedure for rectal resection includes the resection of the mesorectal fat and the associated lymph nodes. In patients with TU, extra-mural invasion radio, or chemo therapy is recommended before surgery, to reduce the risk of TU recurrence after surgery. The thickness of the colorectal wall due to TU, extra-tumor invasion, and the presence or absence of resec-

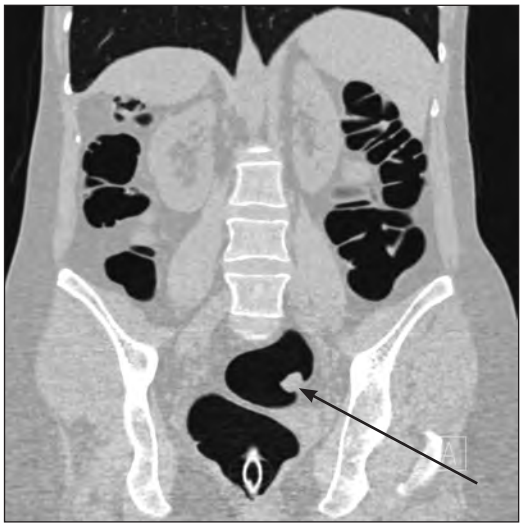

Figure 1 MSCT 2D – adenocarcinoma of recto-sigmoid colon. Black head arrows show the polypoid mass.

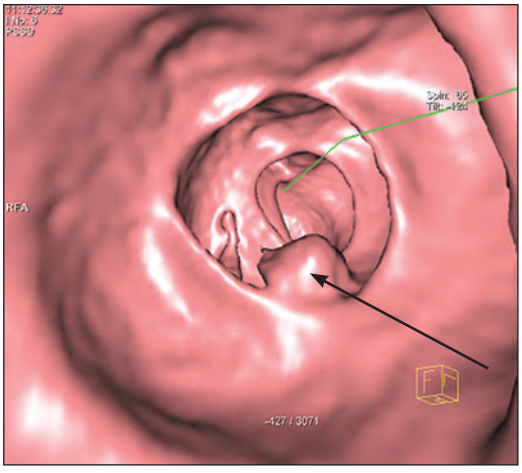

Figure 2 MSCT colonography – adenocarcinoma of rectum. Black head arrows show the polypoid mass.

tion tumor circumferential surface are the main risks for relapse and further post-operative prognosis (17–24).

The identification and staging of rectal cancer by MRI scans is largely based on differences in T2 signal intensity between the tumor, the rectal wall and perirectal fat tissue. The tumor itself has an intermediate signal intensity which is between the high signal intensity of the submucosa, or perirectal fat tissue, and the low signal intensity of the muscular layer (16, 24). The absolute depth of extramural invasion of rectal cancer on MRI scans agrees well with pathological measurement, and the presence or absence of the tumor-effect in the CRM can be predicted accurately, with at least a 1 or 2 mm distance from which there is high risk of postoperative recurrence (16, 24, 25). However, the exact depth of the extra-mural spread of TU is considered to have less importance, since all patients with T3 and T4, and possibly positive lymph nodes, receive preoperative chemo and radio therapy (9).

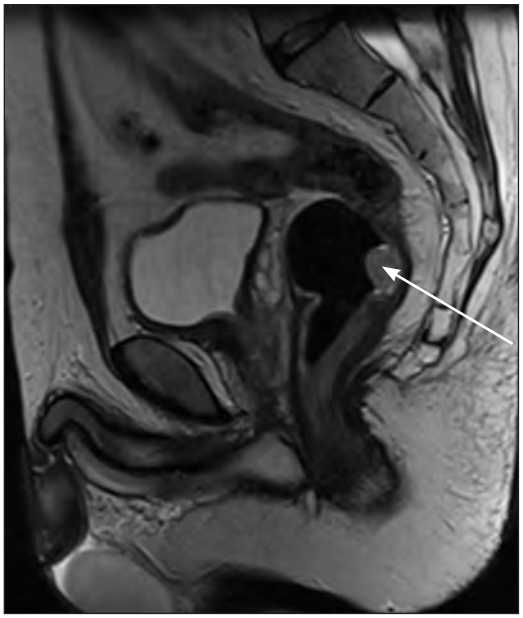

Figure 3 MRI T2WI adencarcinoma of the posterior wall of the rectum. White head arrow shows the polypoid mass.

For successful diagnosis of colorectal carcinoma good bowel distention is crucial, when using either MSCT or MRI scans. In the case of poor distention during CT colonography it is possible to miss larger lesions (9, 25) if the colon is collapsed. In determining the stage of rectal cancer, colonic distension will improve lesion visibility but may alter the distance between the outer margin and mesorectal fascia, reassessing the extramural depth of tumor invasion (9). On MSCT or MRI images, rectal cancer appears

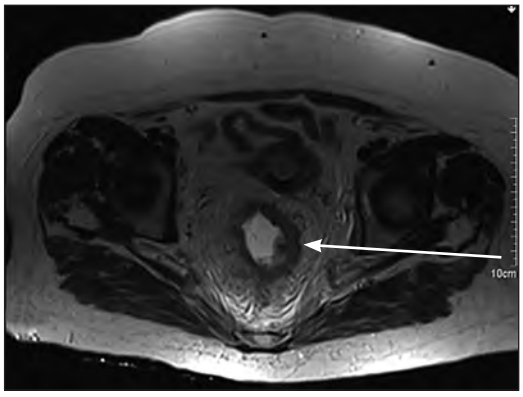

Figure 4 MRI transverse cross-section of the rectum – adenocarcinoma. White head arrow shows asymmetrical wall thickening of rectum with an irregular surface.

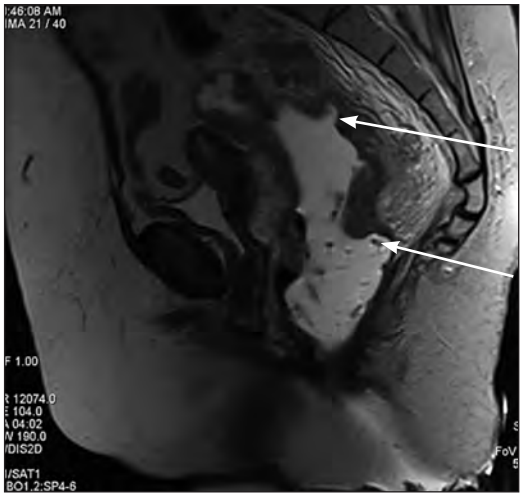

Figure 5 MRI sagittal section of the rectum – adenocarcinoma. White head arrow shows asymmetrical wall thickening of rectum with an irregular surface.

as discrete mass focal or a short-segmented wall thickening (Figure 3).

Asymmetrical wall thickening of rectum, with or without an irregular surface, suggests a neoplastic process (Figure 4, Figure 5) (26).

Assessment of local tumor spread

Diagnosis of tumor invasion beyond the bowel wall can be made with cross – section imaging, but only if the tumor mass extends directly into the surrounding muscle (levator anni, obturator internus, coccygeus, piriformis or gluteus maximus), or organs, obliterating the fat planes and enlarging the individual muscle, or enveloping the neighboring structures.

Accuracy of MSCT and MRI scanning in loco-regional staging has increased considerably thanks to advances in imaging technology in recent years. The diagnostic accuracy for polyps larger than one cm ranges from 60% to 100%, while for cancer it is 100% (9).

Sensitivity of CT imaging in the evaluation of TU and T stage is 78% and 63% respectively, and specificity is 86% and 77% respectively. Sensitivity of the MRI scan in comparison with EAUS is 93% and 78% respectively (27). An MRI of the rectum, when it comes to assessing the extramural spread of the tumor, compared to CT scanning (which has its limitations), has greater diagnostic value (27-30).

The application of an endorectal coil has limitations in the width of field, despite its superior spatial resolution; analysis of the complete tumor expansion to the perirectal structure is insufficient because parts of the mesorectal fascia, fat, and mesorectal lymph nodes, outside the primary field, are not observed. It is similar with TRUS, which is more accurate than CT or MRI scans in evaluating the wall of the rectum and superficial rectal cancer. TRUS is limited in evaluating perirectal and mesorectal fascia due to limited tissue penetration (26, 28). T2 MRI sequences are most suitable for depicting the anatomy of the rectal wall and the spread of cancer in the perirectal space, as well as high-resolution T2 weight sequences imaging, with a non-breath-hold turbo spine echo sequences which was used in most studies (13, 23, 24). Identifying and determining the stage of rectal cancer by MRI scan is based on the differences in T2 signal intensity between the tumor and the rectal wall, and the perirectal fat tissue. There is intermediate signal intensity between the high-signal intensity of the submucosa and perirectal fat, and low signal intensity of the muscular layer (13, 23).

Three dimensional radiation therapy to assess the borders of the tumor is based on MSCT imaging, which is not always the optimal method. The greatest restriction is the low contrast resolution. MRI imaging is more likely to determine smaller lesions, more accurately determining the volume of the tumor compared to CT scanning. The volume of the tumor detected on an MRI scan is smaller and shorter at the distal of the anal sphincter than the volume based on the CT scan. In planning radiotherapy, these results will result in a smaller volume of radiation, leading to dose reduction in the surrounding organs at risk (31).

Rectal cancer grows from mucosa and progressively spreads to deeper layers of the bowel wall. The outer margins of the rectum are smooth on CT and MRI imaging, and perirectal and pericolic adipose tissues have the same density on CT scans and the same signal intensity as on MRI. Spreading of the tumor outside the wall of the intestines is manifested as the irregular outer margin of the bowel wall with expansion to the soft tissue as well as to pericolic and perirectal adipose tissues (Figure 6). A similar expansion may occur in through the desmoplastic response of the peritumor tissues with inflammatory or congestive changes, resulting in a miscalculation of stage T2 and T3 lesions.

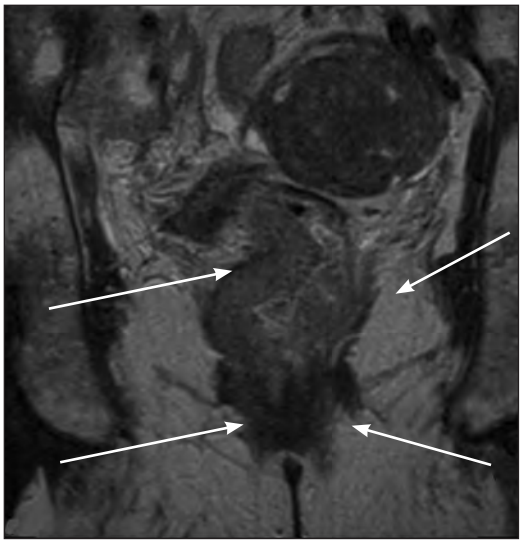

Figure 6 MRI Ca rectum. White head arrows show the spread of the tumor outside the wall of the rectum.

Furthermore, a microscopic cancer invasion into the pericolic adipose tissue cannot be visualized on CT or MRI scans, making the T3 resemble T2 (9).

Involvement of the lymph nodes in rectal cancer

By following the anatomy of the lymphatic drainage of the colon, with present cross-sectional imaging we can predict the spread of colorectal cancer through the lymph. Rectal cancer has two main transmission routes of a tumor by the lymph nodes. For the upper rectum, the tumor extends via the lymph nodes along the branches of the inferior mesenteric arteries. Lower portions of the rectum show the expansion of tumor in the lateral lymphatic flow, along the middle rectal vessels to the internal iliac arteries (26). Careful preoperative evaluation of the internal iliac area is significant for treating rectal cancer, especially in patients with carcinoma of the rectum in the lower portion, because the lymph nodes in that area lie outside the resection margin of the total mesorectal excision. Downward spread along the inferior rectal vessels to the groin is unusual except in very advanced cases, and also when the anal canal is involved (26).

Accuracy of CT and MRI scanning in nodal staging has been studied mostly for rectal cancer. Despite the high spatial resolution of the CT and MRI scans, which allows identification of lymph nodes as small as 2 to 3 mm, reliable detection of nodal metastases is presently not possible. Radiological features, in assessing the involvement of lymph nodes, are mostly related to morphological criteria, such as the size and contour of the lymph nodes.

One meta-analysis showed 52% sensitivity and 78% specificity of CT when it came to detecting nodal metastasis, and 65% sensitivity and 80% specificity for MRI scans. In another meta-analysis, the corresponding sensitivity and specificity were 55% (95% confidence interval: lower limit, 43; upper limit, 67) and 74% (95% confidence interval: 67.8) for CT and 66% (95% confidence interval: 54.76 and 76% confidence interval: 59.87) for MRI scans (28).

The size of lymph nodes is a criterion which restricts analysis, since micro metastases in small lymph nodes are not recorded, and in large lymph nodes it is difficult to distinguish with certainty the presence of reactive hypertrophy from metastases. Rectal cancer is particularly known to have a high frequency of micrometastases in normal sized nodes (13, 30, 32, 33), with 45.4% to 78% of the involved nodes being ≤5 mm in size. Therefore, a lower size criteria in predicting malignant perirectal lymphadenopathy (such as ≥5 mm), compared to the criteria for the other intra-abdominal nodal stations (such as ≥8 mm or ≥10 mm), is recommended in the interpretation of perirectal lymph nodes. Morphological analysis of lymph nodes may help diagnose malignant lymph nodes to a certain degree. Speculated or blurred boundaries of lymph nodes, heterogeneous, mottled, and high signal intensity in the lymph node is characteristic of

malignant lymphadenopathy on T2-weighted MRI imaging (13, 33).

Diagnosis of distant metastases in rectal cancer

Metastatic tumors are very common in the late stages of cancer. The spread of metastases may occur through the blood, the lymphatics, or through both routes. The most common places for metastasis to occur are the liver, lungs, brain and bones. The liver is the most common place of hematogenous dissemination of colorectal cancer with the emergence of a typical focal area of low density on the CT scan or high signal intensity on the T2WI in MRI scan, and low on T1WI, in comparison with normal liver parenchyma, with or without rim enhancement after contrast enhancement. The portal phase is the optimal time for scanning metastasis for lesions, and enables easy detection. The sensitivity of CT and MRI scans in detecting metastases is moderate. A CT scan is highly sensitive in detecting nods in the lungs, so it is preferred in diagnosing lung metastases in colorectal cancer, however it is not overly specific; also, the CT scan is sensitive in the detection of bone metastases. For brain metastasis MRI has been proven more suitable than a CT scan. A CT scan is limited in the diagnosis of the peritoneal spread of metastatic disease in colorectal carcinoma. Sensitivity per patient is 60% - 76%. Per lesion, sensitivity is limited, demonstrating only 9.1% to 24.3% for tumor implants smaller than 1 cm (9, 34, 35).

Fluorodeoxyglucose-positron emission tomography (FDG-PET) in the preoperative diagnosis of rectal cancer

There is controversy surrounding the routine preoperative staging of colorectal cancer with FDG-PET (36), and the role for FDG-PET in determining the stage of colorectal cancer has yet to be established (37). Preoperative FDG-PET imaging may be helpful in detecting distant metastases and could render surgery unnecessary in patients with increased surgical risk. It may be helpful as a baseline evaluation prior to neoadjuvant chemotherapy in patients with advanced stage disease (38).

Several meta-analyses have shown that FDG-PET was superior to CT or MRI in detection of hepatic metastases from various cancers of the gastrointestinal tract, with 90% sensitivity and 85% specificity, or similar to CT or MRI in the detection of hepatic metastases from colorectal cancer, with 75.9% sensitivity (39). This was demonstrated by a meta-analysis comparing non-invasive imaging methods (US, CT, MRI and FDG-PET) for the detection of hepatic metastases from colorectal cancer, gastric, and esophageal cancers, at an equivalent specificity of 85%. FDG-PET had highest sensitivity (90%) compared to MRI (76%), CT (72%), and US (65%) (40). After an economic evaluation, as we see in the literature, we can conclude that FDG-PET and Positron emission tomography CT (PET-CT), as an add-on imaging device, is cost-effective in the preoperative staging of recurrent colon, recurrent rectal and metastatic disease but not in primary colon or rectal cancer. According to the literature, it has low sensitivity in diagnosing FDG-PET lymph nodes metastasis, and it is not recommended in routine diagnosis of metastatic colorectal carcinoma. In the study of Schmidt, et al. it was reported that accuracy for PET-CT was 91% (sensitivity 86%, specificity 96) and 83% for MRI (sensitivity 72%, and specificity 93%) retrospectively. Initial results suggest that differences in accuracy for local and distant metastases detection, using FDG-PET and PET-CT and MRI for integrated screening of tumor recurrences in colorectal cancer, depend on the location

of the malignant focus (41). FDG-PET can detect extra-hepatic metastases and determine whether to go for resection of hepatic metastases, if it would result in a longer benefit to the patient (39).

Post-operative follow-up with the patients with rectal cancer

Recurrences take place 3 to 5 years after treatment. An annual rigid proctoscopy, or barium enema, is used for monitoring patients after surgical treatment, together with radiological imaging methods CT and MRI scans. More than half the patients have post-operative relapse and distant metastases in the liver and lungs (42). A relapse is more likely to occur to the anastomosis as an extraluminal lesion, rather than intraluminal. Therefore, cross-section imaging, such as CT and MRI scans, plays an important role in the post-operative survival of patients after curative operation for colorectal cancer, because it primarily evaluates extra-colic structures. In addition to MSCT imaging, it is necessary to measure carcinoembryonic antigen (CEA) in the serum during the post-operative follow-up (43-45). The American Society of Clinical Oncology has revised the guide for colorectal cancer and suggests an annual abdominal CT control for 3 years after primary therapy, for patients who have a high risk of recurrence. A positive CT finding of local recurrence involves the TU mass with enlargement of the local lymph nodes, or invasion of surrounding structures, as well as ischiorectal fosses. In the diagnostics of pelvic recurrence, CT and MRI are used.

The diagnostic problem of scars and fibrosis after radiation therapy and local recurrence could not be resolved with classical CT scanning. Application of MRI scans is useful in assessing the extension of recurrence or post-operative scarring. Fibrosis has low signal intensity, as opposed to relapse in T2WI, which has high signal intensity. Some studies suggest that high signal intensity on T2WI can be found with a non-neoplastic inflammatory process, or edema and fresh fibrosis (less than 1 year old), while low signal intensity in a TU mass may occur in a recurrence with desmoplastic reaction.

FGD-PET in post-operative follow-up for patients with rectal cancer

Unlike CT and MRI scans, FDG-PET is highly sensitive in diagnosing suspected recurrence of colorectal cancer (41, 43). A number of studies have demonstrated the role of FDG-PET as a metabolic imaging modality for detecting recurrent or metastatic disease. The sensitivity of FDG-PET is in the 90% range, with specificity greater than 70%, both superior to CT scans. A meta-analysis of 11 clinical reports and 577 patients showed that the sensitivity and specificity of FDG-PET for detecting recurrent colorectal carcinoma was 97% and 76% respectively (46). False negative FDG-PET findings have been reported with mucinous adenocarcinoma (47). It is applied in distinguishing a recurrence from postoperative fibrosis in the pelvic area. The residue or recurrence tumor shows accumulation of the radio-tracer (47). It is recommended for patients with elevated CEA, without the presence of radiological signs of recurrence or metastasis (9).

New guidelines for colorectal carcinoma using modern diagnostic imaging in monitoring patients with a tumor

Today, international standards related to the size of tumors are significant but insufficient in indicating the effects of therapy on the biology of the tumor, as well as indicating the success rate of the applied therapy. Modern diagnostic imaging, such as perfused ultrasound imaging, perfused CT or MRI scans,

diffusion MRI, and metabolic imaging with FDG-PET and PET-CT, has an important role in monitoring patients with a tumor (37). In the future, a range of these highly efficient imaging techniques will be applied not only as diagnostic imaging in the early diagnosis of cancer, but also as a routine diagnostic method in each oncology department for chemo or radio therapy. Which methods will be used depends on the CEA in the serum during post operative follow-up. It is recommended that for patients with elevated CEA, without radiological signs of recurrence or metastasis present, metabolic imaging (PET-CT) should be used, which has an important role in monitoring patients with a tumor (43).

Conclusion

In recent years with the improvement of cross-section imaging, the widespread use of MSCT scans, as well as the technologically improved MRI units of 1.5 and 3T**,** the diagnosis of primary rectal cancer and recurrences, or metastases occurrence in postoperative patients with elevated CEA, is safer. Patients are more likely to survive rectal cancer by following the proposed guidelines for diagnosing and post-operative monitoring. MSCT imaging has a primary role in diagnosing and evaluating the process, and monitoring, disease prognosis and monitoring of possible complications. For rectal cancer, the application of TRUS and MRI scanning in diagnosis and differentiating post-operative and post-irradiation fibrosis in relation to relapse, is an advantage compared to MSCT imaging. Today, in the pre-operative stages, rectal MRI becomes mandatory.

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Authors' contributions: Conception and design: LLC and MBČ; Acquisition, analysis and interpretation of data: DV and SVZ; Drafting the article: LLC and MBČ; Revising it critically for important intellectual content: LLC and MBČ.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin. 2009;59:225-9.
2. Winawer SJ, Stewart ET, Zauber AG, Bond JH, Ansel H, Waye JD, et al. A comparison of colonoscopy and double-contrast barium enema for surveillance after polypectomy. National polyp Study Work Group. N Engl J Med. 2000;342:1766-72.
3. McFarland EG, Levin B, Lieberman DA, Pickhardt PJ, Johnson CD, Glick SN, et al. Revised colorectal screening guidelines: Join effort of the American Cancer Society, U.S. Multisocety Task Force on Colorectal Cancer, and American College of Radiology. Radiology. 2008;248:717-20.
4. Punwani S, Halligan S, Tolan D, Taylor SA, Hawkes D. Quantitative assessment of colonic movement between prone and supine patient positions during CT colonography. British Journal of Radiology. 2009;82:475-81.
5. Mehdy Chadi. CT Colonography (virtual colonoscopy). Guerbet. 2005;12:5.
6. Zalis ME, Barish MR, Choi JR, Dachman AH, Fenolou HM, Ferrucci JT, et al. For the working group on Virtual Colonoscopy. CT Colonoscopy Reporting and Data System: A Consensus Proposal. Radiology. 2005;236:3-9.
7. Balchar A, Suosa J. CT colonography (virtual colonoscopy): technique indications and performance. Digestion. 2007;76:34-41.
8. Sofić A, Bešlić Š, Kocijanić I, Šehović N. CT colonography in detection of the colorectal carcinomas. Radiol Oncol. 2008;42(3):136-42.
9. Gollub MJ, Schwartz LH, Akhurst T. Up date on colorectal cancer imaging. Radiol Clin North Am. 2007;45:85-118.
10. Roddie M. CT colonography tools advance in clinical use. Diagnostic Imaging Europe. 2006;10:35-7.
11. Liedenbaum MH, de Vries AH, Gouw CI, van Rijn AF, Bipat S, Dekker E, Stoker J. CT colonography with minimal bowel preparation; evaluation of tagging quality, patient acceptance and diagnostic accuracy in two iodine-based preparation schemes. Eur Radiol. 2010;20:367-76.
12. Luz O, Buchgeister M, Klabunche M, Trabold T, Kopp AF, Claussen CD, et al. Evaluation of dose exposure in 64-slice CT colonography. Eur Radiol. 2007;17:2616-21.

13. Brown G, Daniels IR, Richardson C, Revell P, Peppercorn D, Bourne M. Techniques and trouble shooting in high spatial resolution thin slice MRI for rectal cancer. Br J Radiol. 2005;78:245-51.
14. Salermo G, Daniels IR, Brown G. Magnetic resonance imaging of low rectum. Defining the radiological anatomy. Colorectal Dis. 2006;8(Supp 3):10-3.
15. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, editors. AJCC Cancer Staging Manual, 7th ed. New York (etc.): Springer; 2010 [cited 2012 May 31]. Available from: http://www.cancerstaging.org/staging/index.html
16. Iafrate F, Laghi A, Paolantonio P, Rengo M, Mercantini P, Ferri M, et al. Preoperative staging of rectal carcinoma with MRI imaging correlation with surgical and histopathologic findings. Radiographics. 2006;26:701-14.
17. Kim NK, Kim MJ, Park JK, Park SI, Min JS. Preoperative staging of rectal cancer with MRI: accuracy and clinical usefulness. Ann Surg Oncol. 2000;7:732–7.
18. Burt S, Brown G, Daniels IR, Norman AR, Mason B, Cunningham D. MRI directed multidisciplinary team preoperative treatment strategy: the way to eliminate positive circumferential margins. Br J Cancer. 2006;94(3):351-7.
19. Maier AG, Kersting-Sommerhoff B, Reeders JW, Judmaier W, Schima W, Annweiller AA, et al. Staging of rectal cancer by double-contrast MR imaging using the rectally administered super paramagnetic iron oxide contrast agent ferristene and IV gadodiamid injection: results of a multicenter phase II trial. J Magn Reson Imaging. 2000;12:657-60.
20. Klessen C, Rogalla P, Taupitz M. Local staging of rectal cancer: the current role of MRI. Eur Radiol. 2007;17:379-89.
21. Berman L, Israel GM, McCarthy SM, Weinreb JC, Longo WE. Utility of the magnetic resonance imaging in anorectal disease. World J Gastroenterol. 2007;13:3153-8.
22. Sofić A, Šehović N, Prnjavorac B, Bilalović N, Čaluk J, Sofić D. MR rectum imaging with ultrasound gel as instrumental contrast media in tubulovillous adenoma. Radiol Oncol. 2008;42(3):136-42.
23. Beets-Tan RG, Beets GL, Vliegen RF, Kessels AG, Van Boven H, De Bruine A, et al. Accuracy of magnetic resonance imaging in predicting of tumor-free resection margin in rectal cancer surgery. Lancet. 2001;375:497-504.
24. Wibe A, Rendedal PR, Svensson E, Norstein J, Eide TJ, Myrvold HE, et al. Prognostic significance of the circumferential resection margin following total mesorectal excision for rectal cancer. Br J Surg. 2002;89:327-34.
25. Taylor SA, Halligan S, Goh V, Morley S, Bassett P, Atkin W, et al. Optimizing colonic distention for multi-detector row CT colonography: effect of hyoscine butylbromide and rectal balloon catheter. Radiology. 2003;229(1):99-108.
26. Beets-Tan RG, Beets GL. Rectal cancer: review with emphasis on MR imaging. Radiology. 2004;232:335-46.
27. Kwok H, Bissett IP, Hill GL. Preoperative staging of rectal cancer. Int J Colorectal Dis. 2000;15:9-20.
28. Bipat S, Glas AS, Slors FJ, Zwinderman AH, Bossuyt PM, Stoker J. Rectal cancer: local staging and assessment of the lymph node involvement with endoluminal US, CT and MR imaging – a meta analysis. Radiology. 2004;232:773-83.
29. Geeneu RW, Hussain SM, Cademartiri F, Poley JW, Siersema PD, Krestin GP. CT and MR colonography: scanning techniques, post-processing and emphasis on polyp detection. Radiographics. 2004;24:e18.
30. Martling A, Holm T, Bremmer S, Lindholm J, Cedermark B, Blomqvist L. Prognostic value of preoperative magnetic resonance imaging of the pelvis in rectal cancer. Br J Surg. 2003;90(1):1422-8.
31. O'Neill BD, Salerno G, Thomas K, Tait DM, Brown G. MR vs CT imaging: low rectal cancer tumour delineation for three-dimensional conformal radiotherapy. Br J Radiol 2009;82:509–13.
32. Mönig SP, Baldus SE, Zirbes TK, Schröder W, Lindemann DG, Dienes HP, et al. Lymph node size and metastatic infiltration in colon cancer. Ann Surg Oncol. 1999;6:579-81.
33. Kim JH, Beets GL, Kim MJ, Kessels AG, Beets-Tan RG. High- resolution MR imaging for nodal staging in rectal cancer: Are there any criteria in addition to the size? Eur J Radiol. 2004;52:78-83.
34. de Bree E, Koops W, Kröger R, van Ruth S, Witkamp AJ, Zoetmulder FA. Peritoneal carcinomatosis from colorectal or appendicle origin: Correlation of preoperative CT with intraoperative findings and evaluation of interobserver agreement. J Surg Oncol. 2004;86:64-73.
35. Heunedige T, Teo L, Ang B, Cheong WK, Venkatesh SK. Accuracy of preoperative CT for local staging in colorectal carcinomas. Singapore Med J. 2010;51(6):457-80.
36. Furukawa H, Ikuma H, Seki A, Yokoe K, Yuen S, Aramaki T, et al. Positron emission tomography scanning is not superior to whole body multidetector helical computed tomography in preoperative staging of colorectal cancer. Gut. 2006;55:1007-11.

37. Long Sun, Hua Wu, Yong-Song Guan. Colonography by CT, MRI and PET CT combined with conventional colonoscopy in colorectal cancer screening and staging. World J Gastroenterology. 2008;14(6):858-63.
38. Kiner S, Anatoch G, Bokisch A, Veit-Haibach P. Whole – body PET/CT colonography: a possible new concept - for coloreclal cancer staging. Abdom Imaging. 2007;32:606-12.
39. Bipat S, van Leeuwen MS, Comans EF, Pijl ME, Bossuyt PM, Zwinderman AH, et al. Colorectal liver metastases: CT, MR imaging, and PET for diagnosis – meta analisys. Radiology. 2005;237:123-31.
40. Kinkel K, Lu Y, Both M, Waren RS, Thoeni RF. Detection of hepatic metastases from cancers of the gastrointestinal tract by using noninvasive imaging methods (US, CT, MR imaging ,PET): A meta analysis. Radiology. 2005;224:748-56.
41. Schmidt GP, Bauer-Melnyk A, Hang A, Utzschneider S, Beeker CR, Tiling R, et al. Whole – body MRI at 1.5 and 3 T compared with FDG-PET-CT for detection of tumor recurrence in patients with colorectal cancer. Eur Radiol. 2009;19:1366-78.
42. Manfredi S, Bouvier AM, Lepage C, Hatem C, Dancourt V, Faivre J. Incidence and patterns of recurrence after resection for cure of colon cancer in a well defined population. Br J Surg. 2006;93:1115-22.
43. Flamen P, Hoekstra OS, Homans F, Van Cutsem E, Maes A, Stroobants S, et al. Unexplained rising carcinoembryonic (CEA) in the postoperative surveillance of colorectal cancer. The utility of positron emission tomography (PET). Eur J Cancer. 2001;31:862-9.
44. Marcus J, Morrissey B, deGara C, Tarulli G. MRI of recurrent rectosigmoid carcinomas . Abdom Imaging. 1997;22:338-42.
45. Schaefer O, Langer M. Detection of recurrent rectal cancer with CT, MRI and PET/CT. Eur Radiol. 2007;17:2044-54.
46. Huebner RH, Park KC, Shepherd JE, Schwimmer J, Czernin J, Phelps ME, et al. A meta-analysis of the literature for whole-body FDGPET detection of colorectal cancer. J Nucl Med. 2000;41:1177-89.
47. Whiteford MH, Whiteford HM, Yee LF, Ogunbioyi OA, Dehdashti F, Siegel BA, et al. Usefulness of FDGPET scan in the assessment of suspected metastatic or recurrent adenocarcinoma of the colon and rectum. Dis Colon Rectum. 2000;43:759-67.

Case report

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Multiple pyogenic liver abscesses formed after appendectomy: The role of percutaneous drainage in a critically ill patient

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Multiple pyogenic liver abscesses formed after appendectomy and their percutaneous treatment with multiple catheters have been rarely described. We report a case of multiple pyogenic liver abscesses in a critically ill patient, formed after appendectomy and treated successfully by antibiotics and drainage with six catheters that were introduced simultaneously under ultrasound control. Even though this was a case of liver abscess secondary to appendicitis, today very rare in Western countries, but still a serious complication in developing countries, it was successfully resolved by percutaneous drainage, along with antibiotic therapy. **Conclusion**. We emphasize the advantages of percutaneous treatment compared with surgery regarding the avoidance of perioperative complications and the risks of general anesthesia.

Key words: Interventional ultrasonography, Multiple simultaneous drainages, Antibiotic therapy.

Introduction

Pyogenic liver abscesses are usually caused by infection orginating in the biliary or intestinal tracts. This is a potentially life-threatening disease so that appropriate diagnosis and treatment are very important. The overall mortality is high in patients with multiple liver abscesses (1-3). Abdominal ultrasonografy and computerized tomography are used most frequently in the diagnosis of liver abscess. The diagnosis may be confirmed by image-guided percutaneous aspiration and drainage and then appropriate therapy can be planned according to culture and antibiogram (4, 5).

We report a case of multiple pyogenic liver abscesses formed after appendectomy and treated successfully by antibiotics and drainage with six catheters that were introduced simultaneously under ultrasound control.

Case report

A 52-year-old man was admitted to our hospital with high fever, sweating and right upper abdominal pain that had lasted for seven days. On physical examination, he had marked tenderness in the right upper quadrant of the abdomen, his initial body temperature was 39.2 °C, pulse and respiratory rates were 110 and 31, respectively. His blood pressure was 90/70 mmHg. Two months before admission he had undergone an emergency appendectomy for acute perforated appendicitis. He was in good health after that until 7 days before admission to our hospital. Laboratory tests were not performed during that period.

Laboratory tests performed on admission revealed erythrocyte sedimentation rate 94 mm/hour, C-reactive protein 205 mg/l (normal <3.3), fibrinogen 9.4 g/l (normal 1.8-3.5), WBC 18 600/mm^3, neutrophils 15 240/mm^3, hematocrit 29.5%, platelets 273 000/mm3, prothrombin time 33%, aspartate aminotransferase 33 U/l, alanine aminotransferase 44 U/l, alkaline phosphatase 359 U/l, gamma-glutamyl transpeptidase 541 U/l, total bilirubin 71 μmol/l, conjugated bilirubin 46 μmol/l, total protein 61 g/l, albumin 21 g/l, globuline 40 g/l, BUN 8.8 mmol/l, creatinine 100 μmol/l, glucose 19.5 mmol/l and creatine kinase 206 U/l.

Abdominal ultrasonography revealed that the hepatic parenchyma appeared diffusely heterogeneous and enlarged, with several heterogen-hypoechoic cystic lesions, that looked more like metastases, predominantly in the right lobe. Abdominal CT scan performed few days later confirmed the presence of multiple cystic formations with dense content varying in size, predominantly in the right lobe of the liver (Figure 1, Panel A and B).

Blood culture was performed when the patient had a high fever. The Serratia marcescens strain was found on two occasions.

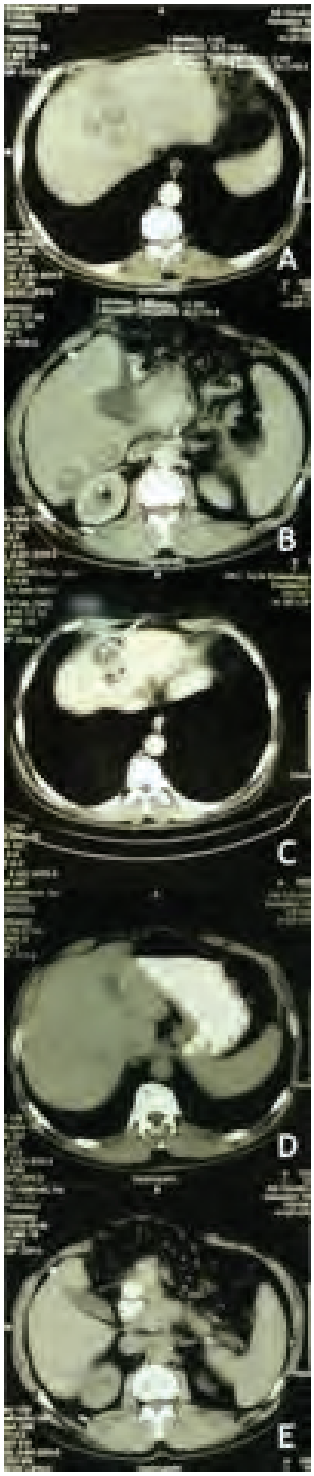

Figure 1 CT scans of multiple liver abscesses before treatment (Panels A and B), catheter inserted into abscess cavity (Panel C) and CT scans of the same parts of the liver three months after treatment (Panel D and E).

Cefazolin 1g tid and gentamicin 1 mg/kg tid therapy was instituted before planned abscess drainage. Ultrasound guided percutaneous drainage was performed 48 h after hospital admission. We used 5 F pigtail polyethylene catheters (PBN Medicals, Denmark) which were inserted under US guidance. The six catheters were simultaneously introduced (Figure 2) into abscesses larger than 30 mm in the longest diameter and 80 ml of frank pus was obtained.

CT scan of one of abscess collection with catheter into cavity was performed, as shown in Figure 1 (Panel C). Escherichia coli and Serratia marcescens were found in cultures of the pus and they were sensitive to the formerly prescribed antibiotics. A total of 200 ml of pus was drained through all six catheters during the following 10 days. The patient's clinical condition improved rapidly and fever subsided 3 days from the beginning of the treatment. Drainage catheters were subsequently removed between the 3rd and 10th days after introduction and the patient was discharged on the 15th day of hospitalization. Antibiotic therapy was changed from parenteral to oral on the 10th day and was stopped on the 28th day from the start of therapy. Ultrasound examination was done every day during catheter drainage, on discharge from the hospital, and 1, 3 and 6 months after the discharge. The patient continued to do well during the follow-up period and a CT scan after 3 months (Figure 1, Panel D and E) and ultrasonography after 6 months revealed that the abscess cavities had disappeared completely.

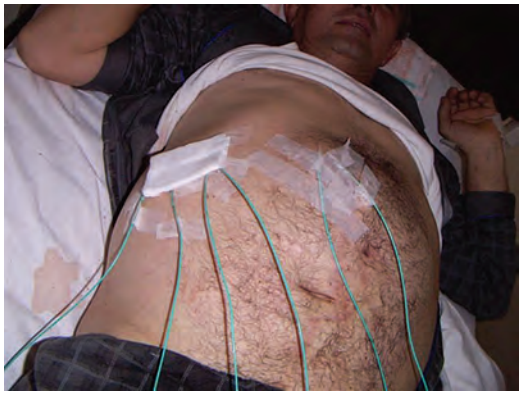

Figure 2 The patient with six catheters introduced simultaneously under ultrasound control.

Discussion

Pyogenic liver abscess is a rare complication after appendectomy. Multiple pyogenic liver abscesses are not frequently reported in the literature, but the overall mortality is high, if left with no treatment or not treated early (2, 6). Pyogenic liver abscess usually develops secondary to biliary infections like cholecystitis, cholangitis, infection of deviscerated liver hydatid cyst (7) or infection of organs that are drained by the portal vein (diverticulitis, inflammatory bowel disease) (8). While studies of patients with infection of organs that are drained by portal vein suggest an increased incidence of portal bacteraemia, the development of liver abscesses in these patients is relatively rare (8). In the majority of cases, more than one organism is isolated from the abscesses (2). In our case two organisms were identified, suggesting that pyogenic liver abscesses could have been possibly caused by infection originating in the intestinal tract. However, we cannot rule out the formation of cryptogenic abscesses, since our patient underwent full recovery after the appendectomy and was free from any symptoms until 7 days before re-admission to our hospital.

In the past, antibiotic therapy and surgical drainage were considered the treatments of choice for liver abscess. Current therapeutic strategies established percutaneous drainage of liver abscesses instead of surgical treatment, with good results. The advantages of percutaneous treatment compared with surgery include external drainage without significant risks of intra-abdominal spillage and avoidance of perioperative complica-

tions, the risks of general anaesthesia, less time and cost, better compliance and easier nursing care. This treatment is indicated especially when patients are in a critical condition postoperatively or when the risks of general anaesthesia or surgical drainage are substantial (9), unless the anatomic location is unsuitable and/or non-liquefied abscesses are present. Timely microbiological diagnosis, the combined use of new and old antibiotics and percutaneous treatment appear to be valuable in managing these serious conditions (10).

Conclusion

Liver abscess secondary to appendicitis, today very rare in Western countries, is still a possible and frightening complication in developing countries. In this case, simultaneous percutaneous drainage with 6 catheters, along with antibiotic therapy, were a safe and effective treatment for abscesses larger than 30 mm in the longest diameter, without complications related to the procedure. Abscess collections smaller than 30 mm in the longest diameter were successfully treated by antibiotics alone.

Authors' contributions: Conception, design and drafting the article: EZ; Acquisition, analysis and interpretation of data: AS.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organization.

References

1. Lee KT, Wong SR, Sheen PC. Pyogenic liver abscess: an audit of 10 years' experience and analysis of risk factors. Dig Surg. 2001;18(6):459-66.
2. Bahloul M, Chaari A, Bouaziz-Khlaf N, Kallel H, Herguefi L, Chelly H, et al. Multiple pyogenic liver abscess. World J Gastroenterol. 2006;12(18):2962-3.
3. Alvarez Perez JA, Gonzalez JJ, Baldonedo RF, Sanz L, Carreno G, Junco A, et al. Clinical course, treatment, and multivariate analysis of risk factors for pyogenic liver abscess. Am J Surg. 2001;181(2):177-86.
4. Seeto RK, Rockey DC. Pyogenic liver abscess. Changes in etiology, management, and outcome. Medicine (Baltimore). 1996;75(2):99-113.
5. Bergert H, Kersting S, Pyrc J, Saeger HD, Bunk A. Therapeutic options in the treatment of pyogenic liver abscess. Ultrashall Med. 2004;25(5):356-62.
6. Alvarez JA, Gonzalez JJ, Baldonedo RF, Sanz L, Carreno G, Jorge JI. Single and multiple pyogenic liver abscesses: etiology, clinical course, and outcome. Dig Surg. 2001;18(4):283-8.
7. Zerem E, Jusufović R. Percutaneous treatment of univesicular versus multivesicular hepatic hydatid cysts. Surg Endosc. 2006;20(10):1543-7.
8. Inoue T, Hirata I, Egashira Y, Ishida K, Kawakami K, Morita E, et al. Refractory ulcerative colitis accompanied with cytomegalovirus colitis and multiple liver abscesses: A case report. World J Gastroenterol. 2005;11(33):5241-4
9. Zerem E, Bergsland J. Ultrasound guided percutaneous treatment of splenic abscesses: The significance in treatment of critically ill patients. World J Gastroenterol. 2006;12(45):7341-5.
10. Di Carlo P, Pantuso G, Cusimano A, D'Arpa F, Giammanco A, Gulotta G, et al. Two cases of monomicrobial intraabdominal abscesses due to KPC-3 Klebsiella pneumoniae ST258 clone. BMC Gastroenterol. 2011;11:103.

Case report

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Ichthyosis vulgaris and pycnodysostosis: An unusual occurrence

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Pycnodysostosis is a rare autosomal recessive disorder whose gene responsible for this phenotype (*CTSK*), mapped to human chromosome 1q21, code for the enzyme cathepsin K, a lysosomal cysteine protease; with an estimated incidence of 1.7 per 1 million births. This clinical entity includes micromelic dwarfism, increased radiological bone density, dysplasia of the skull, acro-osteolysis, straightening of the mandibular angle and in some cases, dysplasia of the acromial end of the clavicle. Oral and maxillo-facial manifestations of this disease are very clear. Herein we reported a case of pycnodysostosis, showing short stature with widening of the sutures, unfused anterior and posterior fontanelles, crowding of teeth with dental caries and typical radiological features associated with ichthyosis vulgaris and palmoplantar keratoderma.

Key words: Pycnodysostosis, Ichthyosis vulgaris, Palmoplantar keratoderma.

Introduction

Pycnodysostosis is an autosomal-recessive disorder of osteoclasts due to mutation in the gene that codes enzyme Cathepsin K causing osteosclerosis (1, 2). The first case was described in 1923 by Montanari, but Maroteaux and Lamy defined the characteristic features in 1962. It has also been named Toulouse-Lautrec syndrome, after the French painter Henri de Toulouse-Lautrec, who suffered from the disease (3, 4). General features include short stature (<150 cm), generalized diffuse osteosclerosis with tendency for fracture, hypoplastic clavicles and acro-osteolysis with sclerosis of terminal phalanges — a feature that is considered essentially pathognomic. Cranial and maxillo-facial features include fronto-parietal bossing, thick calvaria, open fontanelle and sutures, hypoplastic paranasal sinuses, wormian bones in the lamboidal region, relative proptosis, beaked nose and an obtuse

mandibular gonial angle, often with relative prognathism (2).

Ichthyosis is the most frequent inherited disorder of keratinization. Filaggrin, a filament aggregating protein, that plays a role in the formation of the stratum corneum, has been shown to be altered in ichthyosis vulgaris. Mutations in the gene encoding this protein (*FLG*) have been found in patients presenting this skin abnormality (5). Different patterns of inheritance are described in ichthyosis, such as autosomal dominant, autosomal recessive and X-linked. Mutations in *FLG* gene have been associated both with autosomal recessive and dominant modes of inheritance (5).

We report on a patient presenting typical features of pycnodysostosis and ichthyosis vulgaris. This association has never been described before.

Case report

An 8-year-old female child, born by second degree consanguineous marriage presented to us with short stature, dysmorphic facies and ichthyosis since birth (Figure 1).

She evolved with delayed milestones. Her other siblings and parents are normal. On admission child's weight was 14.2 kg (<3rd percentile), height 105 cm(<3rd percentile), upper segment 58 cm, lower segment 47 cm and head circumference 50 cm (normal). The child has a history of recurrent upper airway tract infections and episodes of upper airway obstructions during sleep. She had mid facial hypoplasia with proptotic eyes, fronto-parietal bossing with separated sagittal, coronal and lamboid sutures with widely open anterior and posterior fontanelles. Examination of the mouth revealed

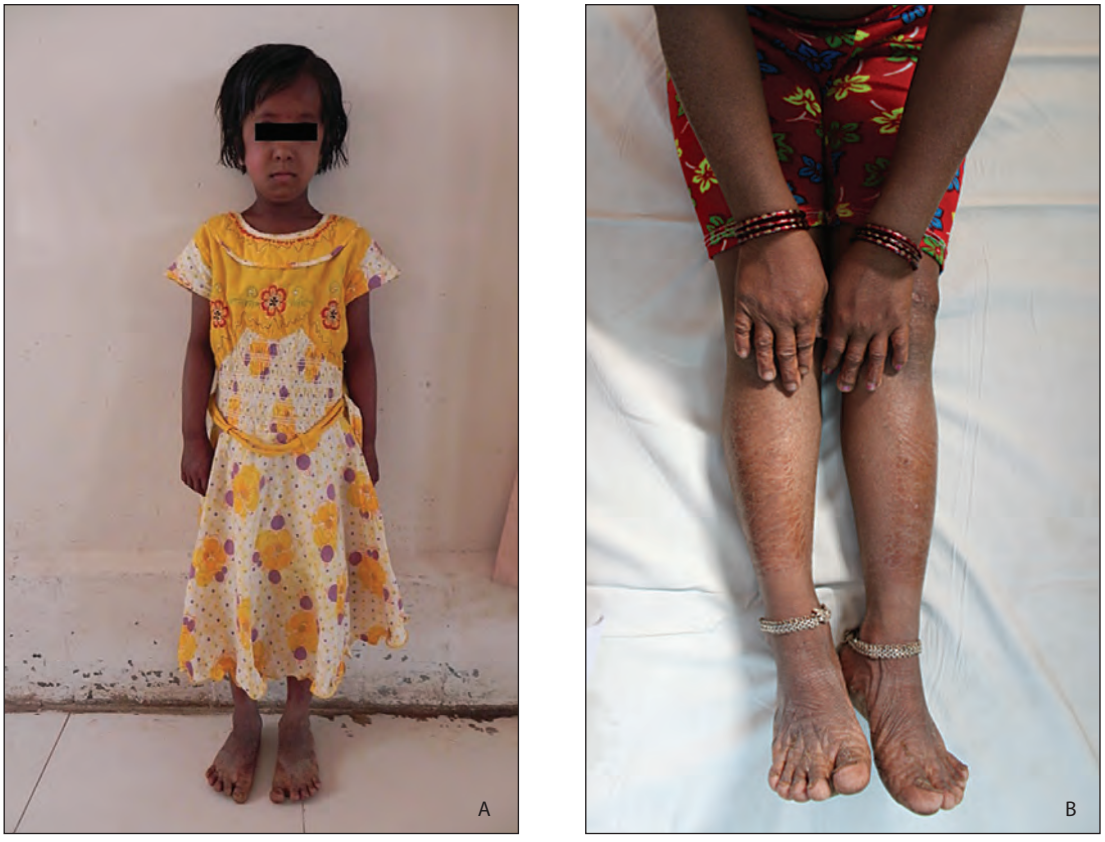

Figure 1 Clinical features of our patient: a) Dysmorphic facial features. b) Ichthyosis.

a narrow high arched, grooved palate with crowding of the lower anterior teeth. Her digits were short, spoon shaped, stubby with no joint laxity, widening of the joints and dystrophic nails. Cutaneous examination revealed ichthyosis with palmoplantar keratoderma. Laboratory investigations including complete blood count, serum calcium, serum inorganic phosphate and alkaline phosphatase were within normal limits. The skin biopsy showed histopathological findings compatible with the diagnosis of ichthyosis vulgaris (Figure 2).

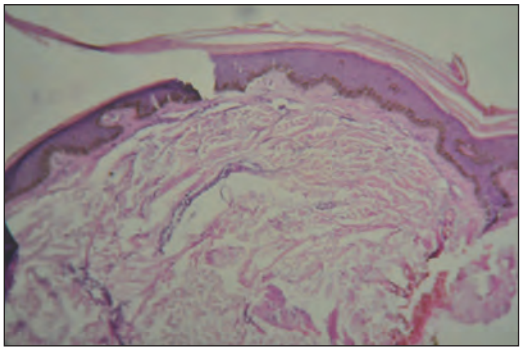

Figure 2 Superficial perivascular lymphocytic infiltrate with slight epidermal hyperplasia, granular layer in thinned with stratum corneum showing mild ortho hyperkeratosis.

The radiographical findings disclosed generalized increase in bone density. Lateral view X ray of the skull (Figure 3) showed open anterior fontanelle with non pneumatized frontal and maxillary sinus and large calvarium while AP view of the skull (Figure 4), failure of sutural fusion with dense sclerosis, obtuse angle of mandible and hypoplastic maxilla. X ray of bilateral hands (Figure 5) showed loss of ungula tufts, tapering of distal phalanges with hypoplastic right thumb and destructive changes of distal phalanges.

Screening for ear defects (otosclerosis), fundus examination and intelligence quotient were normal. She was diagnosed as pycnodysostosis with ichthyosis vulgaris and palmoplantar keratoderma, which has not been reported previously. Genetical confirmation is not available, although the clinical data are strongly suggestive.

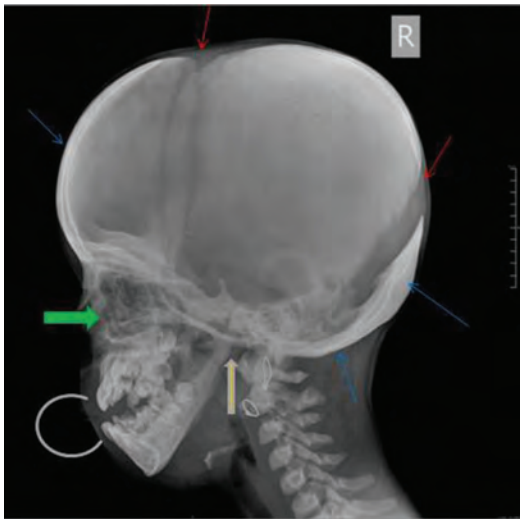

Figure 3 Failure of sutural fusion (red arrow); dense sclerosis of the frontal and occipital bone and base of the skull (blue arrow); obtuse angle of the mandible (thick yellow arrow); hypoplastic maxilla (thick green arrow); defective teeth (grey ring).

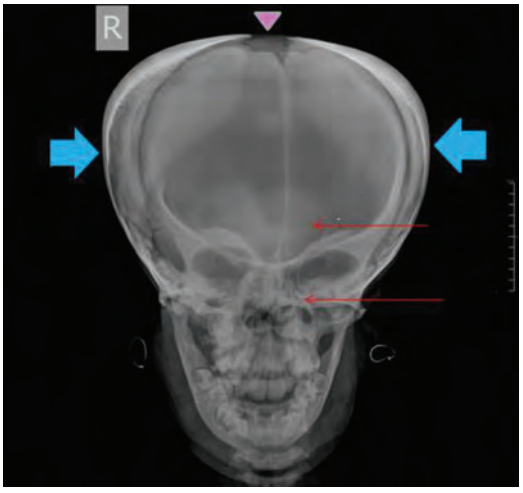

Figure 4 Open anterior fontanelle (pink triangle); non -pneumatized: frontal and maxillary sinus (red arrows); large calvarium (blue thick arrows).

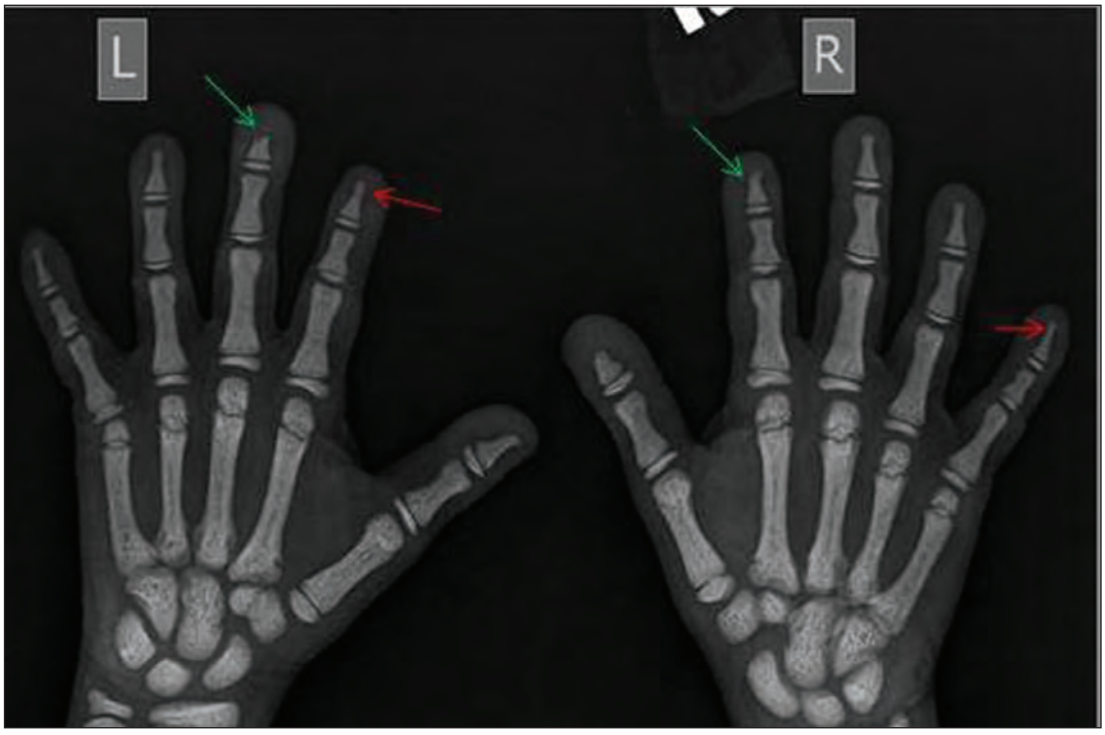

Figure 5 Loss of ungual tuft; tapering of distal phalanges (red arrows); destructive changes in the distal phalanges (green arrow).

Discussion

Pycnodysostosis is a disorder of lysosomal cysteine protease enzyme cathepsin K, which maps to chromosome 1q21. Cathepsin K is highly expressed in osteoclasts and responsible for bone remodelling by degrading collagen type 1 that constitutes 95% of organic bone. Nonsense, missense, and stop codon mutations in the gene have been identified, which results in reduced expression of the enzyme. The bones become abnormally dense and brittle as a result of insufficient reabsorption process (6, 7, 8). Pycnodysostosis is included in the group of disease that is caused by low bone remodeling (9).

Interestingly, this patient with typical features of pycnodysostoisis also presents ichthyosis vulgaris. This skin abnormality is considered a frequent single-gene disorder in humans (10) and, therefore, the co-occurrence of ichthyosis and pycnodysostosis in this case is a real possibility. Some genetic disorders have been associated with ichthyosis vulgaris, such as Refsum disease and multiple sulfatase deficiency, but not pycnodysostosis (11). On the other hand, the gene responsible for the autosomal dominant form of ichthyosis vulgaris was assigned to the same chromosomal region (1q21) of the one responsible for pycnodysostosis. Loss-of-function mutations in the filaggrin (*FLG*) gene have been described in patients presenting ichthyosis. Although the moderate and severe cases showed homozygous or compound heterozygous mutations in this gene, milder conditions were associated with a mutation in a single allele (5). Based on the facts that the genes for pycnodysostosis (*CTSK*) and for ichthyosis vulgaris (*FLG*) are in close proximity and that both disorders are due to loss-of-function mutations, it is tempting to speculate that a microdeletion in the region 1q21 could cause the loss

of both genes in one allele. The association of this microdeletion with a further mutation in the other allele of the *CTSK* gene could be responsible for the association of pycnodysostosis and ichthyosis vulgaris in this patient. Unfortunately, this possibility could not be proved due to unavailability of the molecular tests in our Country.

Various bone diseases should be considered in the differential diagnosis of pycnodysostosis, particularly the ones that evolve with increased bone density and acroosteolysis as well as cleidocranial dysostosis, a disorder which also shows persistence of open fontanelles and cranial sutures at an advanced age. This disorder has an autosomal dominant mode of inheritance and is characterized by absent or hypoplastic clavicles, permitting abnormal facility apposing the shoulders and supernumery teeth with impacted permanent teeth (11). There is no specific treatment for pycnodysostosis for this disorder. Once bone fractures are the primary threat to those individuals affected by pycnodysostosis, a supportive care is important to prevent or minimize fractures that could occur.

Conclusion

This case is reported for its rarity and unusual cutaneous association of ichthyosis vulgaris and palmoplantar keratoderma, as no case has been reported this far. Although we cannot rule out for certainty that icthyosis and pycnodysostosis occured by chance in this patient, there is a possibility that a microdeletion in one allele comprising the genes of these two disorders is responsible for this unusual association.

Authors' contributions: Conception and design: VYK, MA, SN; Acquisition, analysis and interpretation of data: MA,VYK, KS; Drafting the article: MA,VYK, KS; Revising it critically for important intellectual content: VYK, MA.

Conflict of interest: The authors declare that they have no conflict of interest. This article was not sponsored by any external organization.

References

1. Mujawar Q, Naganoor R, Patil H, Thobbi AN, Ukkali S, Malagi N. Pycnodysostosis with unusual findings: a case report. Cases J. 2009; 2:6544.
2. Fleming KW, Barest G, Sakai O. Dental and facial bone abnormalities in pyknodysostosis: CT findings. AJNR Am J Neuroradiol. 2007;28(1):132-4.
3. Clark AR. Two cases of pycnodysostosis (the malady of Toulouse-Lautrec). Postgrad Med J. 1969;45(528):684-7.
4. Maroteaux P, Lamy M. Pyknodysostosis. Presse Med. 1962;70:999-1002.
5. Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet. 2006;38(3):337-42.
6. Elmore SM. Pycnodysostosis: a review. J Bone Joint Surg Am. 1967;49:153-62.
7. Motyckova G, Fisher DE. Pycnodysostosis: role and regulation of cathepsin K in osteoclast function and human disease. Curr Mol Med. 2002;2(5):407-21.
8. Gelb BD, Shi GP, Chapman HA, Desnick RJ. Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. Science. 1996;273(5279):1236-8.
9. Chavassieux P, Seeman E, Delmas PD. Insights into material and structural basis of bone fragility from diseases associated with fractures: how determinants of the biomechanical properties of bone are compromised by disease. Endocr Rev. 2007;28(2):151-64.
10. Wells RS, Kerr CB. Clinical features of autosomal dominant and sex-linked ichthyosis in an English population. Br Med J. 1966;1(5493):947-50.
11. Oji V, Traupe H. Ichthyoses: differential diagnosis and molecular genetics. Eur J Dermatol. 2006;16(4):349-59.

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The developmental venous anomaly associated with the cavernous malformation

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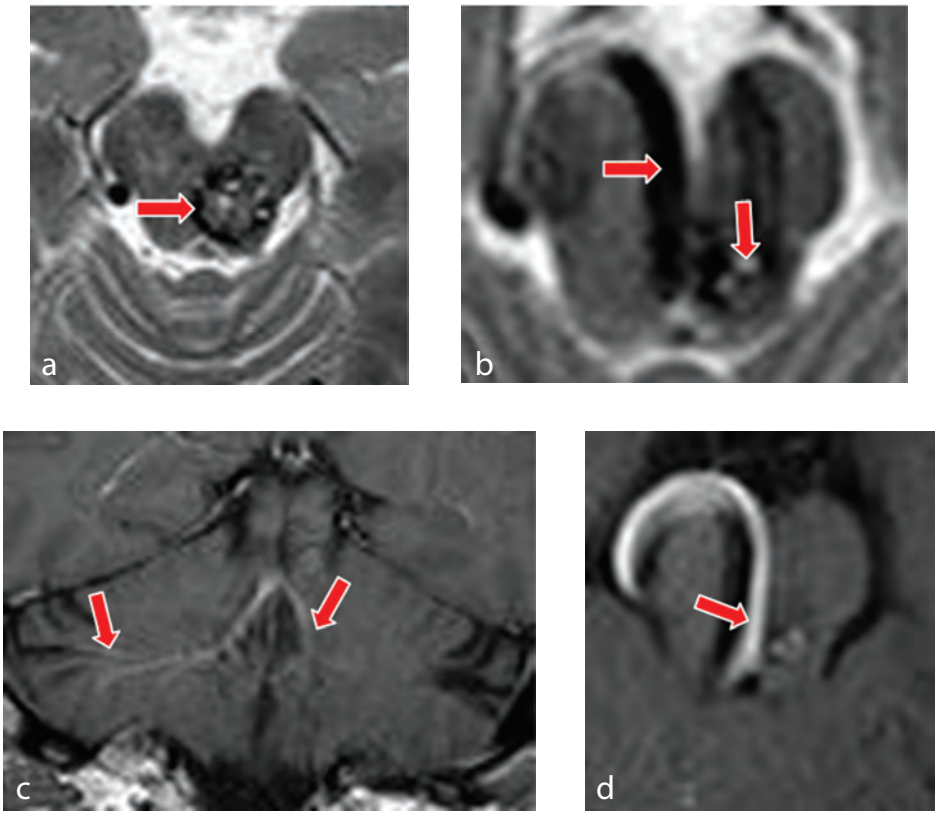
A magnetic resonance imaging (MRI) study was ordered in a 32-year-old man with a 6 month history of double vision and confirmed left abducens nerve palsy on neurological examination. Non-enhanced T1 and T2-weighted images revealed the presence of a cavernous malformation (CM) in the left lateral aspect of the mesencephalon (mostly in the left inferior colliculus), associated with a large blood vessel in the close vicinity of the CM, which corresponded to the collector vein of the developmental venous anomaly (DVA), also known as venous angioma (Figure A, B). The contrast-enhanced T1-weighted images showed small dilated medullary veins of the DVA in both cerebellar hemispheres and a large collector vein draining toward the deep venous system, also visualised on non-enhanced images (Figure C, D). The neurosurgeon suggested neuro-radiological follow-up. The prevalence of DVAs associated with CM can be underestimated even when high field MR is used. Based on MR imaging and intraoperative findings, Wurm et al. identified 25.9% patients with CM who had associated DVA (1). Although CMs have a relatively benign nature, surgery may be recommended when they become symptomatic (most frequently associated with seizures, less frequently with neurological deficit), or if bleeding occurs. In the present case we did not found evidence of recent intracerebellar bleeding. When surgical intervention is ordered and if the DVA is in a close vicinity to the CM, the DVA should be spared in order to prevent the development of venous infarction due to resection of the main collector vein (2). Contrast-enhanced MRI has the most important role in detection of a DVA associated with a CM, since it may be invisible on non-enhanced MRI.



Authors' contributions: Conception and design: SM; Acquisition, analysis and interpretation of data: SM and BIJ; Drafting the article SM; Revising it critically for important intellectual content: SM.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

References

1. Wurm G, Schnizer M, Nussbaumer K, Wies W, Holl K. Recurrent cryptic vascular malformation associated with a developmental venous anomaly. Br J Neurosurg. 2003;17:188-95.
2. Perrini P, Lanzino G. The association of venous developmental anomalies and cavernous malformations: pathophysiological, diagnostic, and surgical considerations. Neurosurg Focus. 2006;21(1):e5.

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Application of conformal radiotherapy in treatment of non-Hodgkin head and neck lymphoma

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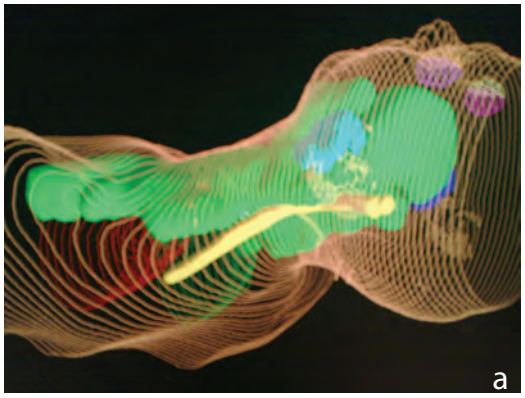
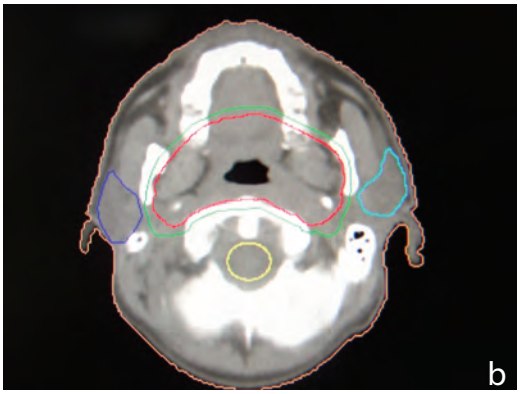
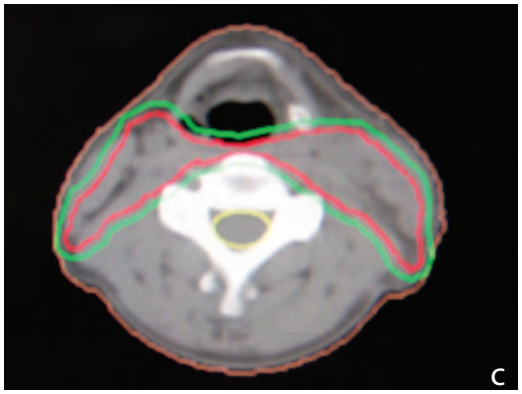
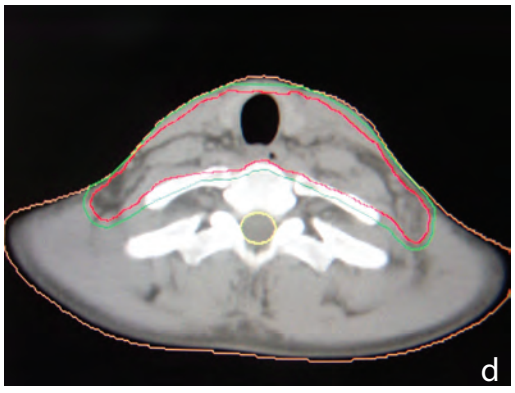
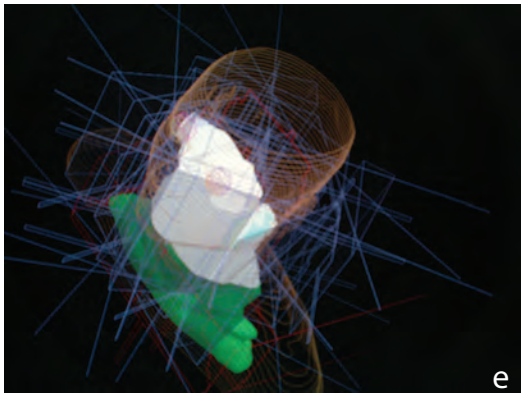
A male patient, aged 33, was treated at the Clinic for Oncology, Hematology and Radiotherapy at the University Clinical Center, Tuzla, with the diagnosis of diffuse large B-cell lymphoma. Conformal radiotherapy was conducted on the region of the epipharynx, oropharynx and lymph nodes of the neck on both sides (1). The target volume included the following: the clinical target volume (CTV) of the tumor, the CTV of lymph nodes and planning target volume (PTV) (Panel A). The CTV of the tumor included: nasopharynx, retropharyngeal lymph nodes, clivus, skull base, posterior part of the sphenoid sinus, pterygoid phosa, parapharyngeal region, the posterior two thirds of the cavum nasi and the posterior two thirds of the maxillary sinus, as well as the soft palate, tongue base and posterior wall of the oropharynx. The CTV of lymph nodes included lymph nodes of the I, II, III, IV, V, and VI levels of the neck. PTV combined both CTVs with a safety margin of 3 mm. The contours were then formed of the organs at risk: the parotid gland, spinal cord, lungs, lenses and retina (Panel B, C, D). Prescription of a therapy dose of TD 40 Gy in 20 fractions was determined at the ICRU referential point – the center of the CTV. The distribution of the 9 conformal fields provided information on the complexity and skill needed in planning conformal radiotherapy for the head and neck region (Panel E). It can be seen how a 95% dose covered the target volume and saved the parotid glands (Panel F and G). Application of 3D planning and conformal radiotherapy by the "involved field"

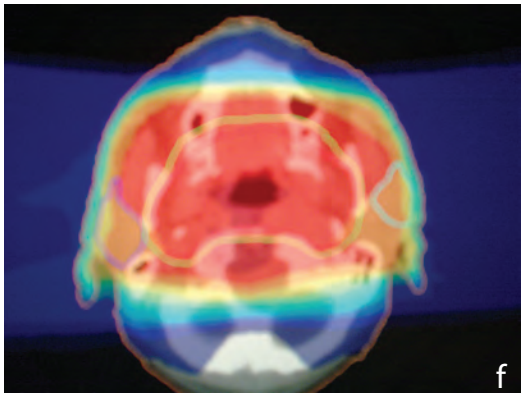


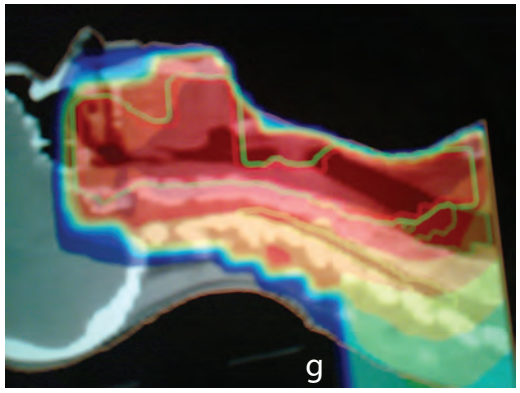












technique enables the precise application of the radiation dose required on the target volume, whilst saving the surrounding healthy tissue (2).

Authors' contributions: Conception and design: GM, SF; Acquisition, analysis and interpretation of data: GM and HO; Drafting the article: GM; Revising it critically for important intellectual content: NO.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

References

1. Gregoire V, Coche G, Cosnard M, Hamoir M, Reychler H. Selection and Delineation of Lymphnode Target Volumes in Head and Neck Conformal and Intensity-Modulated Radiation Therapy. In: Gregoire V, Scalliet P, Ang KK, editors. Clinical Target Volumes in Conformal and Intensity-Modulated Radiation Therapy. Berlin: Springer; 2004. p. 69-90.
2. Kelsey CR, Beaven AW, Diehl LF, Prosnitz LR. Radiation therapy in the menagment of diffuse large B-cell lymphoma: still relevant? Oncology (Willston Park). 2010;24(Suppl 13):1204-12.

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The inframammary dome – a modification of the keyhole pattern for reduction mammaplasty/mastopexy

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Dear Editor,

A modification of the keyhole pattern for reduction mammaplasty and mastopexy is presented. An inframammary dome with a 3-cm diameter and 6 cm base is inserted on the inframammary fold. The authors believe that this modification reduces the incidence of vertical incision separation and minimizes later "bottoming out" of the surgically reduced/lifted breast.

Breast reduction and mastopexy are among the few procedures in plastic surgery that test our ability to achieve superb and long lasting aesthetic results The choice of the appropriate breast reduction technique and attention to fine technical details in planning and execution are very important in determining the outcome of surgery and ultimately, patient satisfaction (1, 2). The inferior pedicle technique and its variations are in wide use thanks to the reliability that the technique offers (3). We present a minor modification of the dependable keyhole pattern with additional technical pearls, proven to be effective in our hands. The senior author has used these modifications in his practice over the last 5 years in 333 patients (161 mastopexies and 172 reduction mammaplasties) with high patient and surgeon satisfaction.

Preoperative markings are made with the patient in the standing position (3). The midclavicular line is marked through the nipple. Standard keyhole markings are made and the inframammary fold is then marked. On the miclavicular line an inframammary dome is designed with a 3-cm radius and 6 cm base in the inframammary fold (Figure 1).

Note deepithelization of the pedicle, except for the inframammary dome, follows the preoperative markings (4). The new nipple position skin resection is left to be

performed at the end of the procedure, similar to the technique suggested by Hester (5). A figure-of-eight 3-0 Vicryl suture is placed through the nipple in a 3^{hr} to 9^{hr} vector at the beginning of the procedure to facilitate the final nipple position, nipple areola delivery and to prevent rotational distortion (Figure 2).

In the final step of the central and inferior skin flap resection, adjustment is made to fit around the inframammary dome. In this way the tension of the skin flap closure is distributed along a 9.42-cm arch instead of a one-point maximal tension juncture, which is standard with the standard inverted T- closure (Figure 3).

The postoperative course in all our patients has been uneventful with no skin flap necrosis and no incisional separation occurring on the vertical and horizontal scar connection. Reduction mammoplasty using the inferior glandular pedicle to perfuse the nipple-areola complex has maintained its popularity and proven its reliability over two decades of widespread use (3, 6, 7). The majority of published series report a 5-10% incidence of incisional separation that causes delayed wound healing (5, 8). Different techniques have been recommended for prevention of insicional skin separation, including a double dermal keyhole technique (9) and the sliding nipple technique (10). Both groups report improved cosmetic appearance and decreased early postoperative wound dehiscence. We believe that with the dome modification of the inframammary incision the incidence of wound dehiscence is reduced.

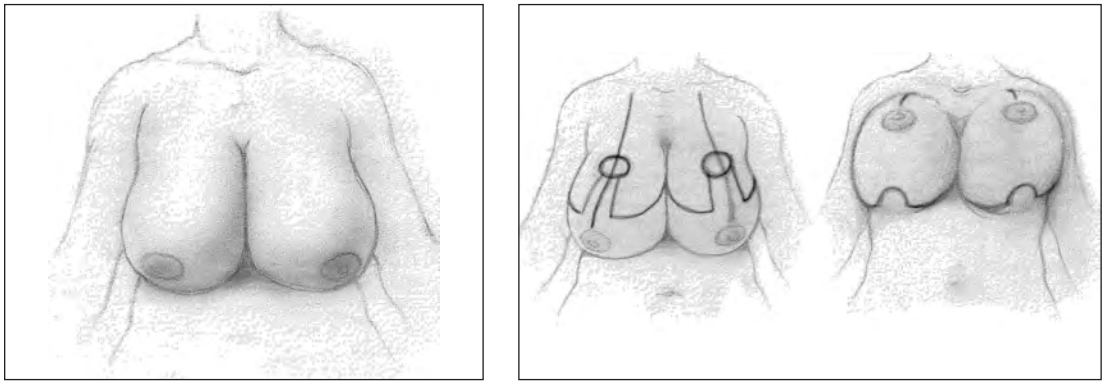

Figure 1 Preoperative markings of the inframammary dome.

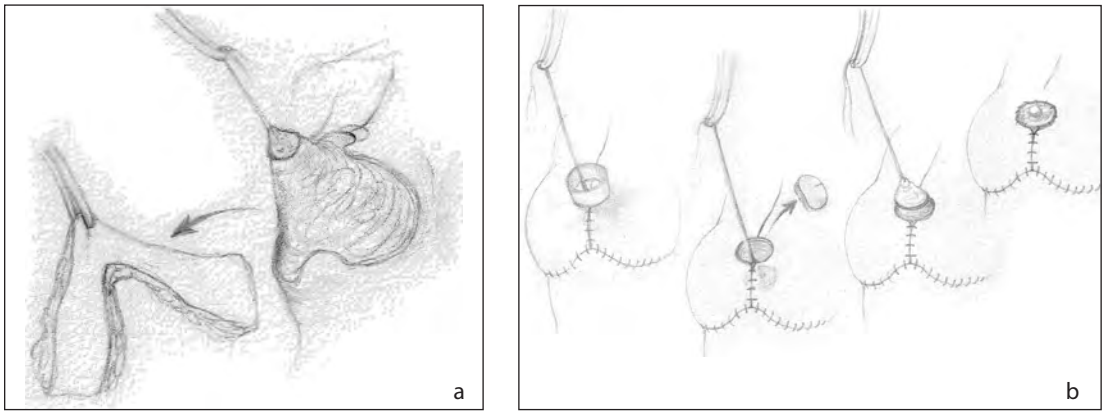

Figure 2 a) Resection; b) Nipple/areola is delivered to the final position.

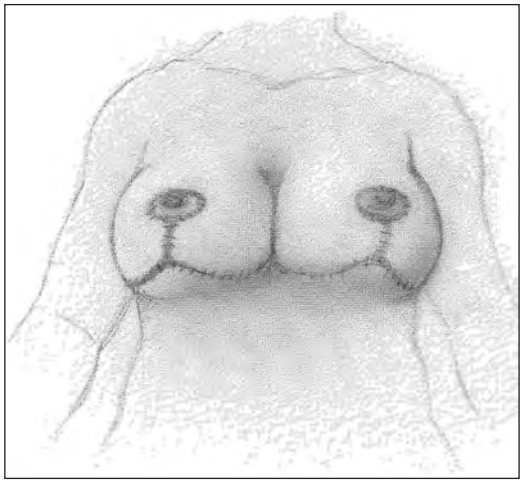

Figure 3 Final inframammary closure.

Authors' contributions: Conception and design: SB and WDL; Drafting the article: SB; Revising it critically for important intellectual content: SB and WDL.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organization.

References

1. McKissock PK. Reduction mammoplasty with a vertical dermal flap. Plast Reconstr Surg. 1972;49(3):245-52.
2. Aufricht G. Mammaplasty for pendulous breast. Plast Reconstr Surg. 1949;4:13.
3. Georgiade GS, Riefkohl RE, Georgiage NG. The inferior dermal-pyramidal type breast reduction: long-term evaluation. Ann Plast Surg. 1989;23:203-11.
4. Kroll SS. A comparison of deepithelialization and deskinning in inferior pedicle breast reduction. Plast Reconstr Surg. 1988;81(6):913-6.
5. Hester TR Jr, Bostwick J III, Miller L, Cunningham SJ. Breast reduction utilizing the maximally vascularized central breast pedicle. Plast Reconstr Surg. 1985;76(6):890-900.
6. Hudson DA. Editorial: Some thoughts on choosing a technique in breast reduction. Plast Reconstr Surg. 1998;102(2):554-7.
7. DeBono R, Rao GS. Vasoconstrictor infiltration in breast reduction surgery: is it harmful? Br J Plast Surg. 1997;50(4):260-2.
8. Krysander L, Bröte L, Ostrup LT. Reduction mammoplasty: comparison of results of plastic and general surgeons. Eur J Surg. 1993;159(5):259-62.
9. Harouche EF. The double dermal keyhole pattern for breast reduction. Plast Reconstr Surg. 1995;96(6):1451-3.
10. Holmström H, Lossing C. Reduction mammoplasty with a sliding nipple technique. Scand J Plast Reconstr Surg Hand Surg. 1990;24(3):245-52.

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Group analysis training for Bosnia-Herzegovina mental health professionals in the aftermath of the 1992-1995 war

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Dear Editor,

After the 1992-1995 war in Bosnia and Herzegovina (BH), the whole population was highly psycho-traumatized (1-4). Mental health therapists did not have enough capacity to meet the needs of the population. They had a long-term need to improve their psychotherapy capacities (5, 6). Group Analysis (GA), or Group Analytic Psychotherapy, was developed in England in the 1940s by the psychotherapist and psychoanalyst Dr Sigmund Heinrich Foulkes, during his work with World War Two veterans. GA originated from psychoanalysis, systems theory, developmental and social psychology and sociology. The hard core of GA presents the belief that the individual is fundamentally social in nature.

The Institute of Group Analysis (IGA) in Zagreb is a full member of the European Group Analytic Training Institutions Network – EGATIN. Established in 1988 by fourteen enthusiastic psychiatrists, the IGA immediately became a fully-fledged Training Institute of EGATIN. Their education methods were developed and modified in accordance with the programs of the IGA London, EGATIN, and the EU requirements related to training. Today, this Institute ranks as one of the best training institutes in Europe.

IGA Zagreb expanded its activities in and out of Croatia, so they began training in Bosnia and Herzegovina. A IGA Training Program took place in the Tuzla University Clinical Centre at the Department of Psychiatry in response to the 1992-1995 war, and in order to help train mental health workers in GA to enable them to treat the

psychological trauma symptoms of war survivors.

The IGA management and its representative Professor Ljiljana Moro, together with Professor Osman Sinanović, decided during the war and in the immediate post-war period that the best way to help BH was by enhancing the expertise of its mental health professionals through training in GA. This was a long-term project which aimed to make them self-sufficient and eventually able to provide training within the country and to found their own GA organisation. Apart from providing training, this entailed providing on-going supervision and support on a regular basis.

The highly dedicated, internationally approved GA trainers: Ljiljana Moro, Tanja Frančišković, Rudolf Gregurek, Gorana Tocilj and Vedran Bilić provided complete training for trainees: neuro-psychiatrists, residents, nurses, psychologists, social workers, special educators, pediatricians, and gynecologists from several different institutions, from seven different cities in BH and Croatia. Training started during the war period, with an introduction course, and continued with Diploma courses. To be accredited GA therapists, all trainees are obliged to practice GA therapy with clients under the supervision of GA supervisors. Today there are 7 graduate Group Analysts in BH: Esmina Avdibegović, neuropsychiatrist from Tuzla; Behzad Hadžić, neuropsychiatrist from Ključ; Mevludin Hasanović, neuropsychiatrist from Tuzla; Izet Pajević, neuropsychiatrist from Tuzla; Nermina Kravić, neuropsychiatrist from Tuzla; Azra Arnautović, pediatician from Tuzla; Zihnet Selimbašić neuropsychiatrist from Tuzla. Three of them: Izet Pajević, Esmina Avdibegović and Mevludin Hasanović are in the process of training for educators (7). Today the IGA conducts continual training courses in Bosnia and Herzegovina in three clinical centers: Tuzla, Sarajevo and Mostar.

Conflict of interests: The author declares that he has no conflict of interests. This study was not sponsored by any external organization.

References

1. Agius M, Butler S, Hasanović M (2012) Re: Post Traumatic Stress Disorder in Bosnia Herzegovina. Published On-line 16 May 2012 in BMJ http://www.bmj.com/content/338/bmj.b1273/rr/585071 (Approached 11 Aug 2012).
2. Hasanović M. Psychological consequences of war-traumatized children and adolescents in Bosnia and Herzegovina. Acta Medica Academica. 2011;40(1):45-66.
3. Hasanović M. Srabović S, Rašidović M, Šehović M, Hasanbasić E, Husanović J, et al. Psychosocial assistance project decreased posttraumatic stress disorder and depression amongst primary and secondary schools students in post-war Bosnia and Herzegovina. Acta Medica Academica. 2011;40(2):122-31.
4. Hasanović M, Pajević I. Religious moral beliefs as mental health protective factor of war veterans suffering from PTSD, depressiveness, anxiety, tobacco and alcohol abuse in comorbidity. Psychiatria Danubina. 201022(2):203-10.
5. Hasanović M, Sinanović O, Pajević I, Agius M. The Spiritual Approach to Group Psychotherapy Treatment of Psychotraumatized Persons in Post-War Bosnia and Herzegovina. Religions. 2011;2(3):330-44.
6. Agius M, Glaurdic J. Remembering wars past. Pediatrics Today. 2012;8(1):1.
7. Hasanović M, Pajević I, Avdibegović E, Kravić N, Moro L, Frančišković T, et al. Training Bosnia-Herzegovina Mental Health Workers in Group Analysis in the Aftermath of the 1992-1995 War. European Psychiatry. 2012:27(Suppl. 1). http://www.em-consulte.com/en/article/729933 (Approached 11 Aug 2012) Doi: 10.1016/S0924-9338(12)75310-5

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Combined pergolide-associated valvular heart disease and achilles tendon contractures

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Pergolide, bromocryptine, and cabergoline are ergot-derived dopaminergic (DA) agonists that have high affinity for the 5-HT(2B) serotonin receptor, which are expressed in heart valves and may mediate mitogenesis and subsequent proliferation of fibroblasts (1). Tendon deformities in Parkinson's disease (PD) patients are an uncommon complication (2) and preceded pergolide use (3). However, we describe a PD patient, who developed both progressive cardiac valvulopathy and severe Achilles tendon contractures after pergolide use. We hypothesize that this case supports the fact that PD patients may be patho-physiologically predisposed due to their inherent DA deficiency and that ergot-derived dopaminergic agonist drugs, such as pergolide cause secondary fibrotic stimulation via the 5-HT (2B) mechanism (4). Images of the fibrotic cardiac valvular pathology and Achilles contractures are demonstrated.

A 68-year old female with Parkinson's disease (PD) with right-sided predominant tremor was taking pergolide, 1mg four times a day orally for ten years. She was referred to our hospital for progressive heart failure, due to tricuspid and mitral valvular insufficiency. At that time, she had mild bilateral Achilles contractures and mild "off" dystonia. The Achilles tendons also appeared thicker than normal. Pergolide was discontinued and levodopa-carbidopa started in its place. The patient's heart failure progressed to require tricuspid and mitral valve replacement with bioprosthetic valves. Pergolide-associated mitral valve pathology (5) is shown (Figure 1).

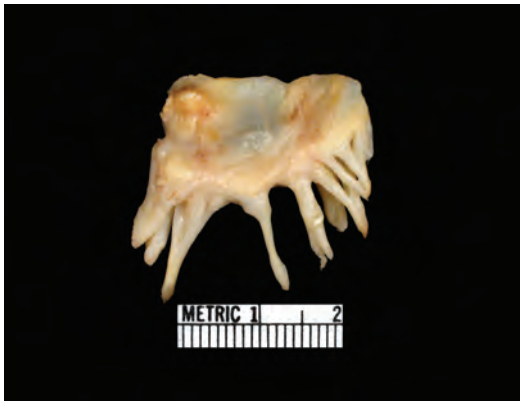

Figure 1 Anterior leaflet excision of the patient's native mitral valve after surgery, showing the diffuse leaflet and chordal thickening, with focal subannular calcification, characteristic for pergolide-associated valvular disease. Underlying valve architecture was intact and undistorted. Microscopically (not shown), the valvular thickening was due to fibroproliferative tissue encasing the tendon and its cords and spreading primarily along the ventricular surface of the leaflet, associated with an abundant extracellular matrix that was rich in collagen and contained lesser amounts of elastin.

However, over the following three years, the patient's Achilles tendon contraction deformities progressed out of proportion to her dystonia (2) (Figure 2), impairing her ambulation, which required a walker. Her Parkinson's tremor, however, was well controlled on levodopa-carbidopa orally.

Physical therapy was attempted for Achilles tendon stretching, and with ankle-foot braces. Botulinum toxin injections into the gastrocnemius were even attempted but did not help release the Achilles tendon contractures. MRI of the brain was normal for age, with only mild small vessel changes (Figure 3). Achilles tendon surgery was ultimately performed to release the tendon contractures and improve her ambulation.

Limb deformities associated with PD were originally reported by Charcot in 1877 (3) and were likely dystonic in nature. However, literature cites that dystonic limb deformities may be accelerated by ergot-derived dopamine agonists, bromocriptine and pergolide (3), but the exact pathologic mecha-

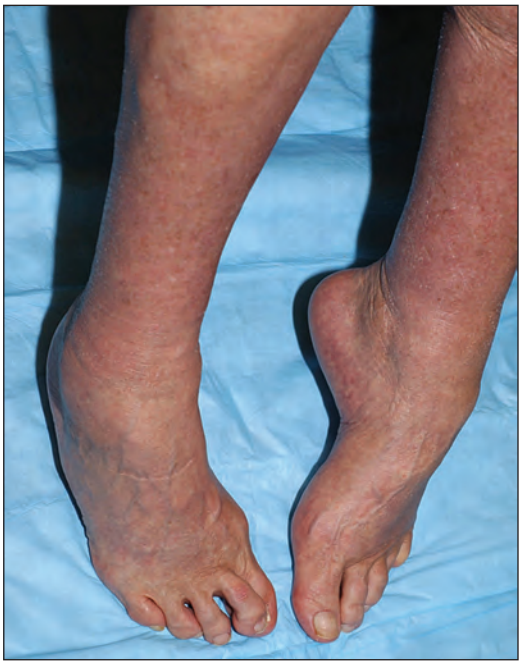

Figure 2 Anterior view just off the midline of the plantar flexor, inversion contractures with shortening of the Achilles tendon and thickening of the overlying skin.

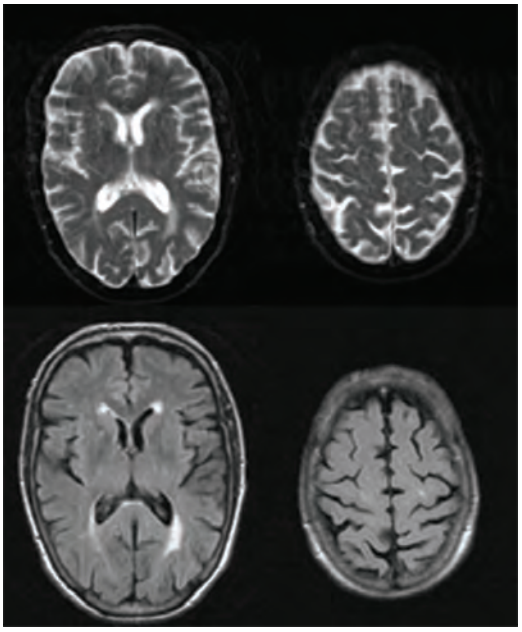

Figure 3 MRI of the brain. The upper images show T2 sequences, and lower images FLAIR sequences, demonstrating periventricular hyperintensities consistent with small vessel disease. Otherwise the MRI brain study, and in particular the basal ganglia, were normal.

nism has remained unclear. To our knowledge, this is the first case report of combined fibrotic disease of the heart (valvular) and exacerbated limb contractures associated with an ergot-related dopamine agonist (pergolide). The Food and Drug Administration voluntarily removed pergolide from the US market due to increasing reports of valvular disease. Fibrotic complications are sometimes reversible after discontinuation of the ergot-derived dopamine agonists, but not in all cases (6), such as some cases of retroperitoneal fibrosis and our case with Achilles tendon contractures.

Further, we feel these findings are particularly important given the known fibrotic complications of these dopaminergic ergot agents, and other retroperitoneal, pleural, pulmonary interstitial fibrosis may be overlooked and attributed to dystonia alone, which was not the sole cause of our patient's severe Achilles tendon contracture. This case may yield further insight into the poorly understood fibrotic pathophysiology between serotonin 5-HT2 and dopaminergic pathophysiology (1, 4).

We hypothesize that Parkinson's disease is an inherent dopaminergic state, which when exposed to non-selective ergot-derived dopaminergic agonists which secondarily stimulate 5HT(2B) leads to fibrotic stimulation. As further support of this hypothesis, the dopaminergic agent lisuride has been shown to be an extremely potent 5-HT(2B) antagonist, where no cases of cardiac valvulopathy have ever been reported, in more than 360,000 patient years (4). The molecular basis for this requires further study.

Authors' contributions: Conception and design: WDF, RES, PTD, JVG; Acquisition, analysis and interpretation of data: WDF; Drafting the article: WDF; Revising it critically for important intellectual content: WDF, RES.

Conflict of interests: Authors declare that they have no conflict of interests. This study was not sponsored by any external organization.

References

1. Antonini A, Poewe W. Fibrotic heart-valve reactions to dopamine-agonist treatment in Parkinson's disease. Lancet Neurol. 2007;6(9):826-9.
2. Hu MT, Bland J, Clough C, Ellis CM, Chaudhuri KR. Limb contractures in levodopa-responsive parkinsonism: a clinical and investigational study of seven new cases. J Neurol. 1999;246(8):671-6.
3. Charcot JM. Lectures on the diseases of the nervous system (paralysis agitans), lecture V. London: New Sydenham Society; 1877. p. 140-7.
4. Hofmann C, Penner U, Dorow R, Pertz HH, Jähnichen S, Horowski R, et al. Lisuride, a dopamine receptor agonist with 5-HT2B receptor antagonist properties: absence of cardiac valvulopathy adverse drug reaction reports supports the concept of a crucial role for 5-HT2B receptor agonism in cardiac valvular fibrosis. Clin Neuropharmacol. 2006;29(2):80-6.
5. Zanettini R, Antonini A, Gatto G, Gentile R, Tesei S, Pezzoli G. Valvular heart disease and the use of dopamine agonists for Parkinson's disease. N Engl J Med. 2007;356(1):39-46.
6. Fukae J, Tanaka S, Hattori N. Retroperitoneal fibrosis secondary to pergolide therapy. Intern Med. 2010;49(15):1687.

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DOI: 10.5644/ama2006-124.60

Očna duplja, anatomija i neurohirurški pristup (The orbit: Anatomy and neurosurgical approaches)

Authors: Ilija Nagulić and Mirjana Nagulić. Publisher: "Obeležja", Belgrade, October 2011, 2 chapters, 202 pages, 46 illustrations and one table.

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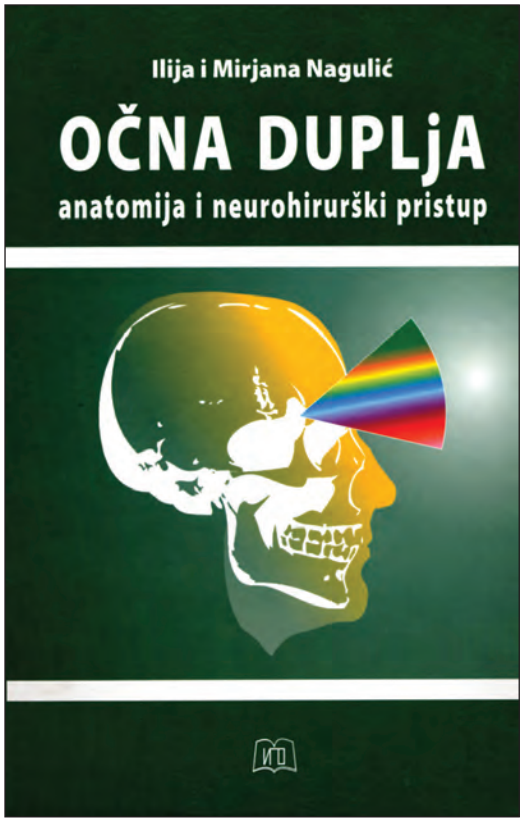

With increasing frequency, the orbit and adjacent areas are being exposed to deal with tumors and other lesions involving the orbit as well as intracranial structures. Difficulties in access to the orbit result from its relatively small volume, irregular quadrilateral pyramid shape and the fact that it is embedded in the craniofacial structures.

It is common for surgeons of different surgical disciplines to come together in the border craniorbito-facial region. The goal of surgery is always providing the optimum approach that involves the least possible functional and aesthetic damage. The selected approach and techniques have to take into account the nature, location and size of the orbital lesion. Usually, a neurosurgeon is asked to perform surgical procedures for lesions of the orbital apex and which are spreading into neighboring regions of the skull and skull base, making it important to gain an understanding not only of orbital anatomy, but also its relationship to the surrounding areas.

This book has two chapters. The first chapter is devoted to the general anatomy of the orbit with an emphasis on the retrobulbar and structures of the so called "orbito-cavernous junction". Readers are provided with descriptions, applied anatomy for neurosurgical approaches by the well-known international authorities, and by the author's own findings obtained from the cadaveric material. The second section of the first chapter is devoted to a description of microsurgical corridors and frequent anatomical variations of the region. The second chapter deals with neurosurgical approaches to the orbit. The first section of the chapter describes the history of access. Here the authorities and their contribution to the field are given, together with a description of the origin and development of the neurosurgical approach to the orbit in this community. The section on modern surgery describes the most common neurosurgical approaches to the orbit used nowadays, as well as the original approach by dr Ilija Nagulić, described as the "Mini orbitotomy drawer". The text is well illustrated with a number of original photographs and sketches.

Based on the authors' long term surgical experience combined with clinical laboratory research, this book was written to satisfy the interest of all who deal with the problems of orbital diseases, specialists of different branches of medical specializations, and why not, curious medical students. I am pleased to recommend the book "The Orbit. Anatomy and Neurosurgical Approaches".

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DOI: 10.5644/ama2006-124.61

International publications of authors from Bosnia and Herzegovina in Current Contents indexed publications in the first half of 2012*

Avdibegović E, Hasanović M, Hodžić M, Selimbašić Z. Psychological symptoms among workers employed in companies undergoing privatization in postwar Bosnia and Herzegovina. Coll Antropol. 2011 Dec;35(4):993-9.

Tuzla University Clinical Center, Clinic of Psychiatry, Tuzla, Bosnia and Herzegovina.

In Central and Eastern European countries, after abandoning communism, significant political, economic and social changes occurred, followed by the increase in income inequality and social disparity. The goal of this study was to examine the relationship between psychological symptoms and monthly income of employees in companies undergoing privatization. The study included 258 workers from seven companies undergoing privatization in the Tuzla Canton region. For the study purposes, the Brief Symptom Inventory (BSI) and a general questionnaire with questions about socio-demographic characteristics, income, and workplace, were used. Monthly income of the majority of workers (207 or 80.2%) was below the monthly income in Bosnia and Herzegovina. Workers with salaries below the average salary for Bosnia and Herzegovina have pronounced somatization, anxiety, paranoia, interpersonal sensitivity and hostility. The BSI scale yielded significant negative correlation between the level of monthly salary and the expression of psychological symptoms ($r = -0.184$, $p = 0.002$) and between the level of family income and the expression of psychological symptoms ($r = -0.123$, $p = 0.024$). Based on the study results, it was determined that socio-economic factors such as the level of salary and total family income and job insecurity, educational level, marital status and gender may be predictors of psychological symptoms.

Beganović A, Sefić-Pašić I, Skopljak-Beganović A, Kristić S, Šunjić S, Mekić A, Gazdić-Šantić M, Drljević A, Samek D. Doses to Skin During Dynamic Perfusion Computed Tomography of the Liver. Radiat Prot Dosimetry. 2012 Jun 22. [Epub ahead of print]

Department of Medical Physics and Radiation Safety, Clinical Centre of Sarajevo University, Bolničkа 25, Sarajevo, Bosnia and Herzegovina.

Many new computed tomography (CT) techniques have been introduced during the recent years, one of them being CT-assisted dynamic perfusion imaging (perfusion CT, PCT). Many concerns were raised when first cases of deterministic radiation effects were reported. This paper shows how radiochromic films can be utilised as passive dosemeters for use in PCT. Radiochromic dosemeters undergo a colour change directly and do not require chemical processing. Prior to their use, they need to be calibrated. Films are placed on top and on the right side of the patient and exposed during the procedure. Readout is performed using a densitometer. Results show that average local skin doses are 0.51 ± 0.07 and 0.42 ± 0.04 Gy on top and on the lateral side of the patient, respectively. Results of the patient dosimetry (local skin doses) are consistent. This is due to the fact that each patient had the

*Data for this survey were collected from PubMed database using the keywords Bosnia and Herzegovina and 2012.

same CT protocol used for imaging (120 kV, 60 mA and C(vol) of 247.75 mGy). Radiochromic films designed for interventional radiology can be effectively used for local skin dose measurements in perfusion CT. Dose values obtained are below the threshold needed for deterministic effects (erythema, hair loss, etc.). These effects might happen if inappropriate CT protocol is used; one that is usually used for routine imaging.

Bralić I, Tahirović H, Matanić D, Vrdoljak O, Stojanović-Špehar S, Kovačić V, Blažeković-Milaković S. Association of early menarche age and overweight/obesity. J Pediatr Endocrinol Metab. 2012;25(1-2):57-62.

Specialist Office in Pediatrics, School of Medicine, University of Split, Trogir, Croatia; Academy of Sciences and Arts of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina; Department for Research and Education, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina; City of Zagreb, Zagreb, Croatia; University Hospital "Sestre Milosrdnice", Children's Hospital Zagreb, Zagreb, Croatia; Department of Family Medicine, "Andrija Štampar" School of Public Health Medical School, University of Zagreb, Zagreb, Croatia; School of Medicine, University of Split, Split, Croatia

AIM: The aim of the study is to assess the association of overweight/obesity and early menarcheal age. PATIENTS AND METHODS: The study comprised 2127 healthy girls aged 9 to 16 years. Menarcheal age was estimated by status quo method. The girls' body weight and height were measured and their body mass index (BMI) calculated. The diagnostic criteria of the WHO were used to define overweight and obesity. Girls with a BMI in the range of 1-2 for age and sex were considered overweight. Girls with a BMI >2 standard deviation (SD) for age and sex were considered obese. Girls with a BMI >1 SD for age and sex were considered overweight/obese. Social and economic status was analyzed according to years of education completed, parents' occupations, and the number of children in the family. RESULTS: Median menarcheal age was 12.83 years; 25% girls had menarche before 11.98 years and 75% by 13.69 years. By 11.21 years, 10% of girls had had menarche, and 95% by 14.91 years. Girls who had menarche before 11.98 years had higher body weight values (48.5 vs. 40.2 kg) ($p<0.001$), height (159.3 vs. 149.2 cm) ($p<0.001$), and BMI (18.9 vs. 17.8 kg/m2) ($p=0.003$) than their peers without menarche. Girls with menarche before 11.98 years had significantly higher BMI values than girls with menarche after 13.69 years (18.94 vs. 17.84 kg/m2) ($p=0.008$). Girls with menarche before 11.98 years and those after 13.69 years differ significantly in distribution of thinness (3.4% vs. 2.54%), normal weight (85.3% vs. 91.8%), and overweight/obesity (11.2% vs. 5.7%) ($p=0.002$). CONCLUSIONS: Girls who experienced early menarche are significantly more often overweight/obese. Overweight/obesity may be considered as one of the predictors for the early occurrence of menarche.

Ćavar M, Sekulić D, Čuljak Z. Complex Interaction of Religiousness with other Factors in Relation to Substance Use and Misuse Among Female Athletes. J Relig Health. 2012 Jun;51(2):381-9.

Faculty of Science, Mathematics and Education, University of Mostar, Mostar, Bosnia and Herzegovina.

Strength of religious faith (SRF) is rarely studied as a protective factor against substance use and misuse in sports. Herein, we studied the potential buffering effect of the complex socio-educational, sports, and religiousness factors in the protection against substance use and misuse, including cigarettes, analgesics, appetite suppressants, potential doping behavior, and binge drinking. The sample of subjects included 40 high-class female athletes (22-26 years of age). Using a strictly anonymous questionnaire, we investigated different social, educational, and sports factors (including SRF measured by the Santa Clara Strength of Religious Faith Questionnaire) in relation to substance use and misuse. Following the calculation of simple correlations, multiple regression analysis revealed that in combination with low sports experience, SRF has a significant buffering effect against binge alcohol drinking and consumption of appetite suppressants. The data are discussed in comparison with previous findings and theoretical background. Future studies should study the topic while observing samples of recreational and competitive athletes of both genders.

De Cock RF, Knibbe CA, Kulo A, de Hoon J, Verbesselt R, Danhof M, Allegaert K. Developmental pharmacokinetics of propylene glycol in preterm and term neonates. Br J Clin Pharmacol. 2012 Apr 27. doi: 10.1111/j.1365-2125.2012.04312.x. [Epub ahead of print]

Division of Pharmacology, LACDR, Leiden University, Leiden, The Netherlands; Department of Clinical Pharmacy, St. Antonius Hospital, Nieuwegein, The Netherlands; Centre for Clinical Pharmacology, University Hospitals Leuven, Leuven, Belgium; Institute of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia Herzegovina; Neonatal Intensive Care Unit, University Hospitals Leuven, Leuven, Belgium.

Aim: Propylene glycol (PG) is often applied as an excipient in drug formulations. As these formulations

may also be used in neonates, the aim of this study was to characterize the pharmacokinetics of propylene glycol, co-administered intravenously with paracetamol (800mgPG/1000mg paracetamol) or phenobarbital (700mgPG/200mg phenobarbital) in preterm and term neonates. Methods: A population pharmacokinetic analysis was performed based on 372 PG plasma concentrations from 62 (pre)term neonates (birth weight (Bbw) 630-3980g, postnatal age (PNA) 1-30days) using NONMEM 6.2. The model was subsequently used to simulate PG exposure upon administration of paracetamol or henobarbitalin neonates (gestational age 24-40 weeks). Results: In a one compartment model, birth weight and PNA were both identified as covariates for PG clearance using an allometric function (CL(i) =0.0849x{(BWb/2720)(1.69) x(PNA/3)(0.201)}). Volume of distribution scaled allometrically with current bodyweight (V(i) =0.967x{(BW/2720)(1.45)}), and was estimated 1.77 times higher when co-administered with henobarbital compared to paracetamol. By introducing these covariates a large part of the interindividual variability on clearance (65%) as well as on volume of distribution (53%) was explained. The final model shows that for commonly used dosing regimens, the population mean PG peak and trough concentrations ranges between 33-144 and 28-218 mg/L (peak) and 19-109 and 6-112 mg/L (trough) depending on birth weight and age of the neonates for paracetamol and henobarbital formulations, respectively. Conclusion: A pharmacokinetic model was developed for PG co-administered with paracetamol or phenobarbital in neonates. As such, large variability in PG exposure may be expected in neonates which are dependent on birth weight and postnatal age.

Jelavić B, Grgić M, Čupić H, Kordić M, Vasilj M, Baudoin T. Prognostic value of Helicobacter pylori sinonasal colonization for efficacy of endoscopic sinus surgery. Eur Arch Otorhinolaryngol. 2012 Jan 12. [Epub ahead of print]

Department of Otorhinolaryngology, Mostar University Hospital, Bijeli brijeg b.b, Mostar, Bosnia and Herzegovina.

Compared with rhinologic patients without chronic rhinosinusitis (CRS), a higher prevalence of sinonasal Helicobacter pylori (HP) in patients with CRS was found. This study investigated if HP sinonasal colonization has a prognostic value for efficacy of functional endoscopic sinus surgery (FESS). Nasal polyps of 40 patients with CRS, undergoing FESS, were analyzed for presence of HP using immunohistochemistry (IHC). Patients were categorized as to whether the IHC was positive (HP+ group) or negative (HP- group). HP+ group and HP- group were compared according to the nasal polyp eosinophil density, and to the improvement (difference between pre- and postoperative scores) of the subjective symptom scores, and the nasal endoscopic scores. Nasal polyps in 28 (70%) patients were positive for HP. There were no significant differences between HP+ group and HP- group comparing the eosinophils, and the improvement of the single symptom and the total symptom scores. HP+ group had significantly greater improvement of the nasal endoscopic scores (F[1.38] = 6.212; P = 0.017). There is no influence of sinonasal HP on tissue eosinophilia and on CRS symptoms. There is a prognostic value for endonasal findings: CRS patients with HP have statistically significant greater improvement of the postoperative endoscopic scores.

Jovanović P, Salkić N, Zerem E, Ljuca F. Gammaglutamyl transaminase in biliary obstruction. Eur J Intern Med. 2012 Apr;23(3):e76. Epub 2012 Jan 5.

No abstract available.

Jurić S, Mišmaš A, Mihić N, Barać AM, Habek M. Newly onset sinus bradycardia in the context of multiple sclerosis relapse. Intern Med. 2012;51(9):1121-4. Epub 2012 Apr 29.

Department of Neurology, University Hospital Mostar, Bosnia and Herzegovina.

Cardiovascular disorders in acute multiple sclerosis (MS) relapse have been infrequently reported. We present a young multiple sclerosis patient with acute onset of cerebellar symptomatology along with sinus bradycardia. Brain magnetic resonance imaging showed one lesion in the left cerebellar hemisphere which showed postcontrast enhancement and one in the midbrain without postcontrast enhancement. No cardiac pathology was found and symptoms gradually improved after a 5-day course of corticosteroid therapy. It is important to bear in mind the possibility of these rare cardiac symptoms in MS patients, because of their timely recognition and appropriate treatment.

Kulo A, de Hoon JN, Allegaert K. The propylene glycol research project to illustrate the feasibility and difficulties to study toxicokinetics in neonates. Int J Pharm. 2012 May 26. [Epub ahead of print]

Center for Clinical Pharmacology, University Hospitals Leuven, Leuven, Belgium; Institute of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

This paper aims to describe our propylene glycol (PG) research project to illustrate the feasibility and the dif-

ficulties encountered to perform excipient studies in neonates. PG is frequently co-administered excipient. PG accumulation potentially results in hyperosmolarity, lactic acidosis or hepato-renal toxicity in adults, reflecting issues related to pharmacokinetics (PKs) and -dynamics (PDs). Consequently, similar observations in neonates are urgently needed. Since newborns display 'physiological' impaired hepatic and renal elimination capacity, description of PG PK in neonates is warranted. The PG PD was assessed based on indicators of renal, hepatic and metabolic (in)tolerance earlier reported in adults and relating to osmolar changes. Based on the PK and PD data collected in neonates, we suggest that there is a lower limit of PG tolerance in neonates. In addition to preliminary data on PG disposition and tolerance in neonates, we mainly focus on the limitations of the current observations and the difficulties encountered during this PG project to further illustrate the specific setting of neonatal research.

Lala C, Framme C, Wolf-Schnurrbusch UE, Wolf S. Three-year results of visual outcome with disease activity-guided ranibizumab algorithm for the treatment of exudative age-related macular degeneration. Acta Ophthalmol. 2012 Jun 14. doi: 10.1111/j.1755-3768.2012.02457.x. [Epub ahead of print]

Universitätsklinik für Augenheilkunde, University of Bern, Bern, Switzerland; Clinical Center of Eastern Sarajevo, Eye Clinic 'Kasindo', E. Sarajevo, Bosnia and Herzegovina.

Purpose: To evaluate 3-year follow-up treatment outcomes with ranibizumab (Lucentis(®)) 0.5 mg administered either monthly or quarterly on a pro re nata (PRN) basis according to a disease activity-guided monitoring and treatment algorithm. Methods: A total of 316 treatment-naive eyes of 316 patients with exudative age-related macular degeneration met the criteria for inclusion in this retrospective, interventional case series. Patients were treated with ranibizumab 0.5 mg according to a disease activity-guided algorithm with monthly monitoring. Optical coherence tomography and fluorescein angiography were routinely used to assess disease activity: active lesions were treated with a series of three monthly injections, whereas inactive lesions were treated with quarterly injections. Results: Mean Early Treatment Diabetic Retinopathy Study best-corrected visual acuity improved from 52 letters at baseline to 59 letters at 12 months, achieved with a mean of 7.1 injections, 61 letters at 24 months with a mean of 5.0 injections administered in the second year and 60 letters at 36 months with a mean number of 5.2 injections. Conclusions: Monthly visits and a morphology-driven PRN regimen with 3 injections in case of recurrence plus quarterly injections in case of inactive CNV resulted in an average VA gain of 7-9 letters that could be maintained over 3 years.

Marković-Peković V, Grubiša N. Self-medication with antibiotics in the Republic of Srpska community pharmacies: pharmacy staff behavior. Pharmacoepidemiol Drug Saf. 2012 Feb 15. doi: 10.1002/pds.3218. [Epub ahead of print]

Ministry of Health and Social Welfare, the Republic of Srpska, Bosnia and Herzegovina.

PURPOSE: Self-medication with antibiotics adds to the global risk of increased spread of bacterial resistance. Attitudes and behavior of health professionals also may reinforce self-medication with antibiotics. The aim of this study was to determine whether self-medication with antibiotics is possible in our community pharmacies and to what extent, and to evaluate the behavior and service of pharmacy health professionals regarding non-prescription antibiotic dispensation. METHODS: An observational, cross-section study was conducted, and pseudo-patient methodology was used to establish the kind of professional service provided in case of patient's explicit demand to buy an antibiotic for treatment of self-diagnosed upper respiratory tract infection. RESULTS: Of the total 318 community pharmacies, 131 (41%) were visited and included in the study. Non-prescription antibiotics were dispensed in 76 (58%) pharmacies. Counseling and symptomatic therapy was offered in 88 (67%) pharmacies. In 25% of pharmacies, no symptomatic therapy was offered; instead, only an antibiotic was sold. Amoxicillin was sold in 85% of cases and, mostly, the one of 1.30 Euro per pack. Both oral and written use instructions were given in 78% cases, whereas none was given in 3% of cases. CONCLUSIONS: Self-medication with antibiotics occurs in our community pharmacies, despite being illegal. Pharmacy staff behavior can be a factor that puts patients at risk for self-medication with antibiotics. Community pharmacies are failing their tasks in enhancing rational use of antibiotics. Such a practice may be a consequence of weak enforcement and control over the legislation and professional standards.

Mitrović J. Jožef Stefan and the dissolution-diffusion phenomena--not only a historical note. Int J Pharm. 2012 Jul 15;431(1-2):12-5. Epub 2012 Apr 13.

Faculty for Production and Management Trebinje, University in East Sarajevo, Republic of Srpska, Bosnia and Herzegovina.

In a series of papers published from 1871 to 1889, Jožef (Josef) Stefan dealt with several diffusion processes, including also multicomponent systems. In his last paper on diffusion, which appeared in 1889, he studied the dissolution-diffusion process with a moving interface, and gave an analytical solution to this problem. However, Stefan's dissolution-diffusion analysis is not mentioned in literature, and its existence seems to be unknown in scientific community. The present paper summarizes the main Stefan ideas on dissolution of solids governed by diffusion of solute in the adjacent solvent phase thus making his results accessible to wider scientific circles.

Mladenović I, Jović N, Čutović T, Mladenović G, Kozomara R. Temporomandibular disorders after orthognathic surgery in patients with mandibular prognathism with depression as a risk factor. Acta Odontol Scand. 2012 Feb 9. [Epub ahead of print]

Department of Prosthodontics, Faculty of Medicine, University of East Sarajevo, Bosnia Herzegovina.

Objective. To examine the prevalence of temporomandibular disorders (TMD) after orthodontic-surgical treatment in patients with mandibular prognathism and analyze psychosocial variables related to TMD. Materials and methods. The case-control study comprised 40 patients with mandibular prognathism who underwent combined orthodontic-surgical treatment (orthognathic surgery group). Forty-two patients with untreated mandibular prognathism served as a control group. Research diagnostic criteria for temporomandibular disorders was used in order to assess the clinical diagnosis of TMD (Axis I) and to estimate depression, somatization and patient's disability related to chronic pain (Axis II). Results. The overall prevalence of TMD was not significantly different between the groups. Myofascial pain was significantly higher, while arthralgia, arthritis and arthrosis was significantly lower in the orthognathic group compared with the controls (90.5% vs 50.0%, 0.0% vs 27.8%, respectively) ($p < 0.05$). Females in orthognathic surgery group showed higher prevalence of TMD ($p < 0.05$) and myofascial pain ($p < 0.01$) and increased level of chronic pain ($p < 0.05$) in comparison with post-operative males. No significant difference in chronic pain, somatization and depression scores was found between investigated groups. With respect to presence of TMD within the groups depression was higher in untreated subjects with dysfunction ($p < 0.05$). Conclusion. Prevalence of TMD immediately after completion of orthodontic-surgical treatment for mandibular prognathism is similar to frequency of dysfunction in untreated subjects, is significantly higher in females and is most commonly myogenic. Furthermore, females show an increased level of chronic pain postoperatively. Somatization and depression levels do not differ between patients with corrected prognathism and untreated prognathic patients.

Pehar M, Vukoja I, Rozić D, Mišković J. Spontaneous diaphragmatic rupture related to local invasion by retroperitoneal liposarcoma. Ann R Coll Surg Engl. 2012 Jan;94(1):e18-9.

University Hospital Mostar, Mostar, Bosnia and Herzegovina.

We report a case of the female patient who was admitted to the hospital because of syncope experienced while climbing stairs. Diagnostic workup raised the suspicion of a right diaphragmatic rupture that was eventually confirmed by surgery (right-sided thoracotomy). Surgery also revealed tissue protruding through the rupture site from within the retroperitoneum that was proven subsequently to be a dedifferentiated liposarcoma. Second surgery was performed to completely remove the liposarcoma tissue and repair a coincident old right lumbar region hernia. The patient recovered fully. Spontaneous rupture of the diaphragm is rare and this is especially true for the right hemidiaphragm. We report the first case of diaphragmatic rupture caused by local infiltration by a retroperitoneal liposarcoma. This and similar reports emphasise that in cases with high clinical suspicion of diaphragmatic rupture, diagnosis should be pursued even in the absence of a preceding traumatic event.

Petrić I, Helić A, Avdić EA. Evolution of process parameters and determination of kinetics for co-composting of organic fraction of municipal solid waste with poultry manure. Bioresour Technol. 2012 Aug;117:107-16. Epub 2012 Apr 26.

Department of Process Engineering, Faculty of Technology, University of Tuzla, Univerzitetska 8, Tuzla, Bosnia and Herzegovina.

This study aimed to monitor the process parameters and to determine kinetics in composting of organic fraction of municipal solid waste (OFMSW) and poultry manure. The experiments were carried out with three different mixtures. The results showed that the mixture 60% OFMSW, 20% poultry manure, 10% mature compost and 10% sawdust provided the most appropriate conditions for composting process. Using nine kinetic models and nonlinear regression method, kinetic parameters were estimated and the models were analyzed with four statistical indicators. Kinetic models with four measured variables proved to be better than models with less number of measured variables. The number of measured experimental variables influences kinetics more than the number of kinetic

parameters. Satisfactory fittings of proposed kinetic model to the experimental data of OM were achieved. The model is more suitable for data obtained from composting of mixtures with much higher percentage of OFMSW than percentage of poultry manure.

Ristić S, Lukić L, Maksimović Ž, Marić S, Marić V, Kovačević M, Trifunović D, Pavlović D, Mijatović S, Marinković J, Đukanović L. High prevalence of risk factors for chronic kidney disease in Balkan endemic nephropathy foci. Ren Fail. 2012;34(4):467-71. Epub 2012 Feb 24.

Foča Medical Faculty, University of East Sarajevo, Sarajevo, Bosnia and Herzegovina.

BACKGROUND/AIMS: The aim of this study was to find out the prevalence of the most frequent risk factors for chronic kidney disease (CKD) and the prevalence of urinary abnormalities in adult inhabitants of three Balkan endemic nephropathy (BEN) villages near Bijeljina, Bosnia and Herzegovina. METHODS: The survey consisted of an interview, blood pressure measurement, and urine dipstick test for proteinuria, hematuria, and glycosuria. RESULTS: The study involved 1625 (739 males, aged 51 ± 16 years) subjects: 319 (19.6%) with positive family history for BEN, 585 (36%) with hypertension, 604 (37.2%) above 60 years, 146 (9%) with diabetes, and 566 (34.8%) with none of these risk factors. Proteinuria was present in 6.2-7.1% of the subjects with risk factors for CKD but in 3.4% of those without risk factors. Systolic blood pressure and BEN in brother/sister were found to be significant variables associated with proteinuria, but female gender and history of kidney disease with hematuria. CONCLUSION: In addition to a family burden for BEN, other risk factors for CKD were highly prevalent in BEN villages of the Bijeljina municipality. The frequency of proteinuria was higher in the at-risk group than in the group without risk factors and increased with the number of risk factors.

Sekulić D, Ostojić M, Ostojić Z, Hajdarević B, Ostojić Lj. Substance abuse prevalence and its relation to scholastic achievement and sport factors: An analysis among adolescents of the Herzegovina-Neretva Canton in Bosnia and Herzegovina. BMC Public Health. 2012 Apr 5;12(1):274. [Epub ahead of print]

BACKGROUND: Substance abuse among adolescents is a major public health and social problem. However, studies rarely investigate the relationships between substance abuse, educational achievement and sport factors. Substance abuse is an even more significant problem in societies that have experienced trauma, such as Bosnia and Herzegovina, which have had recent wars. The aims of this study were to investigate substance abuse among adolescents in Bosnia and Herzegovina and to study the potential gender-specific relationships between a) sport factors (physical activity/exercise/athletic participation) and substance abuse and b) scholastic achievement and substance abuse. METHODS: Our sample consisted of 1,032 adolescents who were 17 to 18 years old (435 boys and 597 girls) and who were in the final grade of high school. These subjects were randomly selected from the territory of Herzegovina-Neretva Canton of Bosnia and Herzegovina. Retrospective testing was performed using an extensive self-administered questionnaire. The questionnaire included questions involving topics such as sociodemographic variables, scholastic variables, sport factors, and substance abuse data (smoking habits, drugs consumption and alcohol consumption using the AUDIT questionnaire). Descriptive statistics, frequencies, analyses of the differences and correlational analyses were performed. RESULTS: Our results found that greater than one-third of the boys and one-fourth of the girls were daily smokers, and almost half of the boys and one-fifth of the girls practiced harmful drinking; other drugs (i.e. heroin, cocaine, amphetamines, etc.) were rarely consumed. Boys dominated in sport factors, whereas girls were more successful in scholastic achievement. Approximately 23% of the boys and 6% of the girls reported that they practiced harmful drinking and smoked simultaneously. Educational failure, which was defined as having one or more negative grades at the end of the last two school years, was identified in 20% of the boys and 9% of the girls. In both genders, substance abuse was negatively correlated with educational achievement, and half of those students who failed educationally reported daily smoking. Among the girls who experienced education failure, 33% were smokers, and 22% practiced harmful drinking. Sport factors were weakly correlated with substance abuse in boys; thus, we could not support the hypothesis that sports are a protective factor against substance abuse among male adolescents. In girls, participation in team sports was related with a higher incidence of smoking, but there was no evidence of sport factors having an influence on the consumption of alcohol. CONCLUSION: In this study, the incidence of smoking and the consumption of alcohol were alarmingly high. These findings demonstrate the need for intervention programs to address these issues. These problems are particularly important, considering that substance abuse has a negative impact on educational achievement among boys and girls, and sport factors have not been found to be protective factors against substance abuse.

Smajlović L, Davoren J, Heyman P, Cochez C, Haas C, Maake C, Hukić M. Development and optimization of a PCR assay for detection of

Dobrava and Puumala hantaviruses in Bosnia and Herzegovina. J Virol Methods. 2012 Jun;182(1-2):37-42. Epub 2012 Mar 13.

International Commission on Missing Persons, Sarajevo, Bosnia and Herzegovina.

Hantavirus-specific serology tests are the main diagnostic technique for detection of hantavirus infection in Bosnia and Herzegovina. In order to enhance hantavirus infections monitoring a sensitive PCR based assay was developed to detect Dobrava (DOBV) and Puumala (PUUV) hantaviruses. Nested primer sets were designed within three different regions of the viral RNA (S and M segment of DOBV and M segment of PUUV) based on highly similar regions from a number of different European hantavirus strains. Assay conditions were optimized using cell cultures infected with DOBV Slovenia, PUUV Sotkamo and PUUV CG 18-20. This sensitive and specific assay has proven to be useful for detection of both Puumala and Dobrava hantaviruses.

Šimić D, Šitum M, Marijanović I, Hadžigrahić N. Most common skin tumours in correlation with solar ultraviolet radiation in the area of West Herzegovina. Coll Antropol. 2011 Dec;35(4):1129-34.

University of Mostar, Mostar University Clinical Hospital, Department for Dermatology and Venerology, Mostar, Bosnia and Herzegovina

Incidence rate of skin tumours, both, non-melanoma and melanoma, is increasing nowadays. Various etiological factors are of relevance for the occurrence of the diseases. The solar radiation, as well, long-term exposure to ultraviolet (UV) radiation, have the greatest impact on development of these skin tumours. Non-melanoma skin tumours, Basal Cell Carcinoma (BCC) and Squamous Cell Carcinoma (SCC), are the most common skin tumours in humans, and usually develop on the chronically photo-exposed areas. As for the Malignant Melanoma (MM), one of the most aggressive skin tumours, the exposure to solar radiation also plays an important role. This study investigates the correlation between the skin tumours and UV radiation in the area of West Herzegovina, on the sample of 1676 patients. It presents the occurrence of skin tumours in the period from 1997 to 2003. The study investigates the incidence and the risk factors separately for every skin tumour which can be etiologically related to the occurrence of skin tumours and UV radiation: occupation, exposure to UV radiation, skin type, and family history on malignan tumours within the patient's family. The exact incidence rate of non-melanoma and melanoma skin tumours in Bosnia and Herzegovina is still unknown, for the reason that the united National Cancer Register does not exist yet.

Tahirović E, Begić H, Tahirović H, Varni JW. Quality of life in children after cardiac surgery for congenital heart disease. Coll Antropol. 2011 Dec;35(4):1285-90.

University of Sarajevo, Sarajevo University Clinical Center, Heart Center, Sarajevo, Bosnia and Herzegovina.

The aim of this study was to assess the quality of life children after cardiac surgery for congenital heart disease (CHD) and to compare these results with healthy children. To assess the quality of life children after surgery for CHD we performed a cross-sectional study of 114 patients who were patients at the Department of Paediatrics in Tuzla, between the ages of 2 and 18 years, of both sexes, and with one of their parents. We used the "PedsQL 4.0 Generic Core Scales", with both child self-report and parent proxy-reports. By self assessment, the PedsQL total scores for quality of life were statistically significantly different between children after cardiac surgery for ages 13 to 18 years and healthy children, while by parent report PedsQL total scores were statistically significantly different between children after cardiac surgery for ages 5 to 7 years and healthy children. By self assessment, children after cardiac surgery for ages from 5 to 7 and 13 to 18 years reported that they have a statistically significantly lower quality of life in the segment school functioning compared to the healthy children. By parental assessment, children after cardiac surgery for ages 2 to 4, 5 to 7 and 8 to 12 years have a statistically significantly lower quality of life in the segments of physical and psychosocial health, emotional, social and school functioning. The results of our study indicate that children after cardiac surgery for CHD by self and parent assessment have a lower quality of life than healthy children.

Tahirović H, Toromanović A, Tahirović E, Begić H, Varni JW. Health-related quality of life and metabolic control in children with type 1 diabetes mellitus in Bosnia and Herzegovina. Coll Antropol. 2012 Mar;36(1):117-21.

University Clinical Centre Tuzla, Department for Research and Education, Tuzla, Bosnia and Herzegovina

The primary objective of the study was to examine the relationship between generic and disease-specific HRQOL scores and metabolic control in children with Type 1 Diabetes Mellitus (T1DM). This cross-sectional study included 65 consecutive children between ages 5 and 18 years with T1DM. According to their values of glycosylated hemoglobin (HbA(1C)), the children were assigned to one of two groups. In Group 1 (N = 21) were the children with HbA(1C) values < 8%

(good to moderate metabolic control) and Group 2 (N = 44) were children with > 8% (poor metabolic control). To evaluate generic and disease-specific HRQOL scores in children with T1DM in relation to metabolic control, we used the PedsQL 4.0 Generic Core Scales and the PedsQL 3.0 Diabetes Module. The patients in Group 1, by pediatric patient self-report and parent proxy-report, had statistically better disease-specific HRQOL scores on the diabetes symptoms, treatment barriers, treatment adherence and worry domains in comparison with Group 2. We also found significant correlations between the total generic HRQOL scores and HbA(1C) for both parent proxy-reports' Spearman's coefficient of rank correlation rho = -0.257; p = 0.0412 and pediatric patients' Spearman's coefficient of rank correlation rho = -0.269; p = 0.0313. The current findings suggest that poor glycemic control in children with T1DM is associated with lower generic and disease-specific HRQOL scores in developing and transitional countries.

Vranić S, Bender R, Palazzo J, Gatalica Z. A review of adenoid cystic carcinoma of the breast with emphasis on its molecular and genetic characteristics. Hum Pathol. 2012 Apr 18. [Epub ahead of print]

Department of Pathology, Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

Breast carcinomas that do not express estrogen receptor α, progesterone receptor, or human epidermal growth factor receptor 2 are frequently grouped together as "triple negative" and considered an aggressive type of breast malignancy; however, this group is not homogeneous. Adenoid cystic carcinoma of the breast is a rare type of breast cancer with such triple-negative features and, generally, a more favorable clinical course. This comprehensive review describes diagnostic, molecular, and clinical features of adenoid cystic carcinoma and compares them with those of triple-negative breast carcinomas of no special type.

Zerem E. Percutaneous versus endoscopic approach in treatment of acute cholecystitis. Gastrointest Endosc. 2012 Jan;75(1):226; author reply 226-7.

No abstract available.

Zerem E, Imamović G, Ljuca F, Alidžanović J. What is the optimal treatment for appendiceal mass formed after acute perforated appendicitis. World J Gastroenterol. 2012 Apr 21;18(15):1849-50.

We read with great interest the editorial article by Meshikhes AWN published in issue 25 of World J Gastroenterol 2011. The article described the advantages of emergency laparoscopic appendectomy compared with interval appendectomy as a new safe treatment modality for the appendiceal mass. The author concluded that the emergency laparoscopic appendectomy was a safe treatment modality for the appendiceal mass, and might prove to be more cost-effective than conservative treatment, with no need for interval appendectomy. However, we would like to highlight certain issues regarding the possibility of percutaneous catheter drainage to successfully treat the appendiceal mass, with no need for appendectomy, too.

Zerem E, Imamović G, Mavija Z. Is irrigation necessary during endoscopic necrosectomy of pancreatic necroses? Surg Endosc. 2012 Apr 26. [Epub ahead of print]

Department of Gastroenterology, University Clinical Center Tuzla, Trnovac bb, Tuzla, Bosnia and Herzegovina

No abstract available.

Zerem E, Omerović S. Can percutaneous cholecystostomy be a definitive management for both acute calculous and acalculous cholecystitis? J Clin Gastroenterol. 2012 Mar;46(3):251.

No abstract available.

Zerem E, Pavlović-Čalić N, Bevanda M. Is minimally invasive retroperitoneal pancreatic necrosectomy too aggressive in treating infected pancreatic necrosis. Pancreatology. 2011;11(6):610-1. Epub 2012 Feb 2.

No abstract available.

Zerem E, Sušić A, Pavlović-Čalić N, Haračić B, Jovanović P. What is the optimal treatment for peripancreatic fluid collections? J Gastrointest Surg. 2012 Aug;16(8):1635-6. Epub 2012 Feb 4.

Department of Gastroenterology, University Clinical Center Tuzla, Trnovac bb, Tuzla, Bosnia and Herzegovina.

No abstract available.

by Nerma Tanović

Acta Medica Academica 2012;41(2):240
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Academician Ladislav Ožegović (1921-2011)

Lidija Lincender – Cvijetić

Academician Ladislav Ožegović, born in Split, 1921, graduated from the Faculty of Veterinary Medicine in Zagreb, in October 1947. In November 1947, he was elected as an assistant at the Veterinary Medical Clinic in Zagreb, where he earned his Ph.D. in 1948. Upon his arrival in Sarajevo in 1950, at the newly established Faculty of Veterinary Medicine, he became docent for the subject of internal diseases in solipeds and carnivores. In 1952, he established a Medical Clinic at the Faculty of Veterinary Medicine, which he led until 1958. From 1954 to 1978 he worked at the "Dr. Simo Milošević" Dermatovenerology department as a mycologist, and later as the chief mycologist at the same laboratory. Along with his work at various laboratories, he worked as a translator at the Faculty of Veterinary Studies in Sarajevo, where he became a professor, and, after retirement, professor emeritus. He received his vast education from an international list of universities, from Great Britain to The Netherlands, France, Belgium, the United States, Costa Rica, Venezuela and Guatemala. He was part of a myriad of international conferences on mycotoxins, while publishing his work in various periodicals and academic journals; he was also the author of several books and university textbooks. From 1956 to 1976 he participated in antimycotic projects in suppressing endemic dermatophytes. From 1971, when elected as a fellowof the Department of Medical Sciences at the Academy of Sciences and Arts of Bosnia and Herzegovina, professor Ladislav Ožegović led a number of medical mycology and endemic nephropathy projects; in recognition of his achievements gained through these projects, in 1978 he was elected as a corresponding member and then as a full member of the Academy of Sciences and Arts of Bosnia and Herzegovina. He established a Committee for Medical Mycology and Endemic Nephropathy of the Department of Medical Sciences of ANUBiHwhere he engaged a number of established, as well as young researchers through collective projects, zealously instilling his knowledge into his pupils. In his later years, as the director of the Center for Coordination of Medical Research of ANUBiH, he led a number of academic and research projects. From his professional, academic, directorial, and tautological accomplishments one may trace the career of a true academic visionary of astonishing capabilities, whose research and academic work meant so much to the national and international medical community. Due to his great enthusiasm, altruism, and an undying pursuit for teaching, Academician Ladislav Ožegović will remain dear to many generations of students, colleagues, and friends. His work will continue to inspire his fellow scholars in their academic and medical endeavors. The death of academician Ladislav Ožegović is a huge loss to the Department of Medical Sciences and the Academy of Sciences andArts of Bosnia and Herzegovina.

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Academician Srećko Šimić (1929-2011)

Lidija Lincender – Cvijetić

Born in Travnik in 1929, academician Srećko Šimić graduated from the Faculty of Medicine, University of Sarajevo in 1954, and completed his specialization exam in gynaecology and obstetrics in 1961 at the Clinic for Gynaecology and Obstetrics in Sarajevo. In 1968 academician Srećko Šimić began his academic career in habilitation, defending his doctorate dissertation in 1972. There followed a period of active training and professional development in the field of gynaecology at clinics in Ljubljana, Graz, Vienna, Munich, Hamburg, Kiel, Stockholm, London and Oxford. He began his teaching career in 1961 when he was elected assistant in the Department of Obstetrics and Gynaecology, then in 1968 he was elected assistant professor, in 1974 associate professor and full professor in 1982. From 1974 to 1994 he worked as the director of the gynaecology/obstetrics clinic in Sarajevo during which period the clinic proved to be among the leading medical centres in Europe, delivering around 10 000 newborns annually and performing thousands of gynaecological surgeries. In his time at the clinic, academician Srećko Šimić introduced modern methods of diagnostics and therapeutics. He introduced a number of abdominal and vaginal operations, of which vaginal surgery for cervical cancer is especially significant. He was the first who introduced culdoscopy and laparoscopy into clinical practice to the territory of the former Yugoslavia (1962). From 1984 his devotion to the artificial insemination project resulted in the clinic's first successful artificial pregnancy in 1990, making the Sarajevo Clinic the third in Yugoslavia to do so. As a member of several gynaecological/obstetrical associations, academician Srećko Šimić actively participated in myriad conferences and symposiums around the world as well as domestic gatherings of international repertoire. Upon his retirement in 1998, after 40 years of active work, he was honoured as professor emeritus by the University of Sarajevo. From 1998 he worked as a consultant at the gynaecologic/obstetrics branch at the "Prim. dr. Abdulah Nakaš" General Hospital in Sarajevo. From 2003 he was the acting head of the department for gynaecology, childbirth, and health care at the Faculty of Health Studies, University of Mostar, where he was active until the very end. In 2002 he was elected as a corresponding member of ANUBiH, and in 2008 he became a full member; during his time in ANUBiH he organized three symposiums on the topics of gynaecology and perinatology. During his extensive career he was the author of seven books, co-author of ten, and published 198 articles in various journals. In addition to his professional activities, professor Srećko Šimić was a good father to his sons, who were his pride. As a doctor-gynaecologist he was always willing to help those patients who had gynaecological problems as well as his colleagues. He left a constellation of other young and now even senior gynaecologists, physicians who had the good fortune and honour to learn from professor Srećko Šimić.

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

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More than six authors:

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

Organization as author:

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension. 2002;40(5):679-86.

No author given:

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

Volume with supplement:

Geraud G, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. Headache. 2002;42(Suppl 2):S93-9.

Issue with supplement:

Glauser TA. Integrating clinical trial data into clinical practice. Neurology. 2002;58(12 Suppl 7):S6-12.

Issue with no volume:

Banit DM, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. Clin Orthop. 2002;(401):230-8.

Letters or abstracts:

Tor M, Turker H. International approaches to the prescription of long-term oxygen therapy [letter]. Eur Respir J. 2002;20(1):242. ; Lofwall MR, Strain EC, Brooner RK, Kindbom KA, Bigelow GE. Characteristics of older methadone maintenance (MM) patients [abstract]. Drug Alcohol Depend. 2002;66 Suppl 1:S105.

Article republished with corrections:

Mansharamani M, Chilton BS. The reproductive importance of P-type ATPases. Mol Cell Endocrinol. 2002;188(1-2):22-5. Corrected and republished from: Mol Cell Endocrinol. 2001;183(1-2):123-6.

Article with published erratum:

Malinowski JM, Bolesta S. Rosiglitazone in the treatment of type 2 diabetes mellitus: a critical review. Clin Ther. 2000;22(10):1151-68; discussion 1149-50. Erratum in: Clin Ther 2001;23(2):309.

Article published electronically ahead of the print version:

Yu WM, Hawley TS, Hawley RG, Qu CK. Immortalization of yolk sac-derived precursor cells. Blood. 2002 Nov 15;100(10):3828-31. Epub 2002 Jul 5.

Books and other monographs

Personal author(s):

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Editor(s), compiler(s) as author:

Gilstrap LC 3rd, Cunningham FG, VanDorsten JP, editors. Operative obstetrics. 2nd ed. New York: McGraw-Hill; 2002.

Organization(s) as author:

Royal Adelaide Hospital; University of Adelaide, Department of Clinical Nursing. Compendium of nursing research and practice development, 1999-2000. Adelaide (Australia): Adelaide University; 2001.

Chapter in a book:

Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113.

Conference paper:

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Dissertation:

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Other published material

Newspaper article:

Tynan T. Medical improvements lower homicide rate: study sees drop in assault rate. The Washington Post. 2002 Aug 12;Sect. A:2 (col. 4).

Dictionary and similar references:

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

Electronic material

CD-ROM:

Anderson SC, Poulsen KB. Anderson's electronic atlas of hematology [CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

Audiovisual material:

Chason KW, Sallustio S. Hospital preparedness for bioterrorism [videocassette]. Secaucus (NJ): Network for Continuing Medical Education; 2002.

Journal article on the Internet:

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Monograph on the Internet:

Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/0309074029/html/.

Homepage/Web site:

Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

Part of a homepage/Web site:

American Medical Association [homepage on the Internet]. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2 screens]. Available from: http://www.ama-assn.org/ama/pub/category/1736.html

Database on the Internet:

Who's Certified [database on the Internet]. Evanston (IL): The American Board of Medical Specialists. c2000 - [cited 2001 Mar 8]. Available from: http://www.abms.org/newsearch.asp

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