UDK 611(082)

ISSN 1512-8245

AKADEMIJA NAUKA I UMJETNOSTI BOSNE I HERCEGOVINE
АКАДЕМИЈА НАУКА И УМЈЕТНОСТИ БОСНЕ И ХЕРЦЕГОВИНЕ
ACADEMY OF SCIENCES AND ARTS OF BOSNIA AND HERZEGOVINA

RADOVI

KNJIGA XCII

Odjeljenje medicinskih nauka

Knjiga 31

Centar za medicinska istraživanja

Knjiga 2

SARAJEVO 2003

UDK 611(082) ISSN 1512-8245

AKADEMIJA NAUKA I UMJETNOSTI BOSNE I HERCEGOVINE
АКАДЕМИЈА НАУКА И УМЈЕТНОСТИ БОСНЕ И ХЕРЦЕГОВИНЕ
ACADEMY OF SCIENCES AND ARTS OF BOSNIA AND HERZEGOVINA

RADOVI

KNJIGA XCII

Odjeljenje medicinskih nauka

Knjiga 31

Centar za medicinska istraživanja

Knjiga 2

SARAJEVO 2003

UDK 611(082)

ISSN 1512-8245

AKADEMIJA NAUKA I UMJETNOSTI BOSNE I HERCEGOVINE
АКАДЕМИЈА НАУКА И УМЈЕТНОСТИ БОСНЕ И ХЕРЦЕГОВИНЕ
ACADEMY OF SCIENCES AND ARTS OF BOSNIA AND HERZEGOVINA

WORKS

VOL. XCII

Department of Medical Sciences
Vol. 31
Centre of Medical Research
Vol. 2

SARAJEVO 2003

CONTENT

Works

Reports

Case report

Book Rewiew

WORKS

ANU
BiH
АНУ
БиХ

POPULATION GENETICS OF ABO BLOOD-GROUPS SYSTEM IN A SAMPLE OF PREGNANCIES WITH AN ELEVATED RISK OF FAILURE – FREQUENCY OF THE MATING TYPES.*

Ljubomir Berberović[1], *Amira Redžić*[2]

Abstract

Study concerning the possible impact of ABO antigens relations between parental and filial generations has been studied using population genetics analysis of mating system in the three samples of pregnancies(births), different with regard to the estimated «a priori» pregnancy risk. The evidence obtained supports the view that the ABO incompatibilities do not affect significantly the fertiliity of the mating pairs.

Introduction

There are many reasons why it is important to assess clinically, as objective as possible, the risk level of every individual given pregnancy. In order to quantify the risks there are many parametres, coming mainly from the personal and familial anamnesis. In the last few years attempts have been made to involve the data regarding the genetics of the local populations in the evaluation of the individual pregnancies risk. An attempt was made to study different bioreproductive and hereditary traits of the population as possible elements in the procedures of risk quantitative assessment the individual pregnancies.

Materials and methods

A sample of 755 pregnant women, who applied for genetic counseling in the period 1989-1992, or have been diagnosed as pregnancies at risk in the University Clinical Center of Sarajevo, have been studied (sample A). Two subsamples have been observed

---

* This work was registered for presentation at the Congress of Yugoslav Anthropologists, Tivat (Montenegro), 2002. The authors wish to thank the authorities of the University Medical Centre Sarajevo, for their collegial cooperation.

[1] Faculty of Science, University of Sarajevo.

[2] Faculty of Medicine, University of Sarajevo

separately, according to the clinical «a priori» genetical risk level estimate: subsample A1 - pregnancies at high risk (amniocentesis ordered), and subsample A2 – pregnancies at lower risk (amniocentesis not ordered). A group of 265 cases of disputed paternity, treated at the courts of Bosnia and Hercegovina (Bućić 1966), has been taken as the control group representing a random sample of the general population. All the individuals comprised in the (sub)samples have been tested with regard to their belonging to one of the four basic phenotypes of the ABO blood-groups system (A, B, AB and O). The composition of the material used for the study is shown in table 1.

Starting from the previously established basic population genetic parametres (Berberović & Redžić 1996), this study involves computing of the mating types frequency, and the elementary statistical comparison techniques (chi-square).

Table 1. *General properties of the studied sample*

			(SUB)SAMPLE SIZE (N)
PREGNANCIES INDICATED FOR GENETIC CONSULTATION	SAMPLE A	SUBSAMPLE A1	409
		SUBSAMPLE A2	346
GENERAL POPULATION SAMPLE (BUĆIĆ 1966; "CONTROL GROUP")			265

Results

Basic population genetics parametres in the studied samples (frequencies of genes and genotypes, and the significance of the differences between them) have been published elsewhere (Berberović& Redžić 1996). No significant differences among the (sub)samples have been revealed in this respect.

In the present study the same samples have been studied with regard to the mating system patterns concerning basic ABO blood groups. The absolute and relative frequencies of the mating types are presented in the tables 2 and 3. A corporate view of the presented data point to the conclusion that there are no significant differences between the observed and expected mating pairs frequency in the studied samples (table 4).

It can be seen that the clinically estimated risk of failure varies prominently between the observed groups of pregnancies, which is reflected in the different clinical treatment prescribed for the inidividual

pregnancies (see table 1). The observed three samples of mating pairs exibit an obvious gradation in this respect (A_1, A_2, and Bućić's sample – starting from the highest risk group).

Table 2. *Frequency of mating types*

	FREQUENCY		
MATING TYPES	*A1 SUBSAMPLE*	*A2 SUBSAMPLE*	*BUĆIĆ 1966*
A × A	62	54	41
A × B	64	54	39
A × AB	19	26	23
A × O	133	112	73
B × B	12	9	11
B × AB	13	17	7
B × O	45	22	24
AB × AB	4	6	2
AB × O	21	16	9
O × O	36	30	36
TOTAL	**409**	**346**	**265**

Table 3. *Proportions of mating types*

TYPE OF MATING	*SUBSAMPLE A1*	*SUBSAMPLE A2*	*BUĆIĆ 1966*
A × A	0.15	0.16	0.15
A × B	0.16	0.16	0.15
A × AB	0.05	0.07	0.09
A × O	0.32	0.32	0.27
B × B	0.03	0.03	0.04
B × AB	0.03	0.05	0.03
B × O	0.11	0.06	0.09
AB × AB	0.01	0.02	0.01
AB × O	0.05	0.05	0.03
O × O	0.09	0.08	0.14

Table 4. χ^2 *test*

DISTRIBUTIONS (%) COMPARED			χ^2	*p*
OBSERVED		*EXPECTED*		
OUR SAMPLE	A1	BUĆIĆ 1966	6.59	> 0.50
	A2		7.12	> 0.50

Discussion and conclusions

There is a long lasting dispute about the consequences of the ABO blood groups mother-child incompatibility as reflected in the genetic aspects of the reproduction processes (e. g. mating pairs frequency) in the populations. While some researchers affirm that there is evidence for these reflections (Matsunaga & Itoh 1958, Matsunaga et al. 1962), others reached the opposite conclusions (though with slight reserves; Reed 1956, Reed & Ahronheim 1959). Despite the well known fact that the clinical manifestations of the newborn haemolythic anaemia due to ABO incompatibility are generally mild and have not an important clinical significance (Erak 1970), one could not exclude the possibility that the ABO blood groups relationships still play a role in the complex mother-child immunological interactions (Thompson et al. 1991). Our results could be interpreted as a contribution to the evidence supporting the formerly mentioned negative views expressed in the literature.

A simple conclusion seems to appear from the evidence drawn from the study of the corporate data on mating pairs frequency in the three observed samples, i. e. – the inidividual adherence to the different ABO blood groups have no impact to the chances of success of a given pregnancy.

Apstrakt

Posljednjih nekoliko godina poduzima se analiza bioreprodukcijskih i populaciono – genetičkih pokazatelja u uzorcima trudnica, s ciljem utvrđivanja pokazatelja povišenog rizika za neuspjeh trudnoće. Ranije su utvrđeni osnovni populaciono-genetički parametri u posmatranim grupama (Berberović & Redžić 1996).

Raspoloživi podaci omogućavaju pristup nešto detaljnijoj populaciono-genetičkoj analizi, s obzirom na fenotipski sistem osnovnih ABO krvnih grupa. Posmatrani uzorak je formiran od 755 slučajeva trudnica koje su na Ginekološko-akušerskoj klinici u Sarajevu zatražile genetičku konsultaciju; radi se, dakle, o trudnoćama koje su iz različitih razloga unaprijed procijenjene kao povišeno rizične (Redžić 1996). Za 409 pripadnica ove grupe je ordinirana amniocenteza (poduzorak A1), dok kod 346 njih (poduzorak A2) nije bilo

indikacija za amniocentezu. Oba poduzorka obuhvataju pretežno žene sa područja sarajevskog regiona.

Bioreprodukcijski i populaciono-genetički pokazatelji praćeni su i u uzorku trudnica koji obuhvata slučajeve spornog očinstva (Bućić 1966), dakle, trudnoća bez primijećenog ili registrovanog genetičkog rizika.

Utvrđena je frekvencija pojedinih tipova parenja u proučenim uzorcima, kao i komparabilni podaci izvedeni računom iz materijala drugih izvora. Komparacijom je utvrđeno da nema statistički značajnih razlika, iako se radi o uzorcima čija je priroda u velikoj mjeri međusobno različita. Odstupanja u frekvenciji pojedinih tipova parenja nisu signifikantno različita u praćenim (specifičnim) uzorcima stanovništva Sarajeva u odnosu na populaciono genetičke podatke za širu populaciju Sarajeva i BiH.

Literature

Berberović Lj. & Redžić A. (1996). *Krvne grupe ABO sistema u uzorku trudnica sa indikacijama za genetičko savjetovanje.* Radovi ANUBiH (Odjeljenje prirodnih i matematičkih nauka), 26:119.

Bućić (1966). *Biološke odlike krvi u dokazivanju očinstva kod nas.* Medicinski arhiv, 20:9.

Erak P. (1970). *Hemolitička bolest novorođenčadi.* Medicinska enciklopedija JLZ, sv., str. 605-607. JLZ, Zagreb.

Matsunaga S., Hiriaizumi Y., Izumiyama H. & Furusho T. (1962). *Studies on selection in ABO blood groups.* National Institute of Genetics, Annual Reports, 13:103.

Matsunaga S. & Itoh S. (1958). *Blood groups and fertility in a Japanese population, with special reference to intrauterine selection due to maternalfoetal incompatibility.* Annals of Human Genetics, 22:111.

Redžić A. (1996). *Rezultati i rizik amniocenteze (analiza 409 slučajeva genetičkog savjetovanja).* Radovi ANUBiH (Odjeljenje prirodnih i matematičkih nauka), 26:125.

Reed T. E. (1956). *Tests of models representing selection in mother-child data of ABO blood groups.* American Journal of Human Genetics, 8:257.

Reed T. E. & Ahronheim J. H. (1959). *An association between ABO blood groups and fertility in a normal American population.* Nature, 184:611.

Thompson M. W., McInnes R. R. & Willard H. F. (1991). *Genetics in Medicine (Fifth edition).* W. B. Saunders Company, Philadelphia-London-Toronto-Montreal-Sydney-Tokio.

ANU
BiH
АНУ
БиХ

ANTI-INFLAMMATORY ACTIVITY OF PLANTS POTENTILLA SPECIOSA VILLD AND POTENTILLA TOMMASINIANA FW. SCHULTZ., ROSACEAE

Jela Grujić-Vasić[*1], *Saša Pilipović*[2], *Irfan Zulić*[3], *Mirjana Mijanović*[3], *Sulejman Redžić*[4]

Abstract

Anti-inflammatory activity of acetone extract of plant root sorts *Potentilla speciosa Villd.* and *Potentilla tommasiniana FW.Schultz, Rosaceae* was examined. The examined material was picked up in autumn in the surroundings of Sarajevo, dried in thin layer and pulverized immediately before the experiment. Swiss albino mice were used as experimental animals. The examinations were performed on mouse ear in groups as presented in the Table 1. As comparing substance 1% hydrocortisone cream was used. The other ear of the same animal was used as control one. It is found that acetone does not influence the process of inflammation. The achieved results are presented by changes in ear appearance after three days from the moment of examined extracts application. The treated ear looked significantly better than untreated ear. The examined mice groups and used substances are presented in Table 1.

This method of local anti-inflammatory activity examination on mouse ear is very suitable for examination because it gives data even for small sample quantities.

Examined acetone extracts of plant sorts *Potentilla speciosa Villd.* and *Potentilla tommasiniana FW.Schultz, Rosaceae* showed to possess anti-inflammatory activity, and the achieved results can be objectively shown by photographs of the examined samples. Comparing the achieved results we can come to the conclusion that acetone extract of the plant root *Potentilla speciosa Villd.* Showed stronger anti-inflammatory activity than the extract of plant root *Potentilla tommasiniana FW.Schultz, Rosaceae.*

* *This work was performed in the frame of scientific – research project Phytopharmacs for 21st Century- Theoretical and Practical Aspects, Federal Ministry of Science, Culture and Sport.*

[1] Academy of Scienece and Arta of Bosnia and Herzegovina
[2] Institute for Quality Control of Meidicines FBiH, Sarajevo, B&H
[3] Pharmacology Institute of the Faculty of Medicine in Sarajevo, B&H
[4] Faculty of Science and Mathematics, Sarajevo, B&H

Introduction

Potentilla plant species are numerous. Over five hundred species of *Potentilla* are known (1), from which 20 are listed in one or more pharmacopoeia (2). Having in mind the necessity of clinical examinations of pharmacological drug and herbal medical products activity, as well as constantly growing consumption of drugs of plant origin, we performed preliminary examinations of the root activities in plant species *Potentilla speciosa Villd. and Potentilla tommasiniana FW. Schultz* on the model of a mouse ear. We hope the results of these preliminary examinations will justify further researches in this direction not only for these two plants but of other plant species as well.

We performed these examinations on Swiss albino mice; inflammation was induced by 3% acetone solution Oleum crotonis using slight changes in legation to the quoted literature (3).

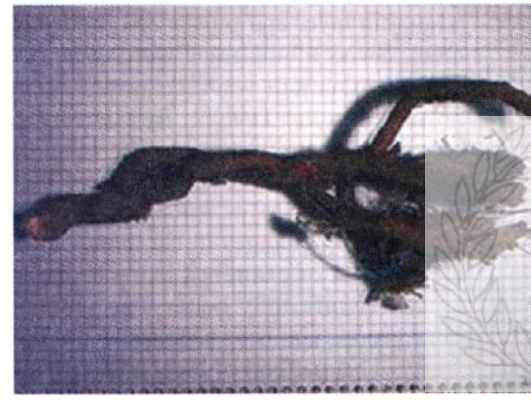


Figure 1: *Potentilla speciosa* Villd.

Figure 2: *Potentilla tommasiniana* FW. Schultz

Material and methods

Newly gathered material – root was cleaned, washed cut and dried. Drying was done in thin layer protected from direct sunlight. Immediately before examinations the root was pulverized and sieved through the sieve of 0,75 mm. The acetone extract was prepared from pulverized drugs (1:1). Extraction was done at room temperature in the course of 24 hours, mixing every 2 hours. Swiss albino mice were used for the examination, brood from the Pharmacology Institute of the Faculty of Medicine in Sarajevo, mass 28±3 grams. In order to induce local inflammation, both mouse ears were smeared by 3% Oleum crotonis solution in acetone, quantity of 10 μL (3, 4 and 5).

Drug extract in quantity of 10 μL was applied only on one ear once (cured L) 2 hours after the beginning of inflammation, while the other ear was used as a control sample (uncured N). 1% hydrocortisone cream was used as comparative substance and it was applied in the quantity of 10 mg (group 2).

Used substances

Acetone BP 1988 Se 64 355 01 Lex Potroroz
Croton oil Sigma C 6719
Hydrocortisone 1% cream (Hydroderm) Splabo, Heist Belgium Se 000 11 OZ/10 200H
Standard extracts prepared in ratio 1:1 acetone solution of dried pulverized plant root sort *Potentilla speciosa and Potentilla tommasiniana*

Identification of anti-inflammatory activity

Mice were divided into groups of three experimental animals and for each mouse in a group 10nL 3% solution Oleum crotonis was applied on the outer side of both ears. After two hours drug extracts were applied once on one (cured) ear (L) in the quantity of 10 μL, then hydrocortisone cream on the ear of comparative group. One group of mice was treated only with acetone on one ear while the other ear was not treated at all in order to examine the influence at the solvent to inflammation process.

There were four groups of mice examined all together, and examinations were repeated.

Identification of anti-inflammatory activity effect was performed by observing changes on mouse ear in the course of seven days (6) and by comparing L and N of the ear. The degree of inflammation is expressed in scores from 0 to 14.

Results

Table 1: *Survey of substances application on examined mice groups**

Group No.	Mouse mark	Mass	L ear 2 hours after Oleum crotonis application
1	1	27 g	Acetonum
	2	28 g	Acetonum
	3	30	Acetonum
2	1	31 g	Hydrocortisone cream 1%
	2	31 g	Hydrocortisone cream 1%
	3	28 g	Hydrocortisone cream 1%
3	1	25 g	Extract Potentilla speciosa
	2	28g	*Extract Potentilla speciosa*
	3	26 g	*Extract Potentilla speciosa*
4	1	27 g	*Extract Potentilla tommasiniana*
	2	26 g	*Extract Potentilla tommasiniana*
	3	29 g	*Extract Potentilla tommasiniana*

*On both ears 10 μL 3% acetone Oleum crotonis solution was applied

Antiinflammatory activity of examined substances

Table 2: *Numerical expressions of mouse ear reaction in the observed period*

Group / Mouse sign	After 24 h		After 48 h		After 72 h		After 7 days	
	L	N	L	N	L	N	L	N
1/1	10	10	12	12	12	14	14	14
1/2	12	10	12	12	14	14	14	14
1/3	12	12	12	12	14	14	14	14
2/1	8	12	8	14	8	14	10	14
2/2	6	10	8	14	8	14	10	14
2/3	8	10	8	14	10	14	10	14
3/1	8	10	8	12	8	12	10	14
3/2	6	12	8	14	10	14	10	14
3/3	8	10	10	12	10	14	12	14
4/1	8	10	10	12	10	14	10	14
4/2	8	10	10	12	10	14	12	14
4/3	8	12	8	14	10	14	12	14

Group 1

Picture 3: *ear appearance 24 hours after croton oil application*

Group 2

Picture 4: *24 hours after hydrocortizone*

Picture 5: *ear appearance 7 days application after hydrocortizone application*

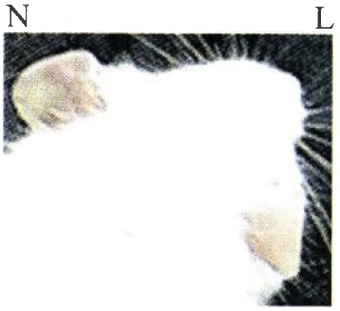
Group 3

Picture 6: *24 hours after application*

Picture 7: *4 hours after extact application*

Picture 8: *7 days after extact application*

Group 4



Picture 9: *24 hours after application*

Picture 10: *7 days after application*

Pictures are shot by Agfa CL 64

Results discusion

Examinations performed after 4, 24, 48 and 72 hours show that the changes on the ear treated by root extract of *Potentilla speciosa Villd.* and *Potentilla tommasiniana FW.Schultz., Rosaceae* are clearly shown for its prevention of inflammation.

Group 1 is the group in which one ear was examined for the solvent influence to inflammatory process; the influence of the solvent to inflammation was not noticed.

Group 2 in which 1% hydrocortisone cream was used. The treated ear showed significant difference in observed appearance parameters, so that the treated ear is less red, transparent, without dark edge, with regular ear rims.

Group 3 the treated ear is apparently thinner, smaller in size, transparent, blood vessels poorly expressed, ear edge pale, while untreated ear has visible suffusions, ear rims darker, 1/3 of ear missing after 7 days. The edge has torn rims, markedly damaged with present bloody dried layer.

Group 4 the ear treated by the extract *Potentilla tommasiniana* has clear blood vessels, the ear remains transparent, and the edge complete. Untreated ear is visibly thicker, turbid with bloody suffusions, and after 7 days big erosions of ear can be noticed.

It can be seen from the above listed facts that this method can be acceptable in the cases when the quantity of examined material in experiments is small. The achieved results are reproducible and the pictures represent objective documentation.

Conclusion

Methods of inflammation and irritation with 3% Oleum crotonis in acetone on mouse ear are simple, quick and reproducible method. The development of the inflammation by 3% acetone solution Oleum crotonis, of mouse ear ranges from 1 hour to 3 days and can be visually followed.

The best application time of anti-inflammatory substance of examined root extracts of *Potentilla speciosa Villd.* and *Potentilla tommasiniana FW.Schultz, Rosaceae* is between 1 and 6 hours after application of solution Oleum crotonis that the inflammation was provoked by.

Very small sample quantities are sufficient for such examination, from 5 to 10 μL of solvent; Acetone extracts of the plant root *Potentilla speciosa Villd.* and *Potentilla tommasiniana FW.Schultz, Rosaceae* visibly reduced inflammation during activity of 24 hours for 30-50% compared with the control ear.

Based on the achieved results we can make a conclusion that acetone extracts of plant roots of *Potentilla speciosa Villd.* and *Potentilla tommasiniana FW.Schultz, Rosaceae* have anti-inflammatory activity. According to observed parameters acetone extract *Potentilla speciosa Villd.* has more expressed anti-inflammatory activity in relation to acetone extract of plant root *Potentilla tommasiniana FW.Schultz, Rosaceae.*

The advantage of this examination method on experimental animals is also shown in the fact that experimental animals don't need to be sacrificed in the process of examination.

Apstrakt

Ispitivana je antiinflamatorna aktivnost acetonskog ekstrakta korijena biljnih vrsta *Potentilla speciosa Villd.* i Potentilla *tommasiniana FW.Schultz..*, *Rosaceae*. Ispitivani biljni materijal ubiran je u jesen u okolini Sarajeva, osušen u tankom sloji i pulveriziran neposredno prije eksperimenta. Kao ogledne životinje korišteni su švajcarski odrasli albino miševi. Ispitivanja su vršena na uhu miša u grupama kako je prikazano u tabeli 1. Kao uporedna supstanca korištena je 1% hidrokortizonska krema. Drugo uho iste životinje korišteno je kao kontrolno. Utvrđeno je da aceton ne utječe na proces inflamacije. Dobiveni rezultati predstavljeni su promjenama u izgledu uha, nakon tri dana od momenta aplikacije ispitivanih ekstrakata. Liječeno uho je izgledalo znatno bolje od neliječenog. Ispitivane grupe miševa i korištene supstance predstavljene su u tabeli 1. Ova metoda ispitivanja lokalnog antiinflamatornog djelovanja na uhu miša je vrlo pogodna za ispitivanje, jer daje podatke i za male količina uzorka.

Ispitivani acetonski ekstrakti biljnih vrsta *Potentilla speciosa Villd.* i *Potentilla tommasiniana FW.Schultz.*, *Rosaceae* pokazali su da posjeduju antiinflamatorno djelovanje, a dobiveni rezultati mogu biti objektivno prikazati i fotografskim snimcima ispitivanih uzoraka. Uporedenjem dobivenih rezultata može se zaključiti da je acetonski ekstrakt korijena biljke *Potentilla speciosa Villd.* pokazao jače antiinflamatorno djelovanje od ekstrakta korjena biljke *Potentilla tommasiniana FW.Schultz.*

Ključne riječi: *Potentilla, protuupalno djelovanje, ekstrakt, uho miaa*

Literature

Evans C.E: *Trease and Evans' Pharmacognosy,* 13nd ed. London: W.B. Saunders Company Ltd.; 1989. p. 40

Penso G.: *Index plantarum medicinalium totius mundi, eorumque synonimorum,* ed.O.E.M.F.-Milano;1982.p. 773-774

Williamso, E.M., Okpako D.T. and Evans F.J,: *Selection, preparation and pharmacological evaluation of Plant material* in: Pharmacological Methods in phytotherapy research: London: ed. John Wiley&Sons; June 1996. p. 131-153

Cristop A.K. et all: *Studies of Anti-inflammatory Activity of Phytopharmaceuticals prepared from Arnica flowers;* Plan.Med 2002;68: 385-391

Perez Castorena A. L, Arciniegas A., Ramirez A.T., Villasenor L. J.and Romo de Vivar A.: *Evaluation of antiinflamatory and antioxydant activities of the plastoquinone derivates isolated from Rolanda barba- johannis;* Plan.Med 2002;68: 645-647

A.Wallace Hayes: *Principles and Methods of Toksicology;* ed.Raven press, New York; 1984. p. 212

BONE DEFECTS OF THE SCULL: APPEARANCE, INDICATIONS FOR REPAIR, AND ANALYSIS OF RESULTS

Faruk Konjhodžić[1]

Abstract

Author presents results of 4.236 consecutive cases of scull bone defects during thirty years period, starting April lst, 1970 to April lst, 2000. These bone defects, representing 2,8% of total operative pathology seen at Department of Neurosurgery, University Medical Center of Sarajevo, were mainly the result of following operative procedures: reduction of impressive (depressive) fracture or osteoclastic trepanation performed in oder to enable interventions on inner structures of the head. In majority of cases, defects were repaired by alotransplantation (polyacrilamid) although vast number of impressive fracutres were treated by plastic surgery with bone chips of Homologous bone. In this paper autror presents different plastic procedures and cites the number of treated cases.
Key words: *scull bone defects-repairing-bone chipspolyacrilamid-alotransplanttion-autotransplantation.*

Introduction and material

In thirty years, from April lst, 1970 to April lst 2000, Department of Neurosurgery, University Clinical Center, Sarajevo, treated 4.236 consecutive bone defects of scull, which amounted to 2,8% of ist total operative pathology. Majority of edefcts was of iatropic origin as a result of strenuous operative procedures by surgeon: either during reduction of depressed fracture or by osteoclastic trepanation performed in order to approach inner structures of the head (see Table l.)

[1] Academy od Sciences and Arts of Bosnia.and Herzegovina

Table 1. *Origin of the bone defects of the scull*

	Number of cases	percentage
Operation of depressed fracture	2223	52
Osteoclastic trepanation	1998	47
Congenital defect	1	0,023
Other and unknown	14	0,33

Craniectomy is the best and safest way to approach intracranial structures, specially in depressive fractures, but it is performed rutiniously only during Aggression on Bosnia and Herzegovina, because we had a lot of injuries and it was according to treatment doctrine. But all the time the question was aroused what one could have done with defects after the reduction of depressed fracture.

In these operations neurosurgeon was faced with several options. One could have performed repairement with bone chips (plastic with homologous autograft), using material from the bone which surgeon would have to remove. It is the best way probably: healing is good and in case of graft mixture with antibiotics they become very resistent to different infections which is of major benefit seeing that one of the biggest complications is a possible infection. This method we used befor Aggression on Bosnia in all compliated and non-complicated depressed fractures. This method we performed immediately during the operation of depressed fracture, and the patient did not need a second operation. Our patients disliked the second operation of repairment of defects and all patients disliked the material used from crissta ilica. Removal of the bone from crista ilica is even more painful than the operation of the head itself. In some cases the procedure included six hours period arrival of the fracture with delayed plastic with heterogous homo and heterotransplantation (Kiels bone). All others were parformed using alotransplantation (polyacrilamid mass). See Table 2.

It is of importance to mention techniques not often performed in our seria. These were the techniques performed at Department of Plastic Surgery: plastic with unique lamina of calvarious bone, plastic with the rib, reduction of depressed fracture and fixation of fragments with wire, reduction of depressed facture with Kiels bone (sterilized and deeply frozen bone of the calf). Concerning the last one, our experience is scarse since it was performed only once but with good operative outcome. Rare case was plastic procedure with unique lamina from calvaria: removal of normal bone, separation of lamina and fixation of both parts on newly established defect and existing bone defect. Reduction of depressed fractures exclusively or with a fixation of fragment with wire were rarely performed. Operator made bur-holes, lifting the whole bone with depressed fracture and scrubing nurse by the

use of hammer thus reducing the bone. Following the suspension of dura, operator places whole bone on its place. Plastic with rib was performed by fixation of fragments following the removal of twentieth rib done under same anesthesia. Operation was disliked by our patients thus representing main reason of our scarse performance of latter.

Table 2. *Kinds of the operations*

	Number of cases	percentage
Bone chips of homologous bone (autograft)	2358	55,66
Bone chips of heterologous bone (autograft)	91	2,19
Plastic with a lamina of calvarious bone (autograft)	18	0,42
Plastic with rib (auograft)	5	0,11
Reduction of depressed fracture and fixation with a wire	9	0.21
Reduction of depressed fracture only	4	0,09
Plastic with Kiel s bone (heterograft)	1	0,02
Polimerized isoacrilic acid	1748	41,26
Total	4236	approx. 100,00

In case of delayed plastic, method used was the same used in peaceful conditions. For this reason we used alotransplantation mixing the sterilized isomers and polymers of isoacrilic acid during the operation. First of all, we prepared the defect, and after that we put acrilic mass on it, trying to adapt the mass on defect till it was soft. As soon as the mass hardened, salin was used to lower the temperature liberated. But bad condition aroused when the alotransplantation came in contact with air sinuses because of the infection. Skin above the alotransplantat must be in good condition, with good vascularisation, and sutures without a tension. This transplant could be in function without any possibilities of infection. Otherwise it is very good, it funcioned properly and it could be done in course of the same operation with removing the osseous tumors.

This is full indication for filling the defect with bone chips made from homologous and heterologous bone in closed injuries of the head in course of same anesthesia. And in open injuries of the head if the skin is good and the patients arrives within 6 hours to our Emergency Department. It is also indicated to perform the second operation for filling the defect in every case. Poor conditions in use of bone chips aroused because of the time patient had to wait till osseous graft hardened. In performing the second operation, we have to consider the conditions and the pain patient has to underwent taking into

consideration painful use of crista ilica. Also, we have to consider a new scar which is of big importance to some occupations.

Use of polyacrilamid is very useful. It is good for the patient and shortens the time of operation itself. It is the easiest method for the patient, because there is no new scar on the post where we took the graft, it closes the defect sufficiently, it hardens in a very short time, and patient could perform any activity, even shoot ball. And it is cheap.

Complications

Concerning indications for different plastic procedures, the number of complications are pretty small: infection of bone ships and polyacrilamid and uncoverage of the graft without visible infection (UGWVI) during the use of polyacrilamid. Treating complication is different and it depends on situation, sometimes we performed the drainage only, but sometimes we had to remove the graft. Use of polyacrilamid is vast and offers various possibilities following the removal of the graft.

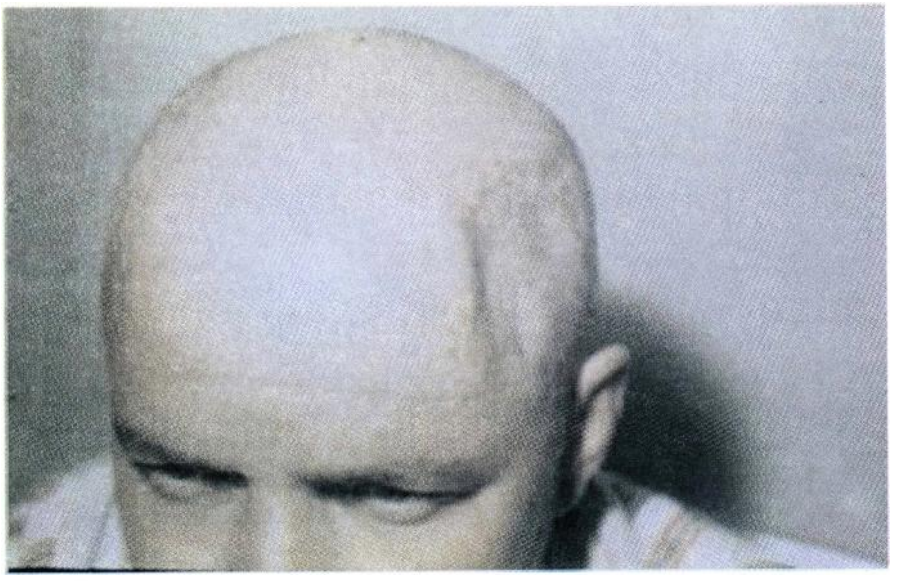
During the filling the defect we used bone chips and always we used it in closed injuries, immediately in same operation if the skin is good and within period od six hours after injury. Finally we used it in second operation if the defect was in connection with air sinus because it is very hard against infestion. In these situations we did not have any complications.

Use of polyacrilamid is a method of choice, but has its limits in aneurysm and tumor when it is a case of first and second operations following osteoclastic trepanation. We always thought that graft in this situation is not in contact with air sinuses, and that skin above is very good. These facts we considered crucial.

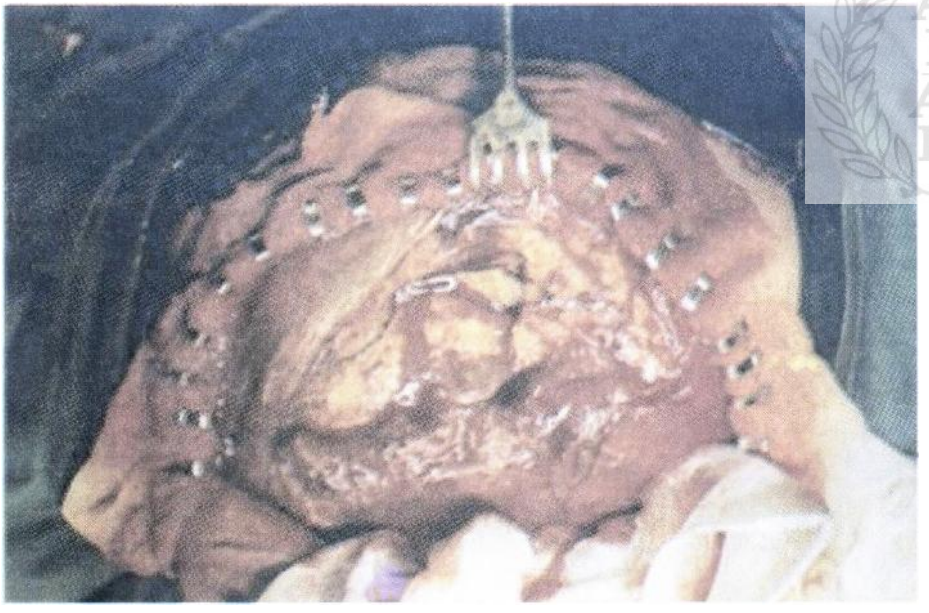
Plastic with bone chips leaves minor cosmetic defects and is more functional in patients with lot of hair. Also bone could be put in bone bank for second operation.

Conclusions

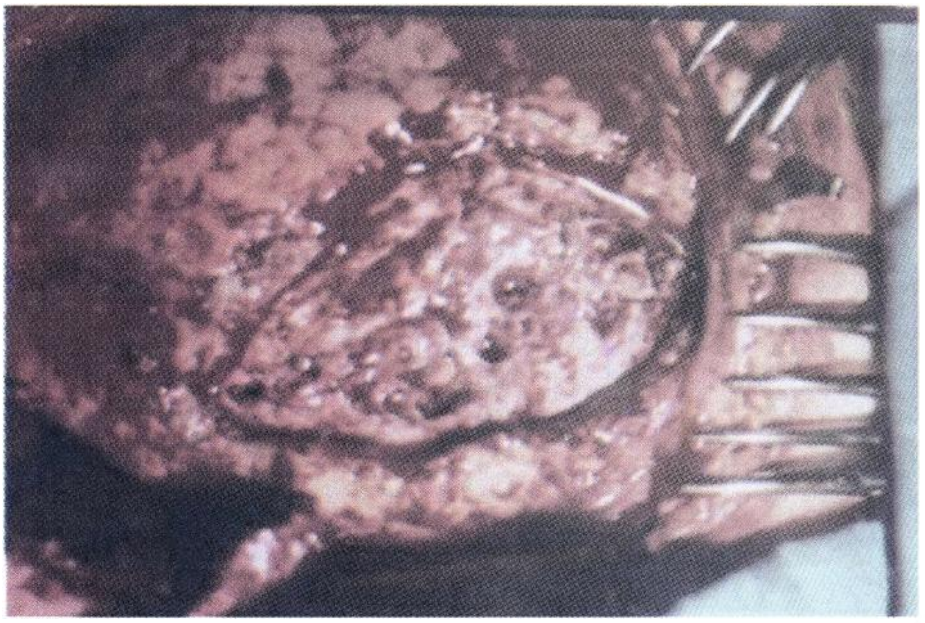
1. Plastic with bone chips and plastic with polyacrilamid are excellent methods for covering the defect of the scull. Complications are rear, if surgeon follows indications.
2. Plastic with bone chips could end with minor cosmtic defects and is more suitable in patients with lot of hair.
3. In all closed injuries of the head, and in second operation one could perform plastic with bone chips.
4. Material for acrylamid is very cheap and is always available.
5. In case one follows indications there are no complications.



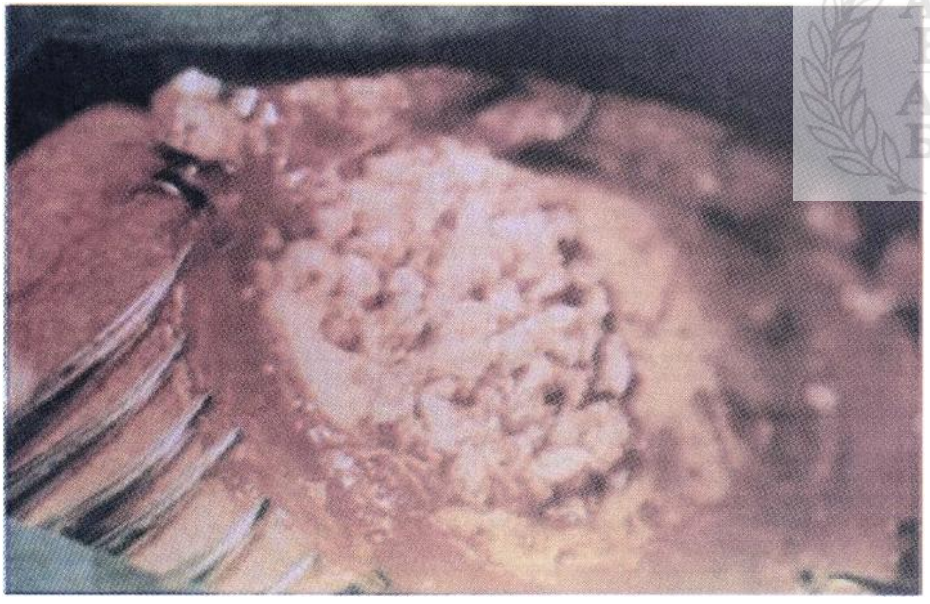
Defect of the scull visible clinically, after depressive fracture



Fresh depressive fracture on operation.

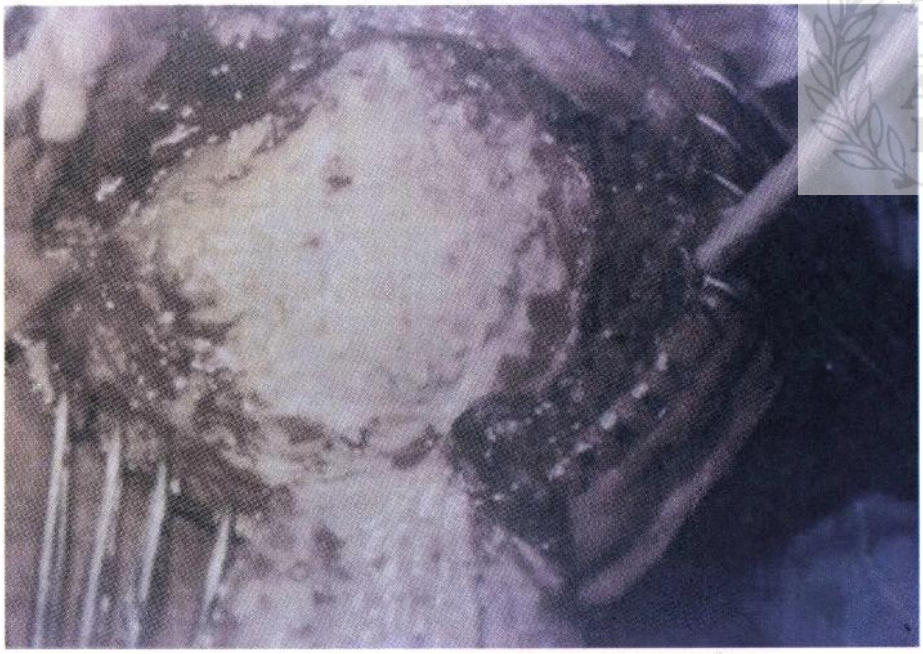


Prepared defect if the scull is visible during operation.

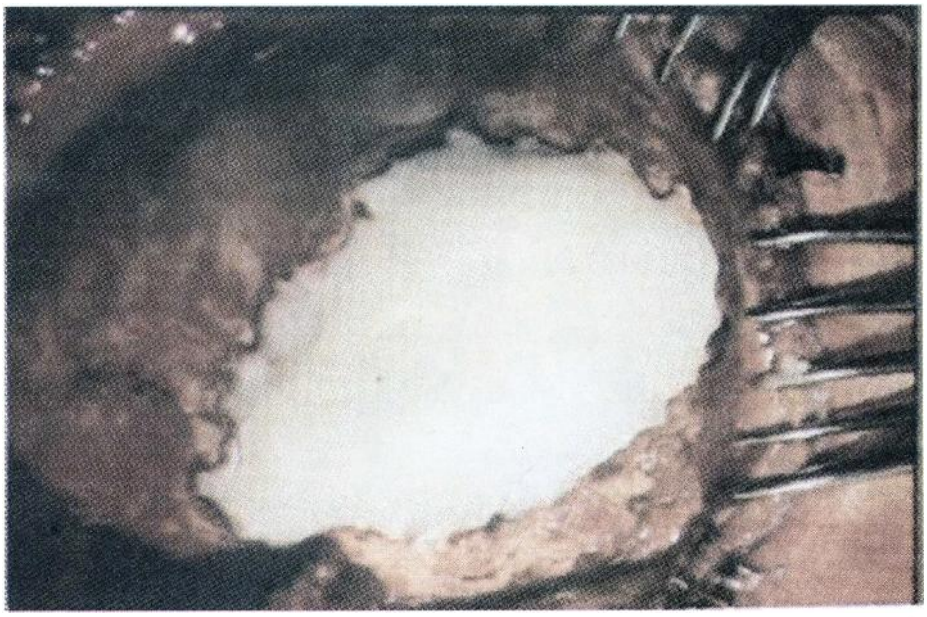


Filling the bone chips, immediately after reduction of depressed fracture.

Plastic with polyacrilamid and its fixation.



Growing the tissue throughout polyacrilamid holes, and in that situation we need no fixation.



Plastic with alograft.

Radiology post operational.

Apstrakt

Autor iznosi rezultate 4.236 uzastopnih defekata na koštanom dijelu skalpa u toku tridesetgodišnjeg perioda, počevši od prvog aprila 1970. do istog datuma 2000. godine. Ovi defekti su nastali uglavnom operativnim zahvatom, redukcijom impresivne (depresive) frakture ili osteoklastičnom trepanacijom da bi se omogućio rad na unutrašnjim strukturama glave i čine oko 2,8% od ukupne patologije na Klinici za neurokirurgiju Kliničkog centra Univerziteta u Sarajevu. U velikome broju slučajeva defekt je riješen plastikom sa alotransplantatom (poloakrilamidom) mada su, posebno kod impresivnih fraktura bile veoma zastupljene i plastike sa isitnjenim koštanim autotransplantatom (bone chips) homolognoga tkiva. U ovome radu autor iznosi indikacije za vrstu plastike i broj tretiranih slučajeva.

Ključne riječi: *defekti koštanog dijela skalpa-plastike-isitnjeni koštani transplantati-ploakrilamid-alotransplantacija-autotransplantacija*

References

Konjhodžić F.: *Neurosurgical Injuries in Defensive war of Bosnia and Herzegovina,* Sarajevo : OKO 1995.

ANU
BiH
АНУ
БиХ

REFORM OF MENTAL HEALTH SERVICES IN THE FEDERATION OF BOSNIA AND HERZEGOVINA

Slobodan Loga[1], *Ismet Cerić*[2]

Abstract

Pre-war psychiatric services in B&H were one of the best-organized in former Yugoslavia. The psychiatric care system was based on psychiatric hospitals and small neuropsychiatry wards within general hospitals, accompanied by psychiatric services in Community health center.

The war disaster brought demolition of numerous traditional psychiatric institutions. Moreover, psychiatric morbidity increased with massive psychological suffering of the whole civilian population. Already during the war, and even more so after the war, the reconstruction and reorganization of the mental health services was undertaken.

The basis of mental health care for the future is designed a system where majority of services are located in the community. The key role is assigned to primary health care (Family practitioners). Community based mental health centers (MHC) will be responsible for prevention and treatment of psychiatric disorders.

Chronic mental patients who are not able to live independently will be accommodated in designated homes and other forms of supporting living arrangement within the communities.

The principal change in mental health policy in B&H was a decision to transfer psychiatric services from traditional facilities into the community, much closer to patients.

Basic elements of the mental health policy in B&H are: decentralization and sectorisation of mental health services, intersectorial activity, comprehensiveness of services, equality in access and utilization of psychiatric service resources, nationwide accessibility of mental health services, continuity of services and care, together with the active participation of the community.

[1] Clinical of Psychiatry - Medical Faculty of Sarajevo and Correspodent Member of Academy of Sciences and Arts of Bosnia and Herzegovina

[2] Clinical of Psychiatry - Medical Faculty, Sarajevo

This paper will discuss the primary health as the basic component of the comprehensive mental health care in greater detail, including tasks for family medicine teams as well as for each individual member.
Key words: *mental health, reconstruction of health care system.*

Introduction

Before the war (1992-95), the psychiatric services in Bosnia and Herzegovina were relatively well developed and represented one of the best organized such services in the republics of the former Yugoslavia.

There were a significant number of qualified professionals from various professions working above all in numerous in-patients psychiatric and other relevant institutions wherein the treatment of the mentally ill was effective and did not differ much from the treatment of such patients in some other European countries.

The basis of the whole system of psychiatric services was psychiatric hospitals and small neuropsychiatric wards of general hospitals accompanied by specialized psychiatric services in community health centers.

In general, Bosnia and Herzegovina psychiatric services system as a whole was until April 1992 organized based on the following principles:

a. In the majority of community health centers, there were psychiatric services staffed by neuropsychiatrists and nurses with psychologists and social workers as consultants. The psychiatric services in community health centers were in their work very closely related to primary health care services and dealt with treatment of psychotic and non-psychotic disorders. Activities aimed at prevention were given but a little attention, whereas the role of the community in promotion of mental health was almost completely neglected.
b. Within general hospitals on the territory of Bosnia and Herzegovina, there was a trend of establishing of small neuropsychiatric wards that treated acute psychotic and other mental disorders within a certain short time period.
c. On the territory of Bosnia and Herzegovina, there were at the same time big psychiatric hospitals (Sokolac, Jagomir, Domanovići) and a psychiatric colony (Jakeš near Modriča) that comprised a classic psychiatric hospital for chronic hospitalized psychiatric patients, a very developed occupational and work therapy, and accommodation for patients with hetero-families in

village households in surrounding villages (Garevac, Jakeš). Each psychiatric hospital had the average of about 300 severely disturbed chronic patients, while Jakeš near Modriča had between 800 and 1000 clients in treatment.

d. The treatment of alcoholism and drug addiction was organized through the Institute for the Treatment of Alcoholism and Other Addictions and the Center for the Treatment of Drug Abuse in the Psychiatric Hospital in Sarajevo. The primary and tertiary prevention of alcoholism was performed within 120 clubs of treated alcoholics.

e. More severely mentally retarded persons were treated in special institutions within the system of social welfare, whereas less severe cases of mental retardation were treated and rehabilitated within their families and educated in numerous specialized schools.

According to the data of the Republic Institute for Public Health, there were on December 31, 1991 in the neuropsychiatric services in Bosnia and Herzegovina 237 specialists neuropsychiatrists, 56 residents in specialistic study, 100 employees with two-year post-secondary school qualifications (senior nurses), 896 employees with secondary school qualifications (nurses) and 36 employees with lower educational background, whereas the total number of beds in all in-patient facilities was 2822 (1).

The war and destruction of psychiatric services in Bosnia and Herzegovina

During the first months of catastrophic events brought about by the war turmoil in Bosnia and Herzegovina, devastation and destruction was aimed at all spheres of life, including the closing and destruction of psychiatric hospitals and services. Psychiatric hospitals such as Jagomir and Domanovići were closed. Severely ill chronic mental patients were expelled from Jakeš. Many patients that had until then spent up to 20 or more years in hospitals were suddenly left on their own without any support. Some of them went missing and were never found, others were killed or wounded. Not a long ago, in the settlement Koprivna close to the Psychiatric Hospital Modriča, the exhumation of the mass grave of patients from Jakeš and Garevac killed in the spring of 1992 was performed. Found in the mass grave on the bank of the River Bosnia were remains of 33 bodies, out of which there were 16 female bodies. At another location, six more bodies were exhumed, while yet another mass grave with remains of 45 bodies of the patients from the same hospital was located. Such an inhuman treatment of such a handicapped group of people is unprecedented in the newer European history (2).

The precise data on the true quantitative consequences of the war catastrophe in Bosnia and Herzegovina do not exist and will probably never be known, by means of using the data of all three parties in the conflict, as well as the data of international organizations and institutions, and their mutual comparison, it is possible to get the numbers that are not far away from the truth.

Let us here, without having any pretensions to and at the same time being unable to describe all consequences of the war and its far-reaching implications, mention only some of the key ones that are mostly of a local character:

- Demographic losses in Bosnia and Herzegovina (in terms of the numbers of the slaughtered, killed, exiled that did not return, those that emigrated, that were not born and that died of consequences of war) in the period until the end of the year 2001 amount to 1.4 million persons (living in Bosnia and Herzegovina today are 3.1 million people, whereas there would have been around 4.5 million people if it had not been for the war);
- During the war in Bosnia and Herzegovina, approximately 236,500 persons were slaughtered or killed, out of which there are around 164,000 Bosniacs (about 126,000 civilians and around 38,000 soldiers), about 31,000 Croats (around 17,000 civilians and 14,000 soldiers), around 27,500 Serbs (about 6500 civilians and about 21,000 soldiers) and around 14,000 others (about 9000 civilians and 5000 soldiers);
- During the war in Bosnia and Herzegovina, approximately 225,000 persons were wounded;
- Approximately three-fourths of the total population of Bosnia and Herzegovina experienced immense suffering and humiliation and above one half of the population was forced to leave their homes;
- During the war in Bosnia and Herzegovina, the material goods worth about 35 billion dollars were destroyed or plundered.
- The losses in Bosnia and Herzegovina in terms of unrealized income until the end of the year 2001 amounted to about 125 billion dollars, and are estimated to amount to the additional 220 billion dollars until the year 2020;
- In Bosnia and Herzegovina and its neighboring countries, there is on the one hand a huge retrogression in the field of legislation, education, science, technology and many other areas, and on the other hand a true collapse in morale and internal security system.

Although it may be possible to establish how many people were killed or permanently physically debilitated, there is no simple way to

estimate the prevalence of psychological disorders that will deeply imbue the present and future generations and influence their future lives.

The intensity and frequency of the stated war atrocities are far from all normal human experiences, which makes perfectly understandable and fully justified the symbolic estimate of the WHO according to which there are over one million people suffering from war stress related mental disorders only on the territory of the Federation of Bosnia and Herzegovina. The biological defense mechanisms of persons that survived psychological traumas are severely impaired.

The catastrophic war events brought about turbulent and devastating disorders in the general population, and their severe repercussions have affected the whole health care system, including the system of psychiatric services (3).

The consequences of war can, if simplified, be divided into two focus areas that are mutually intertwined, and these are:

- War-induced traumatization of a significant portion of the population, and
- War-induced effect on the traditional system of psychiatric services through destruction of mental health institutions, great deterioration of the quality of mental health protection due to the lack of material resources, decline in the number of available health care professionals, as well as the destruction of social and family network, which limited the possibilities of discharging of patients and their treatment both in psychiatric institutions and outside of them (4).

Reconstruction and reorganization of the psychiatric services after the war catastrophe

During the war in Bosnia and Herzegovina and especially after it ended, the work on reconstruction and reorganization of mental health services began. The new system of organization of psychiatric services is based on the following principles:

- The future basis of mental health protection is conceptualized as a system wherein large part of the services is in the community, as close as possible to the place where the patient lives. The key aspect of the health care system as a whole is the primary health care, and the main role is given to family medicine physicians and mental health professionals working in the community.
- Large psychiatric institutions are either closed and/or devastated, or suffered a significant reduction in their capacities during the war. There will be no reconstruction and reopening of the old psychiatric institutions, nor construction of new ones.

- The most integral part of the psychiatric system is represented by community based mental health centers. Each of the centers will serve a certain geographic area inhabited by a certain number of people. The centers will be responsible for prevention and treatment of psychological disorders, as well as for promotion of mental health as a whole.
- Chronic mentally ill patients that do not have families and for any given reason can not live on their own within the community, will be accommodated in special houses and other forms of supportive living arrangements in communities where they come from.

Today in Bosnia and Herzegovina, we are trying to move the psychiatric activities from hospitals to communities, as close as possible to the places where people live. By doing that, we are working on transferring the interest that was earlier focused on the illness itself, towards the personality of the patient and his/her social dysfunction resulting from the mental disorder.

In order to be able to realize this, we are organizing ourselves so that we can replace individual activities with collective ones, using the cross-disciplinary approach by means of which we can enable mobilization of values and potentials of the patient himself/herself and his/her family.

At the same time, we are investing our efforts in intensive education of the general population about mental health problems with a special emphasis on alleviating and eliminating of stigmas and prejudice existing in our society against mentally ill persons.

We are trying to assign great importance to activities and cooperation with non-professionals as well as users of mental health services whose organization into associations we encourage and support.

Our efforts are also aimed at establishing the real value of biological and psychological therapies since we are aware that they alone cannot resolve the complex problems following from mental illness.

All our activities are directed towards maintaining, that is achieving the best possible quality of life of our patients. Therein we are especially taking care of all the legal and administrative conditions that are to serve as a guarantee of human rights of the patients.

In order for any of the above stated goals and intentions to be realized, we are attempting to enable a broad cooperation of mental health services with other sectors, especially social services, educational institutions and other important institutions in the community.

Helped by the international community, mental health services are going through a very slow but steady process of recovery, and so on

a new basis. The network of community based mental health centers is physically established within the deadlines defined in 1996 in the contract signed by the Government of Bosnia and Herzegovina and the World Bank.

However, it should be pointed out that we are still rather far away from the realization of the original plans for the reconstruction of mental health services and establishment of the full function of mental health centers network on the territory of the Federation of Bosnia and Herzegovina (5).

Mental health policy in the Federation of Bosnia and Herzegovina

Perhaps the most important change in mental health policy in the 20th century was the decision to move mental health services from in-patient psychiatric institutions to the community.

Until 1960s a general consensus was reached that milieu of big psychiatric hospitals did not enable satisfactory treatment and rehabilitation of patients with mental disorders, whereas their life in such institutions was not worthy of human beings.

It is for this reason that the number of psychiatric hospitals in Europe with over 1000 beds was significantly reduced between 1972 and 1983, for example in Denmark and Ireland from 4 to 2, in Sweden from 10 to 4, in Spain from 14 to 0, in Italy from 55 to 20 and in England from 65 to 23. Ever since, the trend of shifting the care of people suffering from psychological disorders from psychiatric hospitals have gradually but constantly moved the care of such patients as close as possible to the places where they live, so that the last few decades showed a clear commitment to this choice in many western countries (6).

The term "deinstitutionalization" was used to define this trend. Saraceno believes that, instead of deinstitutionalization, the term "dehospitalization" should have been used so as to avoid many traps and controversies following the concept of deinstitutionalization.

The term deinstitutionalization in his opinion primarily means the process of ending the rule and utilization of institutional psychiatric logic manifested through various forms of institutionalization, like institutionalization of diagnostics, of social invalidity, of forced hospital admission and treatment by new or old ready-made methods used regardless of the needs of patient and the nature of his/her illness. If only discharge from hospitals and prevention, i.e. prohibition of admission to psychiatric hospitals is set as the main and only "objective" of deinstitutionalization, the psychiatry will not change but will go on "in the old way" with the only change being its becoming a unique decentralized institution without walls. It is therefore logical to

look at dehospitalization as a fragment and a beginning of an infinite process that will serve to both patients and psychiatrists as a guarantee of constant critical reexamination of treatment and research in mental health domain. The process of deinstitutionalization continues nowadays in a more rapid way, although it is no longer going on within the walls of hospitals, whereas the dehospitalization process has in many countries ended or is in the final phase (7).

There are numerous and diverse examples of dehospitalization and continued deinstitutionalization in European countries. Establishing of community based mental health services in different countries and even different regions of the same country is done in different ways depending on the existing circumstances.

One of such models of organization of psychiatric services is the Italian model. It is based on the specific legislation representing the legal basis and "framework" of the organization of psychiatric care and its full reform.

Following the recommendations of the WHO, the basis of our mental health policy is contained in decentralization-sectorization of psychiatric services, intersectoral cooperation, comprehensiveness of services, equality in exercising rights, availability of all forms of mental health services in community, continuity of provision of services and active participation of the community.

In the end, it needs to be stressed that we have all the necessary conditions in order to realize, within the given deadline and on the whole territory of Bosnia and Herzegovina, the overall health care policy of the WHO stated in the 6th objective pertaining to the promotion of mental health and reading:

Until the year 2020 the psychosocial wellbeing of peoples shall be improved by means of developing better comprehensive services available to all persons with mental health problems regardless of very unfavorable demographic, social and medical indicators in Bosnia and Herzegovina (8).

The basic elements of the mental health policy in the Federation of Bosnia and Herzegovina

Decentralization-sectorization of mental health services

The basic concept in planning of mental health services in Bosnia and Herzegovina today is establishing of regional or sectoral responsibility. Therefore it is necessary to determine the sphere of responsibility for services in mental health domain, as well as to design services for a given population in order to enable people to be appropriately treated at places close to their place of living, which best

meets the needs of the patient, his/her family and the community. Establishing of sectors (or spheres of responsibility) is the basis of the new organization of mental health services in our circumstances at present.

Given the political and administrative organization of the Federation of Bosnia and Herzegovina, it is not simple to establish a new territorial organization of psychiatric services. The area of the sector is defined, as a geographic area including social structures that satisfy physical, psychological and social needs of the majority of people in the community. We should herein stress that people in a sector live together in a form of social organization and cohesion. Members of a community share to different extent the political, economic, social and cultural characteristics, i.e. interests and aspirations, including those pertaining to health. The community is, therefore, represented by a group of individuals mutually related by common experience, philosophy, moral, social beliefs, opinions and prejudice. The community is defined in terms of space but its exact boundaries are often impossible to determine since they are most frequently set due to political or administrative reasons. The area of the community is defined as a sector, i.e. a geographic area containing social structures that satisfy physical, psychological and social needs of the majority of community members. Each community has necessary resources of critical importance, like human (professionals and experts, laypersons, volunteers, ill and people with disabilities and their families), physical (buildings, equipment, services, economic resources, private property, business and industrial resources, etc.) and structural resources (municipal and other government services, local club organizations, business and industrial organizations, religious communities, etc.) (9).

Intersectoral action

The practical policy of mental health services must be integrated in the general health care policy of the Federation of Bosnia and Herzegovina that is its 10 cantons/counties. Therefore it is necessary at the level of the Ministry of Health and the Government of the Federation of Bosnia and Herzegovina to revise the programs of a number of various sectors in order to ensure a more purposeful and different promotion of mental health. In such different system of the organization of mental health services a special role and importance belongs to the sector of social welfare and social aspect in general.

This is all the more so since it is in the present circumstances almost impossible to realize and work on the promotion of mental health, prevention of mental disorders and psychosocial rehabilitation of chronic mental patients without adequate social care and professional

services of social work that certainly bring a new dimension into medical treatment of the patient enriching it with a more human, different and specific approach.

Although the presence of social work in the area of health care, i.e. in various types of medical institutions in the world, has been there for almost nine decades, getting its confirmation in the everyday practice of social workers, (unlike the developed countries of Europe and the world) the social work practice has in our country not yet been given the due position and appropriate professional dignity. It therefore appears that the new approach to organization and reform of mental health services in the Federation of Bosnia and Herzegovina represents a realistic possibility for the social work profession to get a due place in our system, with the main goal being the patient's wellbeing through treatment, more rapid recovery and social and professional rehabilitation. This is all the more the case since the everyday reality imbued with dreadful consequences of the war (affecting not only the mental health of the population), absolute poverty and social distress, imposes a need for a special kind of help to the patient. The fact proved in practice that there are, in the area of mental health, an increasing number of patients that, in addition to psychological difficulties, suffer from threatening social circumstances, undoubtedly speaks for itself about the close connection between the medical condition of the patient and numerous unfavorable social aspects and problems surrounding him/her. This all implies the need to include all possible social elements in the treatment of such patients, from the family to a broader environment wherein the patient lives and spends his/her time.

Deeply present awareness of unfavorable economic and other resources of the society should in no way represent an obstacle in providing, at least in the beginning, a legislative basis with a view to giving the social sector and social work profession a necessary position of priority in the reform of mental health, following the example of the countries where this approach has already been established. The number of social workers in a mental health center should thus be proportional to the size of the population of the local community served by the center. Naturally, we are still far away from the established world standard of one social worker per 3,500 inhabitants. It is with this regard necessary to create possibilities for social workers within mental health services to do jobs of various degrees of complexity, from individual and group sociotherapy to management, organizational and leader jobs for example in mental health centers. Such approach implicitly includes additional efforts in permanent education and professional specialization of social workers in particular, but also of other professionals in multi-disciplinary teams, which at the same time imposes the need for necessary changes in socio-health care policy of

the society as a whole that itself represents a basic condition for the transformation of social work.

Comprehensiveness

The comprehensive mental health policy in the Federation of Bosnia and Herzegovina especially promotes the protection of mental health as a whole on the level of local communities in corresponding defined sectors without reduction of function of any psychiatric institution and/or service, and in-patient psychiatric institutions in particular. It is for this reason that there was after the war no, nor there will in future be any reconstruction and/or construction of big or small psychiatric hospitals. Protection of mental health as a whole will be carried on in the community with the help of teams of family medicine physicians and specialized multi-professional teams in mental health centers and psychiatric wards of general hospitals and in-patient wards of clinical institutions. Always encouraged therein is seeking of alternative solutions for hospitalization that will by no means represent breaking of connections with the family and community. All the services are a part of the comprehensive system of differentiated mental health services especially designed so that they can meet the needs of the general population, as well as special age and risk groups.

Equality

Inequality and stratification are the basic characteristics of a modern social life. The modern world is based on the productivity and competition and inequality is a powerful force that drives it by means of money thereby determining the way and quality of life and destiny of the individuals and groups. Income, prestige and education level are nowadays the three most frequent indicators of inequality and social position of people. An important force that is already generating inequality and will be its source even more in future is the power of information since it is obvious that those who can create information have control over those that use it.

However, we need to bear in mind that there are other equally important inequalities and distances in human society, measured by other indicators and markers, like, e. g. ethnic or racial status wherein the racial status is a social category indicating a common genetic history, and ethnic status a recognizable social category suggesting a common cultural history. There are numerous indicators showing that social inequality and socioeconomic stratification directly influence the frequency of mental disorders, sometimes even more than cultural and even genetic factors.

Added here should be sex as yet another important indicator of inequality in modern societies that, due to its biological nature has a great role both in the arena of social stratification and in development of mental disorders. Many empirical studies in the field of sociology and social psychiatry are attempting to explain why people from lower social classes develop mental disorders much more frequently than those from higher classes. Numerous analyses showed that persons suffering from severe mental disorders are also severely handicapped on the labor market and are consequently not able to maintain the class status that would be expected based on their personal history.

The factor of belonging to a certain social class, i.e. the consequent social deprivation represents an important risk for developing of mental disorders. Established today are many other corresponding mediators of developing of mental disorders, like so-called "biological causes" (exposure to toxins, infections, allergens and unsafe working environment with high risks of injury), and "non-biological causes" wherein the most frequent ones are social and psychological stressors, that is the identified stress-imbued living environments. Based on the few stated observations, it is clear that, although the material conditions of living are improving, inequality of people in modern society is on the increase accompanied by all the risks following from it.

If we take into consideration the above mentioned "normal" factors of inequality and add to them the indicators of inequality in the present posttraumatic society of Bosnia and Herzegovina resulting from the years of war cataclysm (1992-1995), it becomes clear that equality in distribution of resources and approach to health care and its services is extremely difficult to achieve, especially when it comes to people suffering from mental disorders that represent a very vulnerable segment of population in the community.

Bearing in mind the large number of persons suffering from war stress related mental disorders, large number of the disabled, high percentage of unemployment (up to 40%), increase in the number of suicide, homicide and other violent acts in the community, family violence, occurrence of prostitution and increase in abuse of alcohol and drugs, it is obvious that establishing of equality in using of all segments of mental health services in Bosnia and Herzegovina is very hard to achieve.

At the moment, the main means of establishing some sort of equality and realizing the planned mental health and other policies, especially social policy, is the appropriate legislation. With this respect, first steps were made by introduction of mental health, i.e. mental rehabilitation in the Law on Health Care in Federation of Bosnia and Herzegovina, and particularly by preparation of the Law on Protection

of Persons with Mental Disorders, which was adopted by the Parliament. All needs to be done in order to obtain appropriate legal provisions that need to symbolize the stated and the still unspoken social attitudes and intentions of the society of Bosnia and Herzegovina aimed at maintaining the integrity and dignity of every individual with mental and psychosocial difficulties.

Availability

The right to treatment makes sense only when the treatment is available, and it is up to legislation to ensure the obligation of the health care system to provide it.

In this sense, the legislation represents a declaration of policy and manifestation of principles, i.e. social ideals of a society, whereas the medical indicators are true signs of practical distribution of entire resources of the community, including therein the distribution of resources allocated to health care and mental health in particular.

Continuity

In order for individuals to function within the community it is necessary to have a number of inter-related offices and services, including those services ensuring the fundamental human rights. With a view to ensuring the continuity of mental health protection, we will use the medical model, rehabilitation model and the model of social support aiming at satisfying the majority of needs of persons with short-term and long-term mental disorders. What we need to be aware of herein is that the comprehensive model of community based mental health protection is not represented by the buildings themselves, but by the system enabling persons with mental health problems to move from one level to the other, i.e. from one service in the system to the other without any obstacles.

As we have stressed for a number of times, the whole "new" mental health policy would make no sense at all without a very active participation of the community.

Active participation of the community

In the area of mental health, there are no good results without participation of individuals and the community in defining the policy. Therefore, mechanisms whereby people would be able to express their opinions, wishes, messages and decisions have to be established.

All activities in the area of community based mental health, i.e. in the sector, must be the result of agreement reached between citizens

and mental health care providers. In other words, they have to be accepted by the total population of the sector, that is the community of its inhabitants.

Comprehensive programs of mental health promotion and the very mental health services within community-based programs cannot be provided either by health care, or by the community alone. The effective policy and programs can be developed based on agreements, taking into consideration the needs identified both by those responsible for policy and services, and community members in need of the services. What has to be taken into consideration therein are the prevailing attitudes of the community towards mental illness and persons debilitated by mental disorders, which will ensure that the policy and services meet the specific needs of various communities in the sectors, i.e. mental health centers of the Federation of Bosnia and Herzegovina, as well as the varying needs of specific groups in individual communities. Due to the political and state organization of the Federation of Bosnia and Herzegovina, its mental health policy is specific both in the content and in implementation. The entire general policy of mental health, as well as the health care policy as a whole at the level of the Federation of Bosnia and Herzegovina, is executed by the Federal Ministry of Health. In the Ministry of Health, there is a special advisor for the problems of mental health. In each of 10 cantons, the Federation should appoint a responsible person that will be in charge of the mental health problems and will be employed in the Ministry of Health of his/her canton (county). It is in this way that the needs, demands and resources of cantons (counties) and local communities are being fully respected. It is also only in this way that we can simultaneously take into account the other sectors important for mental health, like education, police system, prosecution and juridical system, social welfare, issues pertaining to ecological problems and working conditions, as well as to special groups like displaced persons, children and adolescents, elderly, women, persons abusing substances and others. The comprehensive system of community based mental health protection, as we have already stressed, can not function without relying heavily on the "third sector", that is non-governmental organizations, associations of volunteers, associations of former patients and their family members, associations of service users, self-help groups, etc. These organizations can give an important contribution in realization of the proclaimed mental health policy and the planned mental health program. In many instances, these organizations can point out at and, by that means, correct the shortcomings, mistakes and ineffectiveness in the comprehensive system of community based mental health protection. In order for this model and the promotion of mental health to be realized, it is necessary, on the basis of the established policy, to

state the general priorities based on the identified needs of the local community, taking into consideration the available resources. Naturally, given the extremely difficult situation in the domain of mental health, it is necessary to first state the priorities based on the identified needs and established resources, and then select those priorities that need to be resolved immediately (10, 11, 12, 13).

General mental health program in the Federation of Bosnia and Herzegovina

Based on the established policy, we have conceptualized the program as a plan describing the priority needs and serving as a basis for organization of a group of activities aimed at achieving certain general and specific goals. Problems related to mental health in the Federation of Bosnia and Herzegovina represent an important priority that might very rapidly become a great burden for the society that has recently emerged from war and is traumatized to the point that it represents a genuine posttraumatic society. There are various sources confirming that this is truly so. For example, according to the already stated data of the Bosnia and Herzegovina Institute for Public Health from the year 2001 showed that, the total number of citizens at that time was 3,690,426. The same source claims that there are still 601,900 refugees from Bosnia and Herzegovina around the world, whereas the total of 487,652 are displaced persons within the country that are, due to various reasons, unable to return to their homes. Over 300,000 citizens are persons with disabilities. There is statistically registered decline in natality whose rate is regressive (in 1981 it was 17.2 and in 2001 – 9.04). At the same time, the mortality rate is on the increase (in 1981 – 6.3, 2001 – 6.84). Other important parameters are given in detail in Table 1.

In the course of the year 2000 and 2001, the Bosnia and Herzegovina Institute for Public Health performed a research study on the whole territory of Bosnia and Herzegovina, i.e. in the both entities and in the Brčko District, in 15 municipalities and on the sample of 2211 families from Bosniac, Croatian, Serbian and other ethnic groups, and by that means estimated the existing situation in respecting of human rights to life and health of the citizens of Bosnia and Herzegovina, with a special emphasis on the rights of displaced persons and returnees.

Table 1. *Demographic, social and health indicators in B&H for the period 1981-2001.*

Social and Health indicators	*1981*	*1991*	*2001*
Population	4.124.256	4.395.643	3.690.426*
Refugees	-	-	607.900**
Displaced persons	-	-	487.562
Natural growth	10,9	7,7	2,19
Biological type of population	*Progressive*	*Stationary-regressive*	*Regressive*
Natality	17,2	14,9	9,04
Mortality	6,3	7,2	6,84
GNP per capita in US$	1.707	2.719	1.230
Social product per capita in US$	1.876$	3.151$	2.106$
Employed: unemployed ratio	5,83:1	3,17:1	1,36:1
Average monthly income in US$	190	299	179
Health care participation in% of GNP	4,6%	11,7%	4,47%
Hospital beds per 1000 citizens	4,1	4,53,7	
Physicians per 1000 citizens	1,1	1,6	4,5
Nurses per 1000 citizens	3,9	4,6	1,4
Immunization coverage	85%	98%	80%

* Preliminary data, the estimation based on the status of January-September, 2001

** Bulletin refugees from Bosnia and Herzegovina and displaced persons – Ministry of Human Rights and Refugees to the B&H Council of Ministers

The study showed the alarming data that 42% of pollees believed that exercising of the right to health care was not ensured; 33% stated that the right to education was not ensured; 34% said the right to work was not ensured; 11% claimed it was not possible to receive medical services in the closest health care institution regardless of the place of living in cases when life was threatened; 50% of pregnancies could not be controlled regularly and free of charge, nor could the deliveries be performed free of charge; 21% of children were not regularly vaccinated; 31% of pollees gave up the prescribed treatment because they were not able to buy the prescribed medication or get it free of charge.

In addition to all the above stated, there is a huge decline in the employment rate (39% of the total labor force is unemployed), as well as the decline in the amounts of monthly salaries of the employed (US $ 299 in 1991 as compared to US $ 179 in 2001). Payment allocations for health care from the national product are on the constant decline and in the year 2001 they amounted to 4.47% (14).

Due to a number of different reasons, the population of Bosnia and Herzegovina lives in a very insecure and dangerous environment, threatened by mines and other unexploded ordnances, residual chemical, biological and radioactive materials that can still, six years after the end of war, cause illnesses and severe injuries to people and especially children.

According to symbolic estimates of the WHO, there are at the moment on the territory of the Federation one million people suffering from war stress related psychological disorders. Some believe that the real number may be much bigger. The complete pre-war structure of the psychiatric services is, both physically and in terms of personnel, destroyed to a significant degree.

Due to all the above stated, the basic strategy in realization of the plan and program of the reconstruction of mental health services on the territory of the Federation of Bosnia and Herzegovina, is establishing of the new model of organization of the comprehensive mental health services, which will enable persons suffering from mental disorders to live and receive treatment in their homes or as close as possible to their homes within the local community, and so in the best and most effective way.

The basic strategy of promotion of mental health, prevention of mental disorders, treatment of acute psychological disorders, psychosocial rehabilitation and protection of chronic mental patients, can be set out in simple terms through the following principles:

1. Primary health care physicians provide comprehensive psychiatric care, specialized community based mental health centers, and psychiatric wards of general hospitals and clinical in-patient institutions providing the "acute" short-term hospitalization.
2. Primary mental health protection was providing by family medicine physicians (primary health care physicians) and their teams.
3. Specialized psychiatric teams of professionals provide care in the community professionals specialized in mental health problems within mental health centers providing services in the given sector.
4. Herein, great importance is given to building and utilizing of connections and establishing of absolute trust between teams of family medicine physicians and specialized teams in mental health centers and psychiatric institutions for acute hospitalization.
5. Psychiatric wards within general cantonal/county hospitals, wards of psychiatric hospitals in Sarajevo, Tuzla and Mostar, as well as the Cantonal Psychiatric Hospital Sarajevo (Jagomir) will provide hospitalization for acute patients, and for chronic patients (in case of any new deterioration of their condition). The treatment in these wards will be short and patients will go back home continuing to

receive treatment from the family medicine physician or in the mental health center.

6. Chronic mental patients, i.e. persons with severe defects of social, psychological or somatic dimension of their personality resulting from mental illness, will, as a rule, live on their own or with their families within the community. Those chronic mental patients that do not have families, nor economic or other necessary conditions to live on their own, will be accommodated in special, supervised houses located in the town where they live, that is in the community that they come from. These supportive living arrangements can be organized in various ways. We have decided to establish the following forms in future:

- Nursing homes intended for chronic patients with serious, severe and permanent dysfunction, with around-the-clock available supervision and care by nurses, and beds for permanent stay of such mental patients.
- Half way houses for patients recovering from acute psychotic episodes resulting in psychosocial breakdown of the personality of the patient. These houses are, as a rule, situated next to the hospitals and are run and supervised by nurses. Patients stay in them for a long time, but the period of their stay is still limited.
- Group homes for permanent accommodation of persons suffering from chronic psychiatric disorders. Patients live in such homes independently, although their autonomy is still limited.

The above stated strategy of the program of mental health protection on the territory of the Federation of Bosnia and Herzegovina has basic and specific tasks.

The basic tasks of the program are:

- Reduction of the incidence and prevalence of some mental disorders and suicide, especially those related to the war stress.
- Reduction in the level of dysfunction resulting from mental disorders by means of improvement of treatment and protection of persons with mental disorders.
- Improvement of psychosocial wellbeing of persons suffering from mental health problems by means of organizing of comprehensive and accessible mental health services based in the community.
- Respecting of fundamental human rights of persons debilitated by mental illness.
- In addition to the basic tasks, the program also has specific tasks the most important of which are the following:
- To detect mental disorders as early as possible and ensure appropriate care and treatment.

- To direct all attention towards promotion of mental health and fighting mental disorders, especially in socially and economically threatened and vulnerable groups.
- To organize and further develop the living and working environment in order to help people from all age groups to develop feeling of closeness and coherence, build and maintain social relationships and cope with stress situations and adverse life experiences.
- On the part of the services for care of persons with mental health problems, to provide care and all forms of high quality treatment, organizing the work of community-based and hospital-based services in a balanced way, and giving a special attention to interventions on persons going through crises, as well as to minority and vulnerable groups.
- To reduce and alleviate other adverse circumstances related to mental disorders (somatic illness, disturbed psychosocial functioning, low social status, family problems and concerns).
- To work on establishing a positive social climate.
- To change the negative attitudes towards mental illness and persons suffering from it.
- To improve the quality of living of people with mental disorders.
- To rehabilitate persons suffering from mental disorders so as to reach the level of their optimal social reintegration.
- To ensure basic and permanent education of professionals working in mental health services.
- To ensure systematic supervision and control of the work of personnel employed in mental health services.
- To establish the information and patient registering system.
- To stimulate research in the area of mental health with a special emphasis on research of the services.
- To provide monitoring and evaluation of the program in a systematic and periodical fashion.

Understanding the practical goals in the broadest sense as quantitatively defined indicators of activities that need to be performed or changed within the given deadline, we have, starting from 1994, changed and upgraded the goals of the program after every two years. Eventually, in the period 1996-1998, after signing of the contract between the World Bank and the Federal Ministry of Health, we directed the practical goals primarily towards the physical reconstruction of community health centers wherein the future community based services will function. In the period 1998-2000, all our efforts were directed towards the implementation of community based mental health services

program in accordance with the principles of comprehensive and equal organization of mental health services, as well as towards education of personnel of various professional profiles, and education of the whole community wherein the services are situated.

The most important goals in the present two-year, that is five-year period 2000-2002-2005, are the following:

Until the year 2002, to implement the program of reform of mental health through ensuring full functioning of all 38 mental health centers on the territory of the Federation of Bosnia and Herzegovina.

Until the year 2002, to perform a ten-day additional education and re-education of at least 50% of all professionals employed in mental health services in the Federation of Bosnia and Herzegovina.

Until the year 2005, to create the conditions for 80% of all mental health problems to be treated by teams of family medicine physicians (primary health care physicians) and specialized mental health services, i.e. community based mental health centers.

Table 2. *Indicators and standards for Program of Mental Health protection program in Federation of Bosnia and Herzegovina*

Area	Criteria	Indicator	Standard
Organization of services	Sectorization	Adoption of sectorizing principles	38 CMHC Psychiatric clinics Jagomir Hospital
	Location of psychiatric beds	Psychiatric beds in general hospital	At least 50%
Accessibility of mental health services	Available hospital beds	Bed/population	0,5/1000
	Distance from nearest mental health institution	Inhabitants that lives one hour drive from the nearest institution	<20%
Staff	Psychiatrists	Psychiatrist/population	1/10.000
	Social workers	Social workers/population	1/10.000
	Psychologists	Psychologists/population	0,3/10.000
	Psychiatric nurses	Nurses/population	4/10.000
	Occupation therapists	Occupation therapists/population	0,1/10.000
Budget	Budget for mental health	% of budget for health care dedicated for mental health	At least 10%
Human rights	UN Charter on Human Rights	Formal approval	Yes
	Compulsory treatment in psychiatric institutions	Compulsory admissions (in%)	<10%

In order to be able to monitor the real level of realization of the defined practical goals of the program, it was necessary to establish criteria, indicators and standards of the program, which we have done to a high extent, following the recommendations of the WHO, as can be seen in Table 2.

The program was from the very beginning monitored and evaluated in a fairly adequate way, primarily by international experts as representatives of the creditor (World Bank) and donors (SWEBiH, HealthNet International, etc.).

Naturally, we have to continue the monitoring and evaluation in an even more strict fashion in order to be able to compare the present "zero" state of the program with the expected results and states in accordance with our detailed program.

We have worked on these activities and will continue to work on them in the course of the realization of the program so that we can make timely and necessary, tolerable corrections as the program implementation develops. What needs to be kept in mind therein is that the main task of the impartial evaluation process is to perform the interventions in the course of the program implementation in accordance with the program tasks, i.e. goals. Moreover, the evaluation needs to clearly determine the influence of the intervention and its connection to the defined activities, as well as the kind of the influence (positive, negative or irrelevant). In the course of the evaluation process, reliable and precise notes need to be kept using certain indicators.

Specialized community based mental health services

Psychiatric services in the community are, together with primary health care teams, responsible for care of the mental health of the community as a whole, and of all patients with mental disorders including those that are "most severely affected".

Community psychiatry, that is public psychiatry today, covers all aspects of care, starting from the usual psychiatric, diagnostic and therapeutic interventions, to partial and full hospitalization, case management, interventions and help in crisis situations, and up to providing of supportive living arrangements. The main organizational structure and prototype of institution of community -based psychiatry are mental health centers (MHC).

MHCs are the integral part of the primary health care and they are from the functional aspect connected with other primary health care services (e.g. family medicine physicians), with secondary health care (wards for acute hospitalization, organization of supportive living arrangements for chronic mental patients, etc.), with centers for social work and other institutions relevant for primary, secondary and tertiary

prevention, that is treatment, rehabilitation and re-socialization of persons with psychological disorders.

MHCs very closely cooperate with the "third sector" of non-governmental organizations and certain service user and volunteer associations.

Community based mental health center is located at the level of primary health care and is responsible for a geographic sector inhabited by the population of 25,000 to 50,000 people. The size of the sector, as well as the number of citizens covered by individual MHCs varies from one center to the other, depending on specific differences in geographical and distribution of settlements and population. This, of course, also depends on various human and financial resources in individual areas.

The MHC is, from the organizational aspect, situated within the primary health care with strong functional connections with all primary health care services, and family medicine in particular. Functional connections are also established with in-patient psychiatric institutions, as well as social and other institutions and areas of work in the given sector.

The basic principle of work is the work in the home of the patient, in his/her family and the wider community.

The partial hospitalization within MHCs represents a day care wherein patients with special needs stay in the facility during the period varying from two to twelve hours, with provided specialized individual and group therapeutic programs and full responsibility of the personnel during the period of the treatment.

If MHCs are functioning optimally and in full cooperation with other institutions and authorities, they can organize a special care for certain risk groups in the general population (children, adolescents, addicts, elderly).

Optimally functioning MHCs, together with primary health care services, take over the responsibility of resolving all mental health problems on a certain territory up to the level of 80% of total needs.

Cooperation and mutual connections among MHCs at entity levels and at the level of Bosnia and Herzegovina as a whole, are necessary with a view to creating a common doctrine of community based mental health care, evaluating the development of the system, as well as introducing certain corrections after a period of their full functioning.

The monitoring and supervision are necessary and include expert and organizational supervision of the system as a whole and at the entity level by entity reference centers or expert groups.

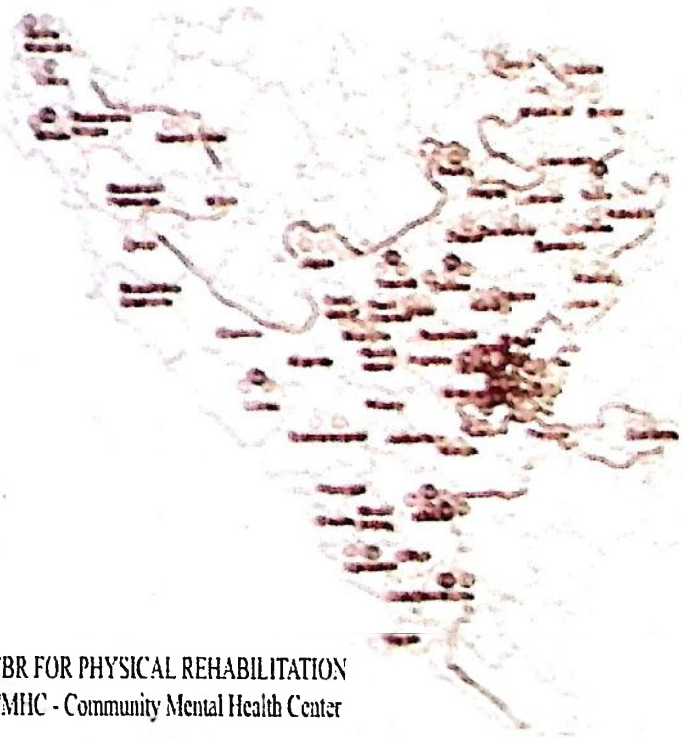
The basic principle of work in the MHC is teamwork performed through permanent and everyday functioning of multi-professional

psychiatric team comprising of the minimum of a psychiatrist, psychologist, social worker, four trained psychiatric nurses and an occupational therapist. In order for such a team to function well, it is necessary that its each individual member applies his/her knowledge and that mutual trust and high professional responsibility always exists. Owing to prejudice, but also to certain objective circumstances in our current situation, the team leader is always a specialist psychiatrist.

On the territory of the Federation of Bosnia and Herzegovina, we have to date established 38 mental health centers. The majority of centers are functioning. At the moment, two more centers that were not planned are being established, their organization being put together by local health care institutions that have understood the importance of such centers and the need for their establishment.

The geographic distribution of the centers can be seen on the enclosed map titled Figure 1.

Figure 1. *Bosnia and Herzegovina, Federation of Bosnia and Herzegovina*



Each mental health center has available 10 psychiatric beds intended for acute hospitalization and located in psychiatric wards of general hospitals and clinical institutions.

The concrete tasks of the specialized multi-professional team could in general be summarized in the following way:

- To provide comprehensive medical and psychological care for all persons suffering from serious mental illness related to severe functional damage and disabilities.
- To diagnose, treat and do the follow-up of patients coming to the MHC for help on they are own, or those referred by team of family medicine physicians or other primary health care practitioners.
- To participate as a team in education and consultation for employees in the primary health care.
- To act as consultants to primary health care providers, which requests a permanent interaction with family medicine physicians, nurses and other personnel, with a view to developing and maintaining mental health related skills in personnel employed in the primary health care.
- To establish the administrative framework for coordination of mental health protection in the MHC's sector of responsibility.
- The population for which a certain MHC is responsible should be in the "authentic" natural ambience and sector.
- The size of the sector's population needs on the one hand to be sufficient so that the MHC activities can be effective in their full range and provide the whole diversity of services, and on the other hand, it needs to be small enough to enable the smooth functioning of the center.
- The services need to be accessible to the population of the sector wherein the given MHC functions.
- 2% of the population in any given year and any given moment is seeking the services of mental health service providers due to mental health problems, and out of this number 80% is seeking the services from family medicine physicians and MHCs.
- To provide immediate care for patients referred by the family medicine physician (primary health care doctor) or coming directly to the center.
- To do the follow-up of patients discharged from psychiatric wards.
- To provide the supervision of personnel in primary health care institutions.
- To provide help to other organizations in the community like schools, prisons, etc.

- To cooperate with the center for social work.
- To cooperate both widely and concretely with non-governmental organizations, associations of former patients, volunteer groups, etc.

Based on the given list of tasks and jobs of the multi-professional psychiatric teams in mental health centers, we can clearly see the range of work that is very extensive but, if done systematically, can be performed fairly easily and without difficulties.

Naturally, in order for the team as a whole to be able to perform all these tasks, its individual members must be aware of their special responsibilities, starting from the team leader that is a specialist psychiatrist, via psychologist, social worker, psychiatric nurses and on to occupational therapist.

The project of reconstruction and reform of the psychiatric services in the Federation of Bosnia and Herzegovina was officially started in 1996, after signing of the contract with the World Bank whereby a long-term credit was given for the physical and psychosocial rehabilitation of the victims of war in Bosnia and Herzegovina. Out of this rather big credit, approximately 5 million dollars have been spent for the reconstruction of mental health services.

In the course of the first two years of the project, areas in community health centers allocated for mental health centers were physically reconstructed according to the unique project.

The premises were reconstructed and equipped. Adequate furniture was purchased and information system established. The necessary literature was also purchased.

The most difficult and most important part of the project of reform of mental health services had already began before the war ended in 1994/95, when the WHO organized one-year post-graduate course in the area of psychological trauma and its treatment. 150 psychiatrists, psychologists, social workers and nurses have successfully completed this important course. This was the beginning of the education process that is necessary for any change in the system, let alone such a radical undertaking as our reform of mental health services. Ever since, the intensive and continuous education has been conducted through seminars, consultation, study trips of our experts to other countries and visits of foreign experts serving as supervisors of the work of our mental health centers in the field.

For two years already we have been publishing the professional magazine "Mental Health in Community" with articles of both local and foreign experts. This has now become an integral element in the reform of mental health services.

In the area of education, there are at the moment several parallel and important on-going projects.

In the Travnik Canton, the Harvard Trauma Center from Boston is completing a very important three-year pilot project of education of primary health care physicians in the area of mental health. This intensive program has proved itself very useful in expanding the knowledge of family medicine physicians in particular. At the moment, the Harvard experts are conducting an identical program in New York, educating in the same way the primary health care physicians for providing help to people suffering from consequences of the catastrophic events.

The University of Sarajevo is, together with the University of Umeö from Sweden, completing a two-year post-graduate study in the domain of child and adolescent psychiatry. 30 students, psychiatrists and psychologists from all parts of Bosnia and Herzegovina are finishing this course.

A multi-disciplinary study attended by 30 psychiatrists, psychologists and social workers have just been completed at the University of Sarajevo. The main theme of this study was community psychiatry. The students attended the lectures of very competent experts and they spent one semester abroad working practically in Great Britain, Italy and Slovenia.

The further education of both professionals in mental health services and non-professionals, as well as the general population, will be continued permanently and intensively since it represents one of the most important segments and conditions of the reform in our country.

This summarized review of the reconstruction, i.e. reform of mental health services on the territory of the Federation of Bosnia and Herzegovina in the war and post-war period and in the country that is in addition going through the period of transition, shows how much energy, will and resources need to be invested in this huge undertaking that is from the temporal aspect unlimited and has numerous problems and obstacles yet to come.

Still, it appears that the most difficult period of reform is now behind us and that the further implementation of this program will go on more smoothly (15, 16).

Apstrakt

Psihijatrijska služba prije ratne katastrofe u Bosni i Hercegovini bila je relativno dobro razvijena i jedna od najbolje organiziranih u republikama bivše Jugoslavije. Osnovu cijelog sistema psihijatrijske zaštite činile su psihijatrijske bolnice i mala neuropsihijatrijska odjeljenja općih bolnica koje su pratile specijalističke psihijatrijske službe u Domovima zdravlja.

Prvih mjeseci katastrofičnih ratnih zbivanja u BiH, došlo je do pustošenja i razaranja u svim domenima života, uključujući i razaranje i

zatvaranje brojnih tradicionalnih psihijatrijskih institucija uz masovnu psihološku traumatizaciju cjelokupne populacije stanovništva.

Tokom rata, a naročito nakon njegovog završetka, započeta je rekonstrukcija i reorganizacija službe za mentalno zdravlje. Planirana osnova zaštite mentalnog zdravlja osmišljena je kao sistem u kojem se veliki dio službi nalazi u zajednici, što je bliže moguće mjestu življenja pacijenta. Ključni aspekt sistema sveukupne zdravstvene zaštite je primarna zdravstvena zaštita, a glavna uloga pripada ljekarima porodične medicine i profesionalcima mentalnog zdravlja koji rade u zajednici.

Velike psihijatrijske ustanove su bile zatvorene, često devastirane, ili su im kapaciteti bili tokom rata znatno smanjeni. Smišljeno, nije došlo do rekonstrukcije i ponovnog otvaranja starih psihijatrijskih ustanova, niti gradnje novih.

U poslijeratnom periodu integralni dio sistema zaštite mentalnog zdravlja predstavljaju Centri za mentalno zdravlje u zajednici. Svaki od ovih centara opslužju pripadajuću mu teritoriju u kojoj obitava određeni broj stanovnika. Centri su odgovorni za prevenciju i liječenje psihičkih poremećaja, kao i unapređenje cjelokupnog mentalnog zdravlja.

Hronični mentalno oboljeli pacijenti koji nemaju porodica i ne mogu, iz bilo kojih razloga, samostalno živjeti u zajednici, biće smješteni u posebne kuće i druge oblike zaštićenog stanovanja u zajednicama iz kojih potječu.

Ključne riječi: *mentalno zdravlje, rekonstrukcija zdravstva.*

References

Loga S, Cerić I. *Razvoj zaštite mentalnog zdravlja u F BiH,* Ministarstvo zdravlja FBiH, april 1999. "Oslobođenje", 11.06.01., str.5.

Cerić I, Oruč L. *Istorijat psihijatrijske službe u Bosni i Hercegovini, International Conference of Mental Health Reform in Bosnia and Herzegovina,* Dubrovnik, Croatia, Book of Abstracts, September 30-October2.,1999:7-9.

Jensen BS, Cerić I. *Community-Oriented Mental Health care in B&H,* Strategy and Model Project WHO. Office, for BiH, Sarajevo, 1994.

Logan S, Ceria I. *Razor ratite minting zdravlja u FBiH,* Ministarstvo zdravstva, April 1999.

Fattore G, Percudani M, Pegnoli C, Contini A, Beecham J. *Mental Health Care in Italy: Organizational Structure Routine Clinical Activity and Cost of Community Psychiatric Service in Lombardy Regional,* International Journal of Social Psychiatry, 2000; 46(4): 250-65.

Saraceno B. *Mentalno zdravlje i primarna zdravstvena zaštita: klinički osvrt na literaturu,* International Conference of Mental Health Reform in Bosnia and Herzegovina, Dubrovnik, Croatia, Book of Abstracts, September 30-October2.,1999.

Health 21 – Health for all in the 21st Century, WHO, Regional Office for Europe Copenhagen, 1999: 40-43.

Peat M. *Community Based Rehabilitation,* WB.Saunders,
Public Mental Health, Guidelines for the Elaboration and Management of National Mental Health Programmes, Division of Mental Health and Prevention of Substance Abuse, WHO, Geneva, 1996.
Consumer Involvement in Mental Health and Rehabilitation Services, Division of Mental Health World Health Organization, Geneva, WHO/MNH/MEP/89.7
Doctor – Patient Interaction and Communication, Division of Mental Health Organization, Geneva, 1993.
A Literature Review on Housing, Division of Mental Health, WHO, Geneva, WHO/MNH/MEP/93.17
Health Status of Population and Health Care System in Transition, Bosnia and Herzegovina; Report for 2001, Institute for Public Health of Bosnia and Herzegovina, Sarajevo, May 2002:8-11.
Cerić I, Loga S, Sinanović O, et al.: *Rekonstrukcija službe za zaštitu mentalnog zdravlja u Federaciji Bosne i Hercegovina,* Med.Arh. 2001;55(1) supl. 1:3-64.
Knesper DJ, Riba MB, Schwenk TL. *Primary Care Psychiatry,* WB. Saunders,1997.
Oakley LD, Potter C. *Psychiatric Primary Care,* Mosby, 1997.

THE PREVALENCE OF PRECANCEROUS LESIONS IN PRIMARY DENTAL HEALTH CARE

Berislav Topić[1]

Abstract

Benign and premalignant states of the oral cavity related to impairments of epithelial keratinization are classified as oral precancerous lesions, and can be hereditary, reactive, immunologic, infectious, idiopathic, or neoplastic. Epidemiologic studies conducted worldwide have identified the following types of oral precancerous lesions to be priorities according to their prevalence and clinical implications: leukoplakia, erythroplakia, oral lichen, actinic cheilitis, candidal leukoplakia, papillomatous lesions, tertiary syphilis, and oral submucous fibrosis. Five of these eight clinical entities, i.e. leukoplakia, erythroplakia, oral lichen, actinic cheilitis and candidal leukoplakia, and their importance in the prevention of oral carcinoma are discussed in detail. General practitioner and polyvalent practicing dental medicine doctor have a major role in the early diagnosis of neoplasms in the head, neck and oral cavity region. Early diagnosis is associated with better treatment options and lower probability of visible mutilations. The occurrence of oral precancerous lesions is influenced by the following factors: cigarette smoking and alcohol abuse, showing positive correlation with both the amount and duration of particular habit; exposure to detrimental factors (occupational and environmental) and radiation (sunlight and ionizing); uncontrolled medication; viruses; and inappropriate dietary habits. Successful prevention of oral precancerous lesions requires regular visits to dentist office, elimination of harmful habits, and healthy lifestyle. In case of clinical manifestation of an oral precancerous lesion, dental doctor should reach an accurate diagnosis by use of laboratory tests and not infrequently biopsy finding, i.e. clinical diagnosis should be verified or ruled out by pathohistology. Therapy and regular control visits are prescribed according to the diagnosis.

Key words: *oral cavity, precancerous lesions, diagnosis, prevention, dental practice*

[1] Academy of Sciences and Arts of Bosnia and Herzegovina

Introduction

Precancerous lesions are pathologic processes that tend to transform to malignancy (1). In general pathology, precancerous lesions are classified as neoplasms, which can be benign, premalignant, or malignant. Benign and premalignant states of oral mucosa that are related to impairments of epithelial keratinization are classified as oral precancerous lesions and are divided into the following types:

- hereditary – white sponge nevus, leukoedema
- reactive – orthokeratosis, parakeratosis, acanthosis, dyskeratosis (mechanical, chemical, thermal, radiation and microbial lesions)
- immunologic – oral lichen, lupus erythematosus (systemic and discoid)
- infectious – keratotic candidiasis, hairy leukoplakia, syphilitic leukoplakia
- idiopathic – lingua villosa, lingua geographica
- neoplastic – epithelial dysplasia (dyskeratosis), carcinomas (*in situ*, squamous cell, verrucous)

Oral precancers include precancerous lesions and precancerous states. Precancerous lesions are erythroplakia, leukoplakia, oral lichen, actinic cheilitis and keratotic candidiasis. Precancerous states are morsicatio buccarum, palatitis nicotinica, discoid lupus erythematosus, white sponge nevus, submucous fibrosis, Plummer-Vinson syndrome, glossitis luetica and xeroderma pigmentosum.

International epidemiologic studies (2) have pointed to the following entities as priorities according to their prevalence and clinical implications of oral precancerous lesions:

- leukoplakia
- erythroplakia
- oral lichen
- actinic cheilitis
- keratotis candidiasis
- papillomatous lesions
- tertiary syphilis
- oral submucous fibrosis

Of these eight clinical entities, the clinical significance of the following oral precancerous lesions in the prevention of oral carcinoma will be discussed in more detail: leukoplakia, erythroplakia, oral lichen, actinic cheilitis and keratotic candidiasis. A general practitioner and polyvalent practicing dental doctor play a major role in the early diagnosis of neoplasms in the head, neck and oral cavity region. Early diagnosis is associated with more favorable treatment prognosis and

lower rate of mutilating interventions in the most visible part of the body.

The occurrence of oral precancerous lesions is associated with cigarette smoking and alcohol abuse (positive correlation with both the amount and duration of these habits), exposure to detrimental noxae (occupational and environmental), radiation (sunlight and ionizing), uncontrolled drug intake, viruses, and inappropriate dietary habits. Dental prevention of oral precancerous lesions includes regular visits to dental office, giving up harmful habits, and healthy lifestyle.

When a patient presents with an oral precancerous lesion, dental doctor should reach an accurate diagnosis by use of laboratory tests and quite frequently biopsy finding for the clinical diagnosis to be confirmed or excluded by pathohistology. The diagnosis will dictate the choice of therapy and regular control visits.

In Europe, health institutions have developed a program of prevention with a motto 'Europe Against Cancer'.

In this paper, literature data and results of our own studies of oral precancerous lesions are presented.

Epidemiologic data

In the USA, the distribution of oral carcinoma in 14253 patients according to the involvement of oral mucosa, i.e. the seat of carcinoma, was as follows: lower lip 38%, tongue 22%, base of the mouth 17%, gingiva 6%, palate 5.5%, tonsils 5%, upper lip 4%, buccal mucosa 2%, and uvula 0.5% (3). In Croatia, the incidence of oral carcinoma in 583 patients in 1999 according to the seat of carcinoma was as follows: tongue 187 (32%), lip 122 (21%), salivary glands 102 (17.5%), base of the mouth 44 (7.6%), and other seats in the oral cavity 94 (16%). The male to female ratio was 441 *vs.* 142 (76% *vs.* 24%) cases (4). In Bosnia & Herzegovina (B&H), there were 6722 patients with malignant neoplasms, and 2986 deaths from malignant neoplasms in 2000 (5). Comparison of the causes of death in B&H and Croatia revealed circulatory system diseases to be the leading cause with 53% in B&H and 52% in Croatia, followed by neoplasms with 17.4% and 22%, respectively (6,7), indicating that about 70% of deaths in B&H were due to circulatory system diseases and neoplasms.

Leukoplakia and erythroplakia

Pindborg *et al.* (8) define leukoplakia as a white lesion of oral mucosa which has no characteristic of any other type of lesion. Leukoplakia is a hyperkeratosis which can be homogeneous or inhomogeneous according to its clinical appearance. Inhomogeneous

leukoplakia is characterized by the occurrence of red and white areas that may be atrophic, nodular, verrucous, or ulcerative (erythroplakia). On differential diagnosis, leukoplakia should be differentiated from friction keratosis, wind keratosis, bite keratosis, chemical mucosal lesions, leukoedema, white sponge nevus, oral lichen, hairy leukoplakia, and pseudomembranous candidiasis.

The prevalence of leukoplakia in the general population of Europe is from 0.9% in Germany to 3.6% in Sweden, 2.9% in the USA, and from 0.7% to 11.7% in India (9). In the epidemiologic study performed in a representative sample of 5552 subjects from B&H, there were 110 (1.8%) subjects with leukoplakia (10). During the 1996 – 2001 period, 6668 new patients were recorded at the Department of Oral Medicine, Zagreb University School of Dental Medicine, 56 of them with leukoplakia. The incidence of leukoplakia among the newly recorded patients was 0.84% (11).

Among 7820 subjects examined in Budapest, mean age 57.1 years, there were 104 (1.33%) subjects with leukoplakia. The percentage of cigarette smokers in the groups of subjects with and without leukoplakia was 86.5% and 29.0%, respectively (12). A study conducted in Nairobi indicated the relative risk of leukoplakia to be 14.7% and 6.7% in those smoking >10 and <10 cigarettes daily, respectively. In those who had been smoking for less than 10 years the incidence of leukoplakia was 7.4% *vs.* 10.8% in those with a >30-year history of smoking (13).

The diagnosis of leukoplakia is made on the basis of personal history (cigarette smoking, alcohol consumption), inspection, palpation, and biopsy finding. Differentiation between benign and malignant leukoplakia cannot be made on the basis of the clinical picture alone but also requires biopsy finding. Pathohistologic diagnosis of leukoplakia is characterized by the basal layer finding, which includes loss of cell polarity, hyperplasia, increased nucleus to cytoplasm ratio, increased number of mitoses, atypical mitoses, irregular epithelial stratification, mitoses in the upper epithelial layers, cellular and nuclear polymorphism, nuclear hyperchromatism, nuclear enlargement, loss of intercellular adhesion, and keratinization in the spinous layer.

Oral lichen rubber

Lichen ruber is a noninfectious, inflammatory disease of the skin and mucosa, characterized by T lymphocyte destruction of the epithelial basal layer. Lichen is classified in the group of chronic mucocutaneous autoimmune diseases of as yet obscure etiology. Genetic predisposition (HLA-DR9), drugs, dental material, chronic diseases of the hepatobiliary system, diabetes mellitus, intestinal

diseases (Crohn's disease) and psychogenic factors have been most commonly reported as factors contributing to the genesis of lichen ruber. Concerning the basic pathogenetic mechanism with unknown antigen, the antigenic structure of epithelial cell is modified. Thus, the autoimmune reaction is triggered, failing to recognize native antigens as its own and leading to epithelial destruction.

Clinically, the disease presents with morphologically diverse manifestations. The basic classification of the various clinical forms is based on the occurrence of efflorescences at various levels of mucosal surface and includes lichen ruber planus, lichen ruber bullosus, and lichen ruber erosivus with the following subtypes: papular, reticular, annular, atrophic, bullous, erosive and ulcerative. Clinical diagnosis should be verified by biopsy, i.e. pathohistologic finding showing hyperkeratosis, acanthosis, epithelium of dentate appearance, liquefaction degeneration of the basal layer, and subepithelial inflammatory cellular infiltrate.

The prevalence of the disease in the general population ranges from 0.5% to 2.5% (14). Its onset usually occurs at 40 to 60 years of age, and shows a 2:1 male to female ratio (15). During a 10-year period (1990-1999), the diagnosis of oral lichen was made in 343 (3.92%) of 8751 patients at the Department of Oral Medicine, Zagreb University School of Dental Medicine. Thus, every 25th patient had oral lichen (16).

The rate of malignant transformation of oral lichen ruber differs among various authors, ranging from 0.4% (17) to 3.3% (18). The number of oral lichen ruber patients in pooled data reported by 11 researchers from different countries ranges from 214 to 2071. During the period of observation that ranged from 2 to 10 years, malignant transformation occurred in 59 (0.9%) of a total of 6529 patients with the diagnosis of oral lichen ruber (19). Patients with a clinical diagnosis of erosive oral lichen ruber showed malignant transformation of the epithelium.

Actinic cheilitis

Actinic cheilitis is labial (more commonly lower lip) alteration due to chronic sun exposure. Ultraviolet (UV) rays of the sunlight spectrum lead to cellular destruction in the labial epithelium and connective tissue. Collagenous and elastic fibrils of the connective tissue are destroyed, resulting in the microscopic appearance known as basophilic degeneration. Longterm continuous sun exposure (20-30 years) is needed for the dysplastic lesion to occur (20). Fair-complexion subjects are at a greater risk of actinic cheilitis than those dark-colored. Individuals aged >60 spending most of the time outdoors for sports or

work (farmers, bricklayers/stonemasons, fishermen, sailors, hunters, mountaineers, skiers, oarsmen, etc.) are usually affected.

Actinic cheilitis shows a male predominance. In women, lipstick is a major protective factor. Smoking, and especially pipe smoking, is a predisposing factor for the development of actinic cheilitis (21,22).

Lower lip mucosa in the region of vermilion is thin, atrophic, homogeneous, with milky-white, smooth, irregular or dentate patches with sharply delineated peripheral margin. Macular pigmentation with scaling may be seen on perioral skin surface. Suspicion of malignant alteration is raised by color heterogeneity, rough thickening, absence of sharp margins, and presence of erosions/ulcerations that would not heal (23). Some 10% of cases of actinic cheilitis show malignant transformation to squamous cell carcinoma (24,25).

Keratotic candidiasis

Keratotic candidiasis or candidal leukoplakia is a unique clinical picture of chronic hyperplastic candidiasis among other forms of oral candidiasis. It is a white plaque-like lesion that can be scraped off. The lesion is well demarcated, white with occasional red patches, thickened or verrucous, mostly localized on the anterior part of buccal mucosa or palate. Clinically, it resembles leukoplakia, whereas histologic finding shows epithelial atypia, especially if biopsy specimen has been obtained from the erythematous area of the lesion.

It is a controversial issue whether the dysplastic alteration in chronic hyperplastic candidiasis is caused by colonization, candidal infection and penetration to the pre-existing plaque-like leukoplastic lesion, or leukoplakia has been induced by Candida. Experimental hyperkeratotic lesion can be reproduced on the dorsal side of mouse tongue if inoculated with *Candida albicans*. *Candida albicans* is known to produce nitrosamine, a carcinogen (26). Nitrosamine is a chemical mitogen. Metabolic activation of nitrosamine proceeds *via* P-450 cytochrome, whereby a potent methylating mutagen, methyldiazohydroxide, is released. In some nitrosamines, potent alkylating intermediaries can be produced. The mutagenic action of nitrosamine manifests by the generation of bonds between DNA molecules and alkylating and methylating metabolites of the compound (27).

In some patients, squamous cell carcinoma developed from candidal leukoplakia in the absence of other risk factors (26).

In dental casuistics, oral mucosa candidiasis is more common than leukoplakia. In each case of leukoplakia, dental doctor should determine the possible presence of Candida as a pathogenic microorganism in the patient's oral cavity.

Discussion

Oral precancerous lesions are pathologic processes that frequently progress to carcinoma. According to a medical axiom, diseases have their causes. If the cause can be eliminated, the disease can be prevented. However, this simple logic does not hold for cancer. Cancer is not a disease that could be ascribed exclusively to one factor but is ultimate result of consecutive or simultaneous activity of a number of factors connected in the causative network. Different factors contribute to the genesis of cancer. Extrinsic factors are smoking habit, alcohol consumption, and UV rays, and intrinsic factors are systemic conditions such as fatigue, malnutrition, sideropenic anemia (Plummer-Vinson syndrome), HIV infection, prior antibiotic therapy, syphilis (glossitis luetica, tertiary stage). Of all these, smoking habit and alcohol abuse require special attention.

There is a significant association between smoking habit and oral carcinoma. The risk of oral carcinoma is proportional to the frequency and duration of smoking habit. The proportion of smokers is on a fast decrease thanks to large-scale health education, ban on cigarette advertising (except at car races), and high tobacco tax rates. However, those who continue smoking do it at a higher rate and with greater passion, so there is no decrease in the rate of oral carcinoma in the general population, although data on oral carcinoma in smokers show 3- to 5-fold figures found in nonsmokers. The more so, alcohol consumption enhances the effect of smoking on the occurrence of oral carcinoma. Studies of the effect of alcohol and its interaction with smoking are hampered, because it is quite difficult to recruit adequate number of subjects in particular groups (e.g., alcoholics non-smokers). According to some literature data, the prevalence of oral carcinoma in heavy smokers and alcohol users is 35-fold that recorded among those neither using alcohol nor smoking.

Ethanol is not carcinogenic in experimental animals, thus it is difficult to explain exactly how alcohol drinks increase the risk of carcinoma. The clue may be some dietary deficiency accompanying longterm alcohol abuse, or contamination (e.g., nitrosamines), or maybe greater mucosal permeability for other carcinogens. The hypothesis of local action appears to be supported by data on the use of oral alcohol containing mouthwash solutions that increase the risk of oral carcinoma (28). The systemic effect of alcohol in the development of liver cirrhosis is crucial. Alcohol consumption and cigarette smoking have a synergistic, cumulative action, resulting in several-fold increase in the incidence of oral carcinoma.

Almost all precancerous lesions and oral malignancies localized on soft tissues of the oral cavity can be detected by properly taken

history, thorough inspection and palpation. Inspection of the oral cavity is a precondition for detection of a risky lesion in its initial stage. Among oral malignancies, oral squamous cell carcinoma is most common. It is for months or even years preceded by the presence of whitish (leukoplakia) and/or reddish (erythroplakia) discoloration known as precancerous lesion. Oral malignancies increase with age, in individuals aged >40. Besides inspection of oral mucosa, palpation with rubber gloved hands is of utmost importance. Resistance to palpation with induration of the lesion area should be a signal to the clinician suggesting a potential malignancy.

The prognosis of patients with oral carcinoma greatly depends on the primary tumor size. Small lesions without metastases have a high percentage of >5-year survival in treated patients. Therefore, early clinical diagnosis when the tumor is still small and free from metastases is crucial for successful therapeutic outcome.

Dental doctors have a major role in the prevention of oral carcinoma by reaching an accurate diagnosis and differential diagnosis of oral precancerous lesions. In suspect cases, biopsy is required to confirm or rule out the diagnosis of precancerous lesion. May some dilemma arise on pathohistologic diagnosis, the methods of immunohistochemistry, immunofluorescence, immunoprecipitation and molecular pathology (polymerase chain reaction, *in situ* hybridization) should be employed.

Biopsy is required in relatively small chronic ulcerative lesions caused by mechanical irritation, if they fail to heal within some 20 days from the cause elimination.

The opinion about the method of treatment of oral carcinoma differs among different centers of head and neck oncology. Some centers primarily perform surgical therapy followed by postoperative radiotherapy (Zagreb), whereas in other institutions radiotherapy is considered the method of choice (Ljubljana). In Sarajevo, a selective approach is used.

Conclusions

1. Benign and premalignant states of oral cavity that are related to impairments of epithelial keratinization are classified as precancerous lesions, and can be hereditary, reactive, immunologic, infectious, idiopathic, or neoplastic.
2. Oral precancers include precancerous lesions and precancerous states (potential precancerous lesions).
   a) Precancerous lesions include erythroplakia, leukoplakia, oral lichen, actinic cheilitis, and candidal leukoplakia.

b) Precancerous states include leukokeratosis palatina nicotinica, morsicatio buccarum, discoid lupus erythematosus, white sponge nevus, submucous fibrosis, Plummer-Vinson syndrome, glossitis luetica, and xeroderma pigmentosum.

3. Precancerous lesions also include paraneoplastic mouth syndromes, which represent the existence of some oral diseases that are in some way associated with neoplastic processes elsewhere in the body.
4. Smoking habit and alcohol consumption have a synergistic, cumulative effect that results in several-fold increase in the risk of oral precancerous lesions and their transformation to oral carcinoma.
5. Dental doctors have a major role in the prevention, diagnosis and therapy of oral precancerous lesions as well as in the diagnosis of oral mucosa carcinoma.

Apstrakt

Udio oralnih prekanceroza u stomatološkoj praksi primarne zdravstvene zaštite

Benigna i premaligna stanja usne šupljine koja su vezana za poremećaje keratinizacije epitela pripadaju oralnim prekancerozama. Oni mogu biti: hereditarni, reaktivni, imunološki, infekcijski, idiopatski i neoplazmatski. Prema svjetskim epidemiološkim istraživanjima, po učestalosti i kliničkom značenju oralnih prekanceroza, važnim za stomatološku praksu prioritetnu listu čine: leukoplakija, eritroplakija, oralni lihen, aktinički heilitis, kandidialna leukoplakija, papilomatozne lezije, tercijarni sifilis i oralna submukozna fibroza. Od navedenih osam kliničkih entiteta, detaljno se raspravlja o pet i to: leukoplakiji, eritroplakiji, oralnom lihenu, aktiničkom heilitisu i kandidialnoj leukoplakiji kao i o njihovom značenju u prevenciji oralnog karcinoma. Liječnik opće prakse i stomatolog polivalentne prakse imaju najznačajniji udio u ranoj dijagnostici neoplazmi u području glave, vrata i usne šupljine. Rana dijagnoza daje veće šanse za liječenje i manje šanse za mutilacijama vidljivog dijela tijela. Na nastanak oralnih prekanceroza značajno utiču pušenje i konzumiranje alkohola, kako količinski tako i vremenski; zatim izlaganje štetnim faktorima (profesionalnim i okoliša), zračenja (sunčana i jonizirajuća), nekontrolirana upotreba lijekova, virusi, i neadekvatna prehrana. Prevencija oralnih prekanceroza traži redovite posjete ordinaciji liječnika – stomatologa, eliminaciji štetnih navika i življenju zdravih stilova života. Kada se klinički manifestira u ustima neka od prekanceroza stomatolog bi morao imati točnu dijagnozu koristeći laboratorijske nalaze, vrlo često i biopsiju odnosno patohistološkom dijagnozom potvrditi ili isključiti postavljenu kliničku dijagnozu. Prema dijagnozi provodi se terapija i redoviti kontrolni pregledi.

Ključne riječi: *usna šupljina, prekanceroze, dijagnoza, prevencija, stomatološka praksa.*

References

Dorland's Illustrated Medical Dictionary. Philadelphia: Saunders Co., 28th ed. 1994.

WHO Collaborating Center for Oral Precancerous Lesions. Definitions of leukoplakia and related lesions. An aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol 1978;46:518-29.

Krolls SO, Hoffman S. *Squamous cell carcinoma of the oral soft tissues.* J Am Dent Assoc 1976;92:571-7.

Hrvatski zavod za javno zdravstvo. *Novi slučajevi raka usta u R. Hrvatskoj 1999.* Bilten 2000;24.

Zavod za javno zdravstvo Federacije BiH. *Mreža, kapaciteti i djelatnost zdravstvene službe u Federaciji BiH u 2000. godini.* Sarajevo, 2001;3.

Zavod za statistiku Federacije BiH. *Uzroci smrtnosti u Federaciji BiH 2000.* Demografski podaci 2001.

Zavod za statistiku R. Hrvatske. *Uzroci smrtnosti u R. Hrvatskoj.* Demografski podaci 2000.

Pindborg JJ, Reichart PA, Smith CJ, van der Waal I. *Histological typing of cancer and precancer of the oral mucosa.* 2nd ed. Berlin: Springer, 2000.

Reichart PA, Philippen HP. *Color atlas of dental medicine.* Stuttgart: Thieme, 2000.

Topić B. *Leukoplakija kod stanovništva BiH.* Projekt: Morfološko-fiziološke karakteristike i patofiziološke promjene orofacijalnog sistema kod stanovništva BiH. Fond za naučni rad BiH 1977.

Topić B, Vučićević-Boras V. *Incidencija leukoplakije kod pacijenata Zavoda za oralnu medicinu Zagreb.* Simpozij Rizične lezije oralne sluznice. Zagreb: HAMZ, 2002.

Banoczy J, Rigo O. *Prevalence study of oral precancerous lesions within a complex screening system in Hungary.* Commun Dent Oral Epidemiol 1991;19:265-7.

Macigo FG, Mwaniki DC, Guthua SW. *Influence of dose and cessation of kiraiku, cigarettes and alcohol use on the risk of developing oral leukoplakia.* Eur J Oral Sci 1996;104:498-502.

Eversole LR. *Immunopathogenic diseases in the oral mucosa.* Michigan: Michigan University, 1995;129-35.

Topić B, Cekić-Arambašin A, Malčić S. *Oral lichen ruber (OLR) epidemiology and clinical findings in 143 cases.* Acta Stomatol Croat 1996;30:111-5.

Topić B, Cekić-Arambašin A. *Incidencija oralnog lihen rubera u desetogodišnjem periodu Zavoda za oralnu medicinu Zagreb.* Simpozij: Rizične lezije oralne sluznice. Zagreb: HAMZ, 2002.

Kovesi G, Banoczy J. *Follow-up studies in oral lichen planus.* Int J Oral Surg 1973;2:13-9.

Barnard NA, Scully C, Eveson JW, Cunnigham S, Porter SR. *Oral cancer development in patients with oral lichen planus.* J Oral Pathol Med 1993;22:421-4.

Scully G, Beyll M, Ferreiro MC, Ficarra G, Gill Y. *Update on oral lichen planus: etiopathogenesis and management.* Crit Rev Oral Biol Med 1998;9:86-122.
Picascia DD, Robinson JK. *Actinic cheilitis: a review of the etiology, differential diagnosis and treatment.* J Am Acad Dermatol 1987;17:255-64.
Lindquist C, Teppo L. *Epidemiological evaluation of sunlight as a risk factor of lip cancer.* Br J Cancer 1978;37:983-9.
Pukkala E, Soderholm AL, Lindquist C. *Cancer of the lip and oropharynx in different social and occupational groups in Finland.* Oral Oncol Eur J Cancer 1994;30B:972-7.
La Riviere W, Pickett AB. *Clinical criteria in diagnosis of early squamous cell carcinoma of the lower lip.* J Am Dent Assoc 1979; 99:972-7.
Main JH, Parone M. *Actinic cheilitis and carcinoma of the lip.* J Can Dent Assoc 1994; 60:113-5.
Blomquist G, Hirsch JM, Alberius P. *Association between development of lower lip cancer and tabacco habits.* J Oral Maxillofac Surg 1991;49:1044-9.
Eisen D, Lynch PD. *The mouth: diagnosis and treatment.* St. Louis: Mosby, 1998:58-91.
Fučić A. *Kemijski kancerogeni.* In: Boranić M, *et al.* Karcinogeneza. Zagreb: Medicinska naklada, 2000;117-44.
Boranić M, *et al.* *Karcinogeneza.* Zagreb: Medicinska naklada, 2000:15-68.

ANU
BiH
АНУ
БиХ

INFLUENCE OF SAMPLE SIZE ON THE RESULTS OF BIOEQUIVALENCE STUDIES

Zulić I, Kusturica J, Potkonjak D, Kapić E. Mulabegović N, Loga-Zec S, Rakanović-Todić M, Bečić F, Krošnjar S.[1]

Abstract

Great number of drugs coming from different manufactures is available on the market.

The biocquivalence studies give substantial evidence if these drugs, given in same doses and under similar conditions, have similar bioavailability. Studies of bioequivalence are performed on healthy young volunteers in crossover designs and artificially controlled environment to minimize factors, other than the drug, which can affect bioavailability. They usually include 24 healthy volonteers with about 20 blood analyzes giving a total of 500.

This kind of research is of big importance for the determination of pharmacokinetic drug characteristics but is very expensive, especially in small countries.

Considering the importance of cost decrement we set the hypothesis that bioequivalence studies can be performed on smaller number of subjects. This hypothesis is confirmed by the results of our analysis (6) included in cross-over study can be an adequate number.

Introduction

A fundamental hypothesis of clinical pharmacokinetics is that a relationship exists between the pharmacological or toxic response to a drug, and the accessible concentration of the drug. This hypothesis has been documented for many drugs, although it is apparent for some drugs that no clear of simple relationship has been found between pharmacologic efect and concentration in plasma (Goodman&Gilman's,1996).

Several alternative and equivalent representations of drug disposition can be used to describe the relationship between dose and concentration, and can be modified to account for the passage of time. In

[1] Institute of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicne, University of Sarajevo, Sarajevo, B&H

the early days of pharmacokinetics, emphasis was placed on half-life to decribe the time course of drug concentration. Now we usually think of clearence and volume of distribution as the essential parameters of pharmacokinetic processes because of close mapping of these parameters to identifiable functional and structural features of the body (Holford, 1992).

Various physiological and morbidity variables that dictate adjustment of dosage in individual patients often do so as a result of modification of pharmacokinetic parameters. Three most important parameters are drug clearence, volume of drug distribution and its bioavailability. These factors coupled with dosage, determine the concentration of a drug at its sites of action and hence the intensity of its effects as a function of time.

When the bioavailability of different preparations, salts of forms of a drug are compared at the same molar dose, under similar experimental conditions, and are found to be the same, the drugs are said to be bioequivalent. Bioequivalence of two drugs means that the rate und extent of absorption are extremely similar, the amount of each preparation eaching bloodstream does not significantly differ, and the preparations are chemically equivalent (Spilker, 1991).

Pharmacokinetic parameters used to establish bioequivalence are the peak plasma concentration achieved (C_{max}), the time to achieve this peak concentration (T_{max}), and the area under the blood (or plasma) time-concentration (AUC_{o-}). Ideally the two curves should be super imposable, so one may conclude that the two drugs are bioequivalent, but in practice the curves may differ. According to FDA, the product is considered bioequivalent if blood levels of two drugs agree within 20%. The FDA also has a princliple that 75% of patients should have plasma levels that are between 75% and 125% of the reference standard (Spilker, 1991).

The most important factors, which can affect bioavailability of a medicine, are: age of patient, food ingested, genetic history physiological capacity of the liver to metabolise, disease, interactions with other medicines, kidney function. To minimise these factors, studies of bioequivalence should be performed on healthy young volunteers in crossover designs, so each patient receives both tretaments, and in artificially controlled environment.

Minimum number of 12 evaluated subjects, should be included in any bioequivalence study (CDER, 2001). Generally, this type of pharmacokinetic researches usually include 24 healthy volunteers. However, these researches are very expensive, and our intention is to demonstrate that the same results can be obtained with smaller number of included subjects. This can be a way to decrease the pharmacokinetic research expense.

In order to obtain reliable results, the bioanalytical methods used to determine active principle and/or its biotransformation product in any suitable matrix should meet requirements of specificity, accuracy, sensitivita and precision.

The knowledge of the active substance quality is also very important. In case that products are prepared according to GMP rules, pharmacokinetic profiles are predictable.

The goal of this meta-analysis is to show that the same results from pharmacokinetic studies could be obtained from 24, 12 or 6 healthy volunteers.

Methods

Study design

In order to prove no significant difference between the pharmacokinetic results compiled from diferently sized groups of subjects we compared data from 3 different bioequivalence studies. Statistical comparison has been made between the compiled results from 24 subject groups, 12 subject groups and 6 subject groups. The groups of 6 and 12 subjects were randomly assigned from the original groups (groups of 23, 24,26 subjects).

Inclusion criteria

Bioequivalence studies to be done according to GCP.
In these studies we included minimum 24 or 12 subjects.

Analyzed studies

Three different bioequivalence studies were included in this analysis: bioequivalence study of sulpiride, bioequivalence study of lisinopril and bioequivalence study of norfloxacin.

Randomisation assignment

A subject number used in the randomisation schedule was assigned to each subject included in the study.

Statistical data analysis

Statistical analysis included data compiled from tabulated pharmacokinetic parameters with summary statistics of the individual study. Statistical analysis was done by statistical programs: Pharma/PCS Version 2.03, Pharmacologic Calculation System; Microsoft Excel 2002; Sigma Stat for Windows Version 2.03, The following paameters were included in the analysis: Cmax, tmax, Ka, Ke, AUC and AUC. Bioequivalence was conformed by Westlake and Hauck tests.

Results and Discussion

BIOEQUIVALENCE OF TWO SULPIRIDE-BASED PREPARATIONS

Study of sulpiride was designed as randomised two-way crossover single blind study with healthy male subjects who received a single oral dose of three 50 mg-capsules (150 mg of sulpiride in total).

Table 1: *Mean values of sulpiride in serum*

Sampling time (h)	DRUG A capsules	DRUG A capsules	Comparative drug capsules	Comparative drug capsules
	26 subjects group I	12 subjects group II	26 subjects group III	12 subjects group IV
T (h)	Mean concentration (ng/mL)	Mean concentration (ng/mL)	Mean concentration (ng/mL)	Mean concentration (ng/mL)
0.0	1.70± 02.46	2.94± 2.18	2.28±02.17	1.57±02.34
0.5	10.48± 8.67	8.25±13.44	11.13±14.18	10.95±09.55
0.75	39.62± 34.64	29.22±20.04	40.61±33.20	36.40±29.36
1	70.90± 48.30	64.37±36.48	72.66±51.96	67.94±37.32
1.25	105.96± 57.87	101.06±59.15	112.48±81.50	105.70±56.60
1.5	123.53± 69.48	137.99±71.47	135.64±86.34	126.63±79.66
1.75	153.88± 79.39	147.75±64.95	140.15±68.73	155.29±94.99
2.0	168.65± 79.56	168.02±73.79	160.42±72.33	163.80±72.23
2.5	186.08± 82.88	206.73±75.44	193.05±77.28	188.31±66.12
3.0	214.92±118.27	254.12±87.82	227.48±92.64	192.32±79.05
4.0	203.53± 81.08	225.65±64.98	206.62±76.54	198.00±68.78
6.0	134.91± 53.09	166.24±36.97	146.62±45.18	135.35±52.45
8.0	102.80± 44.50	124.74 ±16.85	113.96±26.76	99.99±38.99
12.0	70.04± 22.52	81.36±16.29	74.49±20.27	74.74±24.84
24.0	37.62± 11.31	45.80±20.73	42.03±15.68	37.84±11.28
36.0	22.95± 07.01	21.08±06.00	21.52±05.83	21.87±06.35
Statistical significance (t-test)	n.s.			
95 percent C.I. for difference of means group I/III : -54.423 to 47.727 95 percent C.I. for difference of means group I/II : -62.850 to 45.631 95 percent C.I. for difference of means group II/IV : -42.863 to 63.940 95 percent C.I. for difference of means group III/IV : -44.906 to 55.461				

Subjects, aged from 18 to 48 years were screened for inclusion into the trial. In order to acomplish that 24 evaluated male subjects complete the

study, 26 subjects were included in the study. Sampling for the pharmacokinetic evaluation was performed according to the following schedule:pre-dose, 30 minutes, 45 minute, 2 hours, 2 hours nad 30 minutes, 3, 4, 6, 8, 12, 24, 36 hours post dose. The following parameters were calculated from the serum concentration: C_{max},t_{max}, α, β, $t_{1/2}$, KA, KE, AUC and AUC_{∞}.

Statistical analyses did not detect any difference in mean plasma concentrations at any blood collection time point between two products when the sample size was 24 or 12 subjects (Table 1.).

The satistical analysis of non-tranformed data is shown in Table 2. The mean and standard deviation of both formulations in 24 and 12 subject groups were almost same. The t-test was also performed, showing no statistical difference.

Table 2: *Values of pharmacokinetic parameters detected in 26 and 12 subjects*

Pharmacokinetic parameters	**DRUG A** capsules	**Comparative drug** capsules	**Statistical significance 12/6 subjects (t-test)**
	26 subjects	**26 subjects**	
C_{max} (ng/mL)	240±110	258±94	
T_{max} (h)	3.0±0.7	3.1±0.7	
K_e	0.042±0.017	0.048±0.016	
K_a	0.343±0.150	0.347±0.144	
$AUC_{(0-\infty)}$ (ng/mL h)	3278±977	3225±813	
Statistical significance (t-test)	n.s.		
	DRUG A capsules	**Comparative drug** capsules	
Pharmacokinetic parameters	**12 subjects**	**12 subjects**	**n.s.**
C_{max} (ng/mL)	235±78.988	284±77.338	
T_{max} (h)	2.9±0.85	3.0±0.86	
K_e	0.045±0.019	0.053±0.015	
K_a	0.329±0.171	0.338±0.1	
$AUC_{(0-\infty)}$ (ng/mL h)	3261±1140	3267±813	
Statistical significance (t-test)	n.s.		

Mean resistance time and mean absorption time following the calculation of AUMC by trapezoid rule are shown in Table 3 and Table 4.

Table 3: *Values of AUMC calculated by trapezoid method*

Sampling time	DRUG A capsules		Comparative drug capsules	
	26 subjects	12 subjects	26 subjects	12 subjects
t (h)	AUMC (ng/mL)h^2	AUMC (ng/mL)h^2	AUMC (ng/mL)h^2	AUMC (ng/mL)h^2
0.0	1.310	1031	1.391	1.368
0.5	4.369	4.156	4.503	3.933
0.75	12.577	14.511	12.890	11.429
1	25.419	37.476	26.658	24.008
1.25	39.719	77.554	43.008	38.648
1.5	56.826	133.420	56.091	55.405
1.75	75.827	204.815	70.763	71.923
2.0	200.632	418.123	200.872	199.598
2.5	277.498	737.924	291.272	261.941
3.0	729.466	1570.411	754.467	684.000
4.0	1623.642	3470.463	1706.230	1604.100
6.0	1631.922	5465.851	1791.434	1612.036
8.0	3334.464	9414.531	3611.200	3392.000
12.0	10486.512	21869.307	1145.200	10830.672
24.0	10375.776	33019.587	10740.000	10174.248
36.0	42628.000	27110.500	26900.000	25712.359
$AUMC_{0\to 36}$ (ng/mL)h^2	71503.959	60130.087	57665.979	54677.668
$AUMC_{0\to\infty}$ (ng/mL)h^2	72050.482	70671.587	58114.312	65615.168

Table 4: *Mean absorption and mean resistance time in different sample sized groups*

	DRUG A	DRUG A	Comparative drug	Comparative drug
	26 subjects	12 subjects	26 subjects	12 subjects
MRT (h)	21.98	21.67	18.01	20.08
MAT (h)	-1.82	- 0.54	-2.82	- 1.21

Bioequivalence of drug A and comparative drug evaluated in 26 and 12 subjects is confirmed by Hauck test, Table 5.

Table 5: *Hauck test of bioequivalency*

Sample size	Hauck test
26 subjects	N-1=25 T = 3.154602 E-02 δ = 4.561751 E-03 p = 1.18047 E-04
12 subjects	N-1=11 T = .355591 δ= 2.956155 E-03 p = 8.257926 E-04

BIOEQUIVALENCE OF TWO LISINOPRIL-BASED PREPARATIONS

Study of lisinopril was designed as randomised two-way crossover single blind study with healthy male subjects who received a single oral dose of 20 mg-tablet. 12 healthy subjects, aged from 24 to 35 years, were included in the study. Sampling for the pharamacokinetic evaluation was performed according to the following schedule: 0,5, 1, 1,5, 2, 3, 4, 6, 8, 12 and 24 hours post dose.

Table 6: *Mean values of lisinopril in serum*

Sampling time (h)	DRUG B tablets	DRUG B tablets	Comparative drug tablets	Comparative drug tablets
	12 subjects	6 subjects	12 subjects	6 subjects
t (h)	Mean concentration (ng/mL)	Mean concentration (ng/mL)	Mean concentration (ng/mL)	Mean concentration (ng/mL)
0.5	0.95± 0.33	1.11± 0.34	1.08± 0.31	1.07± 0.30
1.0	3.70± 1.76	4.39± 2.13	3.62± 1.56	3.63± 1.95
1.5	9.32± 5.06	11.28± 5.99	8.30± 5.49	9.46± 7.55
2.0	17.51± 8.06	20.30±10.24	15.62± 8.51	17.01± 1.33
3.0	37.19±13,.10	41.95±16.21	34.28±12.03	36.32±13.59
4.0	56.14±16.58	64.13±17.84	49.48±14.30	53.08±15.65
6.0	68.77±23.40	80.71±26.47	69.35±15.71	74.71±17.50
8.0	65.28±22.25	78.24±22.48	65.21±20.80	10.59±27.21
12.0	51.03±20.20	60.67±20.71	53.84±21.31	60.96±24.16
24.0	15.14±10.00	21.27±11.28	15.82±10.32	1.07± 0.30
Statistical significance (t-test)	n.s.			
95 percent confidence interval for difference of means I/II: -32.707 to 20.907 95 percent confidence interval for difference of means III/IV: -30.024 to 20.284 95 percent confidence interval for difference of means II/IV: -38.932 to 15.702 95 percent confidence interval for difference of means I/III: -23.755 to 25.445				

Serum concentrations time profile (means ± SD) following intake of single oral dose is shown in Table 6. The serum concentration time curve was very similar after administration of the two preparations in different size groups.

The mean values (± SD) of pharmacokinetic parameters for the detected evaluation of bioequivalence are shown in Tabele 7. There are no differences of statistical significance.

Tabele 7: *Values of pharmacokinetic parameters detected in 12 and 6 subjects*

Pharmacokinetic parameters	**DRUG B** tablets	**Comparative drug** tablets	**Statistical significance 12/6 subjects (t-test)**
	12 subjects	**12 subjects**	
C_{max} (ng/mL)	70±23	71±18	
T_{max} (h)	6.5±0.9	6.3±0.78	
K_e	0.1±0.02	0.17±0.13	
K_a	0.36±0.06	0.31±0.09	
$AUC_{(0-\infty)}$ (ng/mL h)	1190±498	1188±534	
Statistical significance (t-test)	n.s.		
	DRUG B tablets	**Comparative drug** tablets	n.s.
Pharmacokinetic parameters	**6 subjects**	**6 subjects**	
C_{max} (ng/mL)	82 ±25	79±20	
T_{max} (h)	6.7±1	6.6±1.0	
K_e	0.09±0.01	0.21±0.17	
K_a	0.35±0.06	0.33±0.08	
$AUC_{(0-\infty)}$ (ng/mL.h)	1451±570	1470±623	
Statistical significance (t-test)	n.s.		

Values of AUMC, MRT and MAT are shown in Table 8 and Table 9.

Table 8: *Values of AUMC calculated by trapezoid method*

Sampling time	DRUG B tablets		Comparative drug tablets	
	12 subjects	6 subjects	12 subjects	6 subjects
t (h)	AUMC (ng/mL)h^2	AUMC (ng/mL)h^2	AUMC (ng/mL)h^2	AUMC (ng/mL)h^2
0:5	1.045	1.17	1.039	1.04
1.0	4.422	5.261	4.016	4.447
1.5	12.253	14.379	10.923	12.046
2.0	73.284	83.231	67.044	71.488
3	168.027	191.185	150.384	160.641
4	637.08	740.79	614.004	660.61
6	934.8	1110.198	937.684	1028.558
8	2268.96	1707.8	2335.328	2623.6
12	5852.16	7430.328	6153.984	7302.528
24	5204.36	8722.05	2886.469	2804.97
$AUMC_{0\to 24}$ (ng/mL)h^2	15156.391	21006.392	13160.875	14669.929
$AUMC_{0\to\infty}$ (ng/mL)h^2	16700.268	23841.992	13746.69	15140.439

Table 9: *Mean absorption time and mean resistance time in different sample sized groups*

	DRUG B tablets	DRUG B tablets	Comparative drug tablets	Comparative drug tablets
	12 subjects	6 subjects	12 subjects	6 subjects
MRT (h)	14.03	16.43	11.57	10.3
MAT (h)	3.9	4.9	5.5	5.5

Bioequivalence of the products in differently sized groups is confirmed by Hauck and Westlake test, Table 10.

Table 10: *Test of bioquivalencies*

Sample size	Hauck test	Westlake test
12 subjects	N - 1 = 11 T = 8.168178 E-02 δ = 8.462206 E-03 p = 5.856753 E-04	2.397265 E-22. 6.082929E +24 Kan=3.948
6 subjects	N - 1 = 5 T = 0.2145618 δ = 5.280028 E-03 p = 9.949803 E-04	1,329071 E-33, 1.701412 E +38 Kan=4.893

Study of norfloxacin was designed as randomised two-way crossover single blind study with healthy male subjects who received a single oral dose of 400 mg tablet. 23 healthy subjects were included in the study. Sampling for the pharmacokinetic evaluation was performed according to the following schedule: 0,5, 1, 1,25, 1,5, 1,75, 2, 2,25, 2,5, 3, 3,5,4, 6, 8, 10 and 12 hours post dose.

Serum concentrations time profile (means ±SD) following the intake of a single oral dose is shown in Table 11. The mean both preparation values, after their administrations in 24 and 12 subject groups, are similar.

Table 11: *Mean values of norfloxacin in serum*

Sampling time	**DRUG C** tablets	**DRUG C** tablets	**Comparative drug** tablets	**Comparative drug** tablets
	23 subjects	**12 subjects**	**23 subjects**	**12 subjects**
t (h)	**Mean concentration (ng/mL)**	**Mean concentration (ng/mL)**	**Mean concentration (ng/mL)**	**Mean concentration (ng/mL)**
0.50	187±180	145±105	362±366	270±229
1.00	528±331	546±329	686±519	572±375
1.25	733±442	890±468	829±550	815±505
1.50	886±551	1094±539	852±493	845±465
1.75	970±618	1258±630	886±484	957±480
2.00	985±599	1267±606	877±473	955±462
2.25	936±541	1192±505	863±492	958±492
2.50	924±492	1131±429	851±451	908±404
3.00	697±368	861±331	640±297	672±263
3.50	549±285	636±242	554±269	597±267
4.00	477±243	571±223	465±209	481±191
6.00	324±171	376±155	307±149	320±142
8.00	226±131	266±143	213±109	225±109
10.00	162±109	197±120	159± 90	164±89
12.00	123± 92	157±85	104± 87	122±86
Statistical significance (t-test)	n.s.			
95 percent confidence interval for difference of means I/II: -404.705 to 154.038 95 percent confidence interval for difference of means I/III: -223.797 to 231.664 95 percent confidence interval for difference of means II/IV: -161.178 to 391.311 95 percent confidence interval for difference of means III/IV: -238.083 to 209.683				

The mean values (±SD) of pharmacokinetic parameters for the evaluation of bioequivalence are shown in Table 12. There are no differences of statistical significance. The calculated AUMC, MRT and MAT values are shown in Table 14 and Table 13.

Table 12: *Values of pharmacokinetic paameters detected in 23 and 12 subjects*

Pharmacokinetic parameters	**DRUG C** tablets	**Comparative drug** tablets	**Statistical significance 12/6 subjects (t-test)**
	23 subjects	**23 subjects**	
C_{max} (ng/mL)	1179±453	1204±453	
T_{max} (h)	1.8±0.5	1.8±0.6	
K_e	0.212±0.056	0.215±0.033	
K_a	3.002±1.712	3.261±2.206	
$AUC_{(0-\infty)}$ (ng/mL h)	6244±3539	5399±2290	
Statistical significance (t-test)	**n.s.**		
	DRUG C tablets	**Comparative drug** tablets	**n.s.**
Pharmacokinetic parameters	**12 subjects**	**12 subjects**	
C_{max} (ng/mL)	1267±585	1202±329	
T_{max} (h)	1.9±0.3	1.9±0.1	
K_e	0.212±0.068	0.212±0.039	
K_a	2.824±1.308	3.724±2.215	
$AUC_{(0-\infty)}$ (ng/mL h)	6941±3666	5219±2331	
Statistical significance (t-test)	**n.s.**		

Bioequivalence of drug C and comparative drug, evaluated on 23 and 12 subjects was conformed by Hauck and Westlake test, Table 15.

Table 13: *Values of AUMC calculated by trapezoid method*

Sampling time	DRUG C tablets		Comparative drug tablets	
	23 subjects	12 subjects	23 subjects	12 subjects
t (h)	AUMC $(ng/mL)h^2$	AUMC $(ng/mL)h^2$	AUMC $(ng/mL)h^2$	AUMC $(ng/mL)h^2$
0.50	155	155	217	177
1.00	181	207	215	199
1.25	281	344	289	286
1.50	370	480	354	368
1.75	458	592	413	448
2.00	510	317	462	508
2.25	552	689	509	553
2.50	1100	707	1012	1071
3.00	1003	1202	965	1026
3.50	957	1128	950	1003
4.00	3852	4540	3702	3844
6.00	3752	4384	3546	3720
8.00	3428	4098	3294	3440
10.00	3096	3854	2838	3104
12.00	9696	11377	8066	9678
$AUMC_{0\to12}$ $(ng/mL)h^2$	32159	34075	26830	29427
$AUMC_{0\to\infty}$ $(ng/mL)h^2$	32159	37153	26830	32200

Table14: *Mean absorption time and mean resistance time*

Parameter	DRUG C tablets	Comparative drug tablets	DRUG C tablets	Comparative drug tablets
	23 subjects	12 subjects	23 subjects	12 subjects
MRT (h)	5.149	5.352	4.968	6.168
MAT (h)	0.433	0.947	0.317	1.482

Table 15: *Test of bioquivalencies*

Sample size	Hauck test	Westlake test
23 subject	N-1=22 T = 4.469213E-02 δ = 9.98002E-04 p = 2.658665E-04	0, 5.393475 E + 37 Kan = 0.433
12 subjects	N-1=11 T = 0.7673818 δ = 5.867081 E-04 p = 0.658665 E-04	8.710414 E-39, 1.701412E + 38 Kan = 0.947

Conclusion

On the basis of the above-presented statistical evaluation it is concluded that there are no significant differences in the bioequivalence final results between differently sized sample groups. Pharmacokinetic parameters in bioequivalence studies, when performed on healthy young volunteers and in previously determined conditions, are mostly affected by physic-chemical characteristitcs of the medicine, so significant differences in individual pharmacokinetics can not be expected. This hypothesis is confirmed by the results of our analysis. Considering that the number of dropouts determines sample size, the number of includede subjects has to be adjusted to the circumstances. In the situation when we have a good patient compliance and when severe adverse effects are not expected, the minimal sample size of 12 subjects (approved by FDA) is adequate. Our study results show that even a smaller number of subjects (6) included in cross-over study represents an adequate number. This hypothesis should be considered and explored further more for the reasons of importance of pharmacokinetics investigation costs decrement.

Apstrakt

Na tržištu su dostupni mnogi lijekovi istog sastava proizvedeni od strane različitih proizvodžača. Studije bioekvivalence daju osnovne informacije o tome da li takvi lijekovi, primijenjeni u jednakoj dozi i u sličnim uvjetima imaju približno jednaku bioraspoloživost u organizmu. Da bi se minimizirali faktori koji nisu od strane lijeka, a utiču na njegovu bioraspoloživost, studije bioekvivalence se sprovode sa zdravim dobrovoljcima u *crossover* dizajnu i u kontroliranim uvjetima. U studije se po pravilu uključuju 24 zdrava dobrovoljca za koje je potrebno pojedinačno izvršiti dvadesetak analiza krvi ili ukupno oko 500.

Ovakva ispitivanja su važna za procjenu farmakokinetičkih karakteristika lijekova, ali su i izuzetno skupa, pogotovo u malim zemljama.

Na osnovu temeljnih odredbi bioraspoloživosti, a u cilju smanjenja (za naše uvjete veoma značajnog aspekta) cijene ovakvih istraživanja, postavili smo hipotezu da se studije bioekvivalence mogu sprovesti i sa manjim brojem ispitanika.

Ova teza je potvrdjena rezultatima naše analize. Rezltati su pokazali da čak i manji broj ispitanika (6), u *crossover* dizajnu studije, može biti adekvatan za sigurne studije bioekvivalence.

Literature

Holford, N. (2001): *Clinical Pharmacokinetics and Pharmaco-dynamics: The Quantitative Basis for Therapeutics,* in: Clinical Pharmacology Basic Principles in Therapeutics Ed: K. L. Melmon, H.F. Morrelli, B.B. Hoffman, D.W. Nierenberg, Third Edition 37: 951-964.

Goodman and Gilman' s (1996): *The Pharmacological Basis of Therapeutics,* Ninth edition. Eds.: Hardman, J.G. and Limbird, L.E. McGraw-Hill, Health Professions Division 1-27.

CDER (2001): *Guidance for Industry;* Statistical Approaches to Establishing Bioequivalence. US Department of Health and Human Services; Food and Drug Administration. Centre for Drug Evaluation and Research.

Spilker B (1991): *Guide to Clinical Trials.* Raven Press, New York.

MALIGN CHEST TUMORS

Analysis of cases operated in 1998 through 2001

Budalica M.[1], *Dizdarević Z.*[2], *Žutić H.*[2], *Ćerimagić Z.*[1]

Abstract

Objectives: In our long time practice we have observed occasional changes in the clinical course of malign diseases of chest. Since Bosnia and Herzegovina was in war from 1992 to 1995, we observed more of significant changes in the course and behavior of malign tumors of that area. **Patients and methods:** 532 patients with operated malign tumors of chest in the periods 1988 - 1991 and 1998 - 2001 are processed and 33 patients with operated lung carcinoma in the period 1992 through 1995. Number, localization, patho-histological findings, and TNM classification were analyzed. **Results:** Number of operated malign tumors of chest increased in the post war period (318-214). Number of operated malign tumors of pleura, chest wall, and mediastinum significantly increased. Patho-histological findings show slight turn towards more malign forms. During the war period, operated lung carcinomas showed extremely rapid growth of primary tumor. **Conclusions:** The aforementioned results are evident. But what is the cause of such changes: weapons of war, diet disorder, psychological stress? The task is to scientifically research all the elements that could come into consideration.

Long time experience in monitoring patients with malign tumors of chest points us to periodic differences in many elements related to that disease. While the weight loss was regularly evident earlier (10-15 kg) in the last 15 years it is considerably lower or completely absent. In the last 10 years we discover differences in some other elements of development and form of malign tumors of chest.

In the period from 1992 to 1995 our country, B&H, has been exposed to massive use of instruments of war: projectiles of various gauges and other weaponry, with quantitative and qualitative reduction of groceries, consumption of canned food, and great psychological stresses.

[1] Clinic for of Thoracic Surgery KCU Sarajevo
[2] Clinic for Pulmonary Diseases "Podhrastovi" KCU Sarajevo

In this paper we analyze the matter of malign diseases of chest operated at the Clinic of Thoracic Surgery KCUS in the periods of 4 years before the war, the war period, and 4 years after the war. Processed are: number of cases of malign diseases of chest, surgical findings of malign lung tumors, and staging of lung carcinomas.

Patients and analysis of the material

Annual number of operated malign tumors of chest in our Clinic in 4 years before the war and 4 years after the war is shown in Table 1. Even though the reduction of the territory that gravitates towards our Clinic in the post war period is substantial, the number of cases in that period is evidently increased.

Table 1.

MALIGN TUMORS OF THE CHEST				
	1988	40	1998	75
	1989	57	1999	92
	1990	61	2000	79
	1991	56	2001	72
TOTAL	1988-1991	214	1998-2001	318

Localization of malign chest tumors in pre and post war period is shown in Table 2.

Table 2.

MALIGN TUMORS OF THE CHEST		
	1988-1991	1998-2001
LUNGS	210 (98.13%)	239 (75.17%)
PLEURA	1 (0.47%)	8 (2.51%)
CHEST WALL	2 (0.93%)	35 (11%)
MEDIASTINUM	1 (0.47%)	36 (11.32%)
TOTAL	214	318

It is clear that number of interventions on pleura, chest wall, and mediastinum significantly increased.

Patho-histological findings of malign lung tumors show slight turn towards more malign forms in the post war period. Especially is impressive the percentage of microcellular carcinomas of 8% compared to 0% in the 4 years before the war as shown in Table 3.

Table 3.

PATHO-HISTOLOGICAL FINDINGS OF MALIGN LUNG TUMORS		
	1988-1991	1998-2001
Ca planocellulare	60%	51%
Adenocarcinoma	13%	20%
Ca anaplasticum	13%	9%
Ca microcellulare	0%	8%
Ca bronchoalveolare	4%	2%
Ca gigantocellulare	1%	2%
Carcinoid	9%	8%

During the war (1992-1995), besides huge engagement of the Clinic for Thoracic Surgery in taking care of war injuries of the chest, 33 patients with malign chest tumors were also operated. Considering the number and particular circumstances (the war period), it is impossible to compare this quantum of patients with those in the periods before and after the war. However, we also analyzed these cases and observed odd findings in TNM classification of malign lung diseases during the war (Table 4).

Table 4.

TNM CLASSIFICATION	
T_1NM	0
$T_2N_0M_0$	16
$T_3N_0M_0$	6
$T_4N_0M_0$	3
TOTAL	25 (75%)
$T_2N_1M_0$	2
$T(4\ sl._2,\ 1\ sl._3)\ N_2M_0$	5
$T_2N_0M_1$	1
TOTAL	8 (25%)

We compared it with one of equivalent tables analyzing lung carcinoma from a country in peace (Table 5)

Table 5.

N status in T_1 operated Ca of the lungs			
Group A (Tu up to 1 cm)	N_0 100%	N_1 0%	N_2 0%
Group B (Tu up to 2 cm)	N_0 83%	N_1 5%	N_2 12%
Group C (TU up to 3 cm)	N_0 62%	N_1 12%	N_2 25%

(Ishida: Ann Thorac Surg: 1990,50)

We have observed evident difference. It is clear that there had been incredibly rapid growth of primary tumor before it produced lymph nodes and distant metastasis. As an oddity we point out one case of lung cancer in which after the explorative thoracotomy, there was protrusion of tumor through not yet healed drainage opening on chest wall in the period while there were still no clinical indications of distant metastasis.

Discussion

As long time participants in treatment of malign diseases of the chest we can assert evident increase of the aforementioned, drastically so for pleura and chest wall, and alarmingly so for mediastinum.

Patho-histological findings of lung carcinoma show a turn towards more malign forms.

Staging of lung cancer in the war period differs from that in peacetime. Growth of primary tumor is so rapid that it produces symptoms early, and it is surgically treated without encompassing lymph nodes or appearance of distant metastasis. What is the cause of such differences in our materials? How much has the war period contributed to that? How much influence on this does the employment of the new weaponry have, especially those with uranium content? What is the role of psychological stress in all that?

Conclusion

It is necessary to reevaluate all medical indicators and conditions in periods when these patients' disease occurred, and by means of scientific methods try to reach valid perception on causes of changes in biology of malign diseases of the chest. We have stated and processed facts. What's left for the future is to scientifically process them and find answers.

Apstrakt

Ciljevi: U svojoj dugogodišnjoj praksi primijetili smo povremene promjene kliničkog toka malignih oboljenja grudnog koša. Kako je Bosna i Hercegovina bila od 1992. do 1995. god. u ratnom stanju primijetili smo više značajnijih promjena u toku i ponašanju malignih tumora te regije. **Pacijenti i metode:** Obrađeno je 532 pacijenta operisanih malignoma grudnog koša u periodima 1988. – 1991. god. i 1998 – 2001. god., te 33 pacijenta operisanih karcinoma pluća u periodu 1992 – 1995. god. Analiziran je broj, lokalizacija, pato-histološki nalaz i TNM klasifikacija. **Rezultati:** Povećan je broj operisanih malignih tumora grudnog koša u poslijeratnom periodu (318-214). Značajno je

povećan broj operisanih malignih tumora pleure, zida grudnog koša i medijastinuma. Pato-histološki nalaz pokazuje blago skretanje prema malignijim formama. U ratnom periodu operisani karcinomi pluća pokazali su ekstremno brz rast primarnog tumora. **Zaključci:** Gore navedeni rezultati su evidentni. Ali šta je uzrok ovakvih promjena: ratna oružja, poremećaj ishrane, psihički stres? Zadatak je naučno-istraživački ispitati sve elemente koji bi mogli doći u obzir.

References:

Ishida T., Yokoyama H., Kaneko S., Sugio K., Sugimachi K., *Long-Term Results of Operation for Non Small Cell Lung Cancer in the Elderly,* Ann Thorac Surg, 1990; 50: 919-922.

Shields Th W., Behavior of Small Bronchial Carcinomas, Ann Thorac Surg, 1990; 50: 691-692.

Thomas P., Rubinstein L., and Lung Cancer Study Group, *Cancer Recurrence After Resection T_1N_0 Non Small Cell Lung Cancer,* Ann Thorac Surg, 1990; 49: 242-247.

Budalica M., *Hirurška terapija karcinoma pluća,* Medicinski arhiv, 1995; 49(3-4) supl. 1:59-61.

Budalica M., Hadžismailović A., Ćerimagić Z., Kacila M., *Hirurški tretman karcinoma pluća u ratu,* Medicinski žurnal, 1997; 3(2): 131-134.

ANU
BiH
АНУ
БиХ

WATER QUALITY AND MICROBIOLOGICAL STATUS OF THE DISTRIBUTION SYSTEM EVALUATED USING ESTABLISHED AND EMERGENT PARAMETERS

Mirsada Hukić[1], Fatima Numanović[1], Mahmud Nurkić[1], Nijaz Tihić[1], Snježana Hodžić[2], Avdul Adrović[2]

Abstract

The problem of the lack of good quality and quantity of water for all purposes has been increasing due to the war damage to water supply plants, the effects of the unique phenomena of subsidence of the area as well as flooding caused by recent heavy rain in the area of Tuzla Canton. The flood has resulted in pollution of the drinking water and, in the light of this emergency we carried out a study to determine drinking water quality by two methods: traditional tests required by law and specific laboratory tests.

The aims of the microbiological analysis of water were: to detect evidence of excretal biological pollution as a result of the flooding in the area of Tuzla Canton in 2002; to evaluate the required laboratorial procedures in Bosnia and Herzegovina for the detection of potent pathogens in the drinking water.

The study included the examination of 99 samples of water: 48 samples from municipal water supplies; 13 from closed sources and 38 from open sources. Samples of water were tested by routine bacteriological, parasitological and biological methods. Reverse transcription –polymerase chain reaction (RT-PCR) was applied for the detection of viruses.

Micro organisms were absent in four (4.04%) of the 99 samples of water. Out of 95 samples of water, 240 micro-organisms were isolated as follows: 114 strains of bacteria, 56 viruses, 52 bacteriophages (19 coliphages and 33 Salmonella enteritidis phage), 2 nematodes, 16 algae.

According to traditional tests required by law, water from 35.35% (35/99) sources was found suitable for drinking but using specific laboratory tests, only 10.10% (10/99) of samples were in compliance with the law. There was a significant difference in water quality ($p<0.01$).

[1] University Clinical Centre Tuzla, Department of Microbiology
[2] Tuzla University, Department of Biology

These results call for a revision of water quality guidelines based only on indicator organisms without also making reference to the absence of viruses. We have pointed out the importance of all the parameters, which should be applied during emergencies such as the recent flooding. We also suggest that, along with routine examination of drinking water there should be periodically (per month or per year) incorporated into the current protocol extra measures for detection of enteroviruses and bacteriophages.

Introduction

The biggest problem with the environment in many parts of the world is the lack of water and/or polluted water. As a potential carrier of pathogenic micro-organisms, water can endanger health and life. The World Health Organization (W.H.O.) has estimated that up to 80% of all sickness and disease in the world is caused by inadequate sanitation, polluted water or unavailability of water for all purposes (W.H.O. 1981).

Water-borne pathogens are disease-causing bacteria, viruses, and protozoa that are transmitted to people when they consume untreated or inadequately treated water. The pathogens most frequently transmitted through water are those which cause infections of the intestinal tract; namely, hepatitis A, poliomyelitis, typhoid, paratyphoid, cholera, bacterial dysentery, giardiasis, amoebic dysentery, etc. The causative organisms of these diseases are present in the faeces or urine of an infected person and when discharged may gain entrance into a body of water that ultimately serves as a source of drinking water.

Water is also an important vehicle for extra intestinal infection. A number of pathogens can be acquired through occupational, recreational, and even therapeutic contact with water. Water-borne pathogens can enter the human body via intact or damaged skin, mucous membranes and by inhalation. Extra intestinal infections are primarily superficial infections involving the skin but severe systematic diseases may occur in those who are immunological deficient. The spectrum of microbial agents that can cause such water related diseases includes bacteria, fungi, viruses, and protozoa (Mandell GL, 1995).

Some parts of Bosnia and Herzegovina, especially the area of Tuzla Canton, lack good quality and quantity of water for all purposes. Since 1992 within Tuzla Canton, the problem of the lack of water has been increasing because of the war damage to water supply plants as well as the effects of the unique phenomena of subsidence of the area. Heavy rain has caused flooding in the area recently which, in some places, has resulted in the mixing of different kinds of water with

drinking water. Bacteria and other micro-organisms have polluted drinking-water supplies; sewage spills have occurred, forcing people to boil their drinking water. There was a high risk of an epidemic from water-borne pathogens and therefore it was necessary to carry out intensive controls by quick and simple tests for the detection of intestinal organisms.

In the light of such an emergency we carried out a study to determine drinking water quality. In addition to traditional tests recommended by the W.H.O. and required by "The rule book of hygienic quality of drinking water" (37^0C and 20^0C viable count, total and faecal coliforms and faecal streptococci) Yugoslav Official Register 13/91 we carried out tests for Pseudomonas aeruginosa, other Gram negative bacilli, Gram positive aerobic and anaerobic sporogenic bacilli, Mycobacterium species, enteroviruses, bacteriophages, algae and nematodes.

Goals

The aims of the microbiological analysis of water were:

- to detect evidence of excretal biological pollution as a result of the flooding in the area of Tuzla Canton in 2002.
- To evaluate the required laboratorial procedures in Bosnia and Herzegovina for the detection of potent pathogens in the drinking water.

Materials and methods

The study included the examination of 99 samples of water, which were collected in the area of Tuzla Canton. Three kinds of water sources were examined: 48 samples of municipal drinking water (potable water); 13 from closed sources and 38 from open sources.

Samples of water for bacteriological testing were collected in sterile bottles of 250 ml capacity and for virological, biological and parasitological testing in sterile containers of 10-litre capacity. The samples included the following information:

- Code number of the sample.
- Where the water had been collected (open source, closed source and municipal water supplies)
- Whether the water has been chlorinated.
- Temperature of the source of the sample.
- Date and time when the sample was taken and dispatched.

Residual chlorine detection

Residual chlorine in the water was measured by the DPD (N, N-diethyl-1, 4-phenylenediamine) colorimetric technique (Cheesbrough M, 1992).

Bacteriological examination

The routine bacteriological procedures were applied to detect the presence of: coliform bacteria, Eschericha coli, Enterobacter, Salmonella, Shigella, Klebsiella, Proteus, Serratia, Pseudomonas, other genera of Gram negative, nonsporulating bacillus, Gram positive coccus, Enterococcus faecalis, sulphate-reducing bacteria, Gram positive sporulating bacillus, Mycobacterium species (Poček B, 1990.).

Viral examination

Bacterial viruses (bacteriophages) were isolated and cultivated in young cultures of Salmonella enteritidis and Escherichia coli. Agar culture medium showed a mass of bacterial growth on which bacteriophages produced clear zones or plaques, becoming visible after 8 to 24 hours (Armon R, Kott Y, 1993).

The enteroviruses, which included poliovirus, A coxackievirus, echoviruses, and human enteroviruses types 68 to 71, were detected after concentrating 10 litres of water by a filter absorption method, which resulted in a concentrate containing viruses. All samples were analysed by performing reverse transcription–polymerase chain reaction (RT-PCR) analyses for enteroviruses (De Leon et al 1990; Abbaszadegan et al 1993).

Biological and parasitological examination

Algae and parasites from water samples were concentrated from 10 litres of water by filtration through cellulose acetate filters. They were fixated with 4% formaldehyde and analysed under the microscope. The present micro-organisms were determined according to size, shape and internal morphology by the key for determination (Hindak et al 1978).

Statistical processing

The obtained indicators were processed by counting the percentages of occurrence and numerical values, which then represented their co-relation. The statistical significance of results was analysed by χ^2 test

and Student test (Computers programme SPSS for Windows release). The statistical significant difference between compared results was considered $p<0.05$.

Results

Residual chlorine was detected in 20.83% (10/48) of water samples originating from municipal water supplies (potable water). Residual chlorine was not found in the water from closed or open sources

Micro-organisms were absent in four (4.04%) of the 99 samples of water. Out of 95 samples of water, 240 micro-organisms were isolated as follows: 114 strains of bacteria, 56 viruses, 52 bacteriophages (19 coliphages and 33 Salmonella enteritidis phages), 2 nematodes, 16 algae (Figure 1.).

Figure 1: *The presence of members of different groups of micro-organisms in the drinking water.*

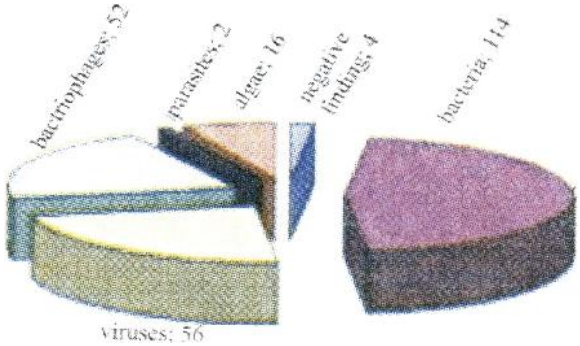
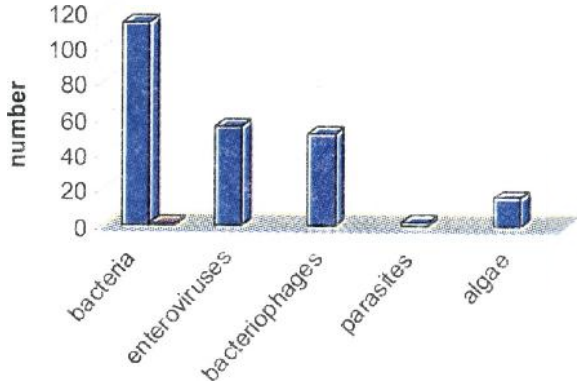


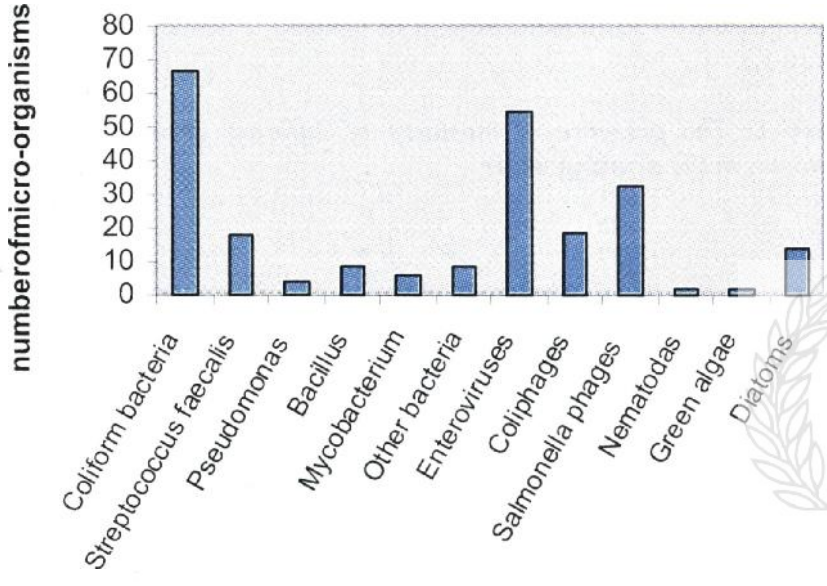
Figure 2: *Number of members from different groups of micro-organisms in the drinking water*



The most frequently detected micro-organisms in the water were bacteria, enteroviruses and bacteriophages. Nematodes were found in 2 samples (Figure 2).

There were found: colifom bacteria (68.69%), viruses (56.57%), bacteriophages (52.52%), parasites (2.02%) and algae (16.16%). Frequency of different genera and/or species of micro-organisms is shown in Figure 3.

Figure 3: *Overview of the micro-organisms present in all the sources of water*



Different genera and species of bacteria were found in all the types of water. The most frequently found bacteria were: Escherichia coli (25.40%), Enterobacter / Klebsiella (18.25%) and Streptococcus faecalis (14.20%), while Pseudomonas aeruginosa and sulphate-reducing bacteria were present in 3.17%. Frequency of genera and/or species of bacteria is shown in the Figure 4.

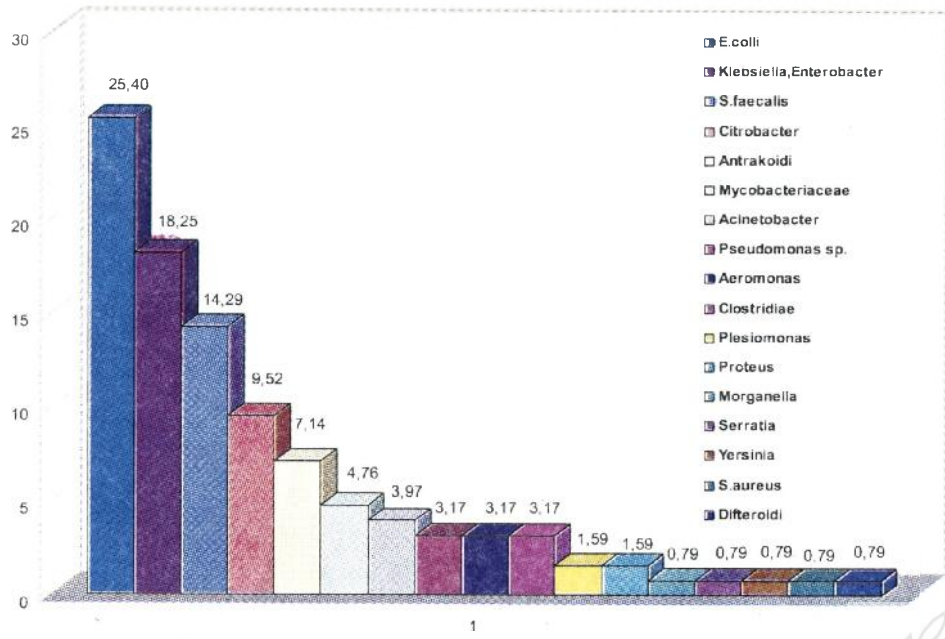
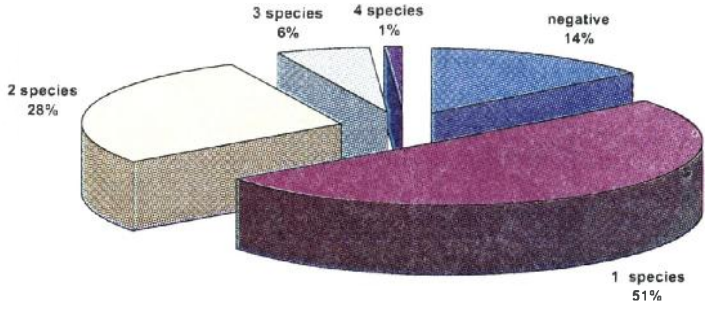


Figure 4: *Frequency of different genera / species of bacteria in the drinking water.*

Between 1 and 4 different kinds of bacteria were present in some samples of water (Figure5).

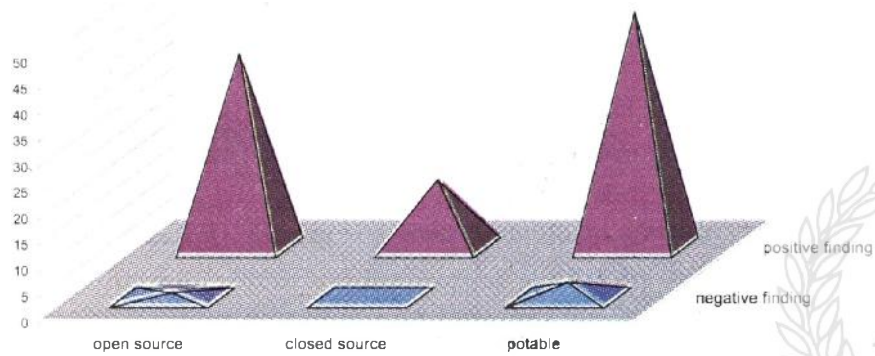
Figure 5: *Overview of isolated bacteria according to number of positive / negative finding per sample.*



In addition, a combination of different kinds of bacteria with other groups of micro-organisms were found: bacteria – bacteriophages 7; bacteria - virus 17; bacteria - virus – bacteriophages 24; bacteria - virus - algae 4; bacteria - virus - bacteriophages – algae 2; bacteria - bacteriophages – algae 3; bacteria - algae 3; bacteria - nematode - algae 1; bacteria + nematode 1.

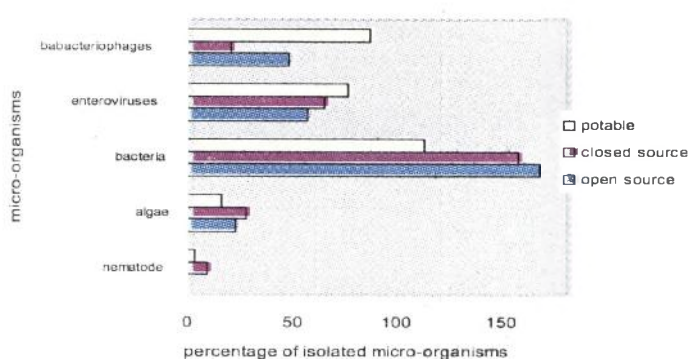
The finding of micro-organisms in the samples of water correlated with the type of water sources (Figure 6). All samples of water from closed sources were positive.

Figure 6: *Frequency of micro-organisms in the drinking water correlating with the type of sources*



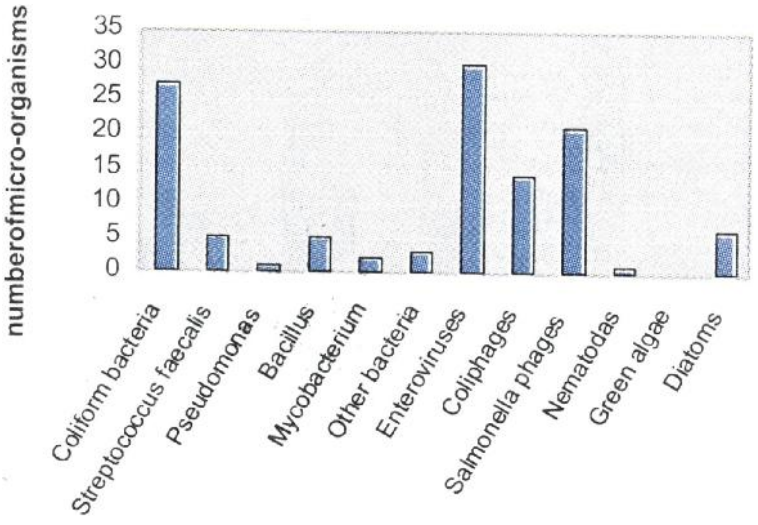
Frequency of different groups of micro-organisms was different in samples originating from the different sources: enteroviruses and bacteriophages were dominant in potable water; bacteria in water from open sources; algae and nematodes in water from closed sources (Figure 7).

Figure 7: *Groups of micro-organisms detected in the different kinds of drinking water.*



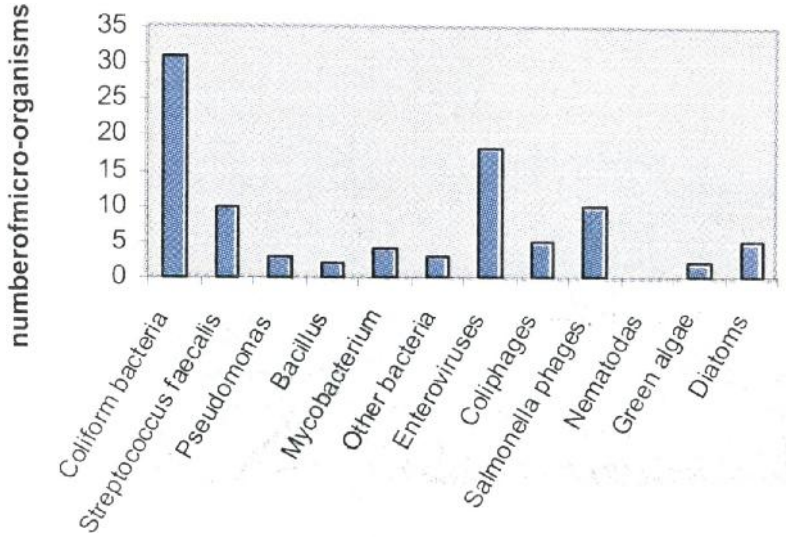
Order of frequency of genera / species of micro-organisms in water from different sources is presented in Figures 8, 9 and 10.

Figure 8: *Overview of the micro-organisms present in potable water.*



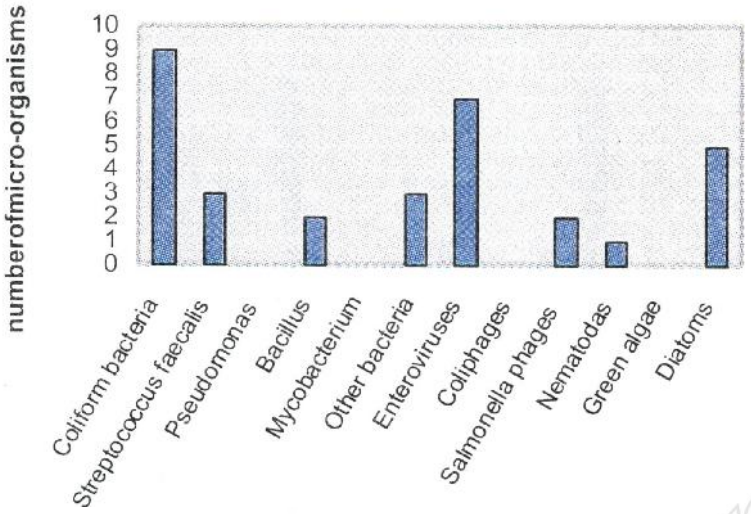
30 (62.5%) enteroviruses, 35 (72.9%) bacteriophages and coliform bacteria 27 (58.70%) were isolated from 48 samples of potable water.

Figure 9: *Overview of the micro-organisms present in open sources of water.*



There were 53 bacteria and 33 viruses (enteroviruses 18 and bacteriophages 5) in open sources (38).

Figure 10: *Overview of the micro-organisms present in closed sources of water.*



Numbers of isolated micro-organisams in closed sources were: bacteria 18, viruses 9, algae 3 and nematode 1.

Rapidly growing mycobacterium (Figure 11), M. fortuitum and M. gordone, were detected in six out of a total of 98 (6.06%) samples; potable water 4.35% and open sources 10.53%.

Figure 11: *Mycobacterium gordonae isolated from drinking water.*

Coliphages were found in 19 samples of water while salmonella-phages were found in 33 samples.

Figure12: *Agar culture medium of E. coli shows a mass of bacteria growth on which bacteriophages have produced visible plaques.*

There were found different combinations of viruses, bacteriophages and bacteria: viruses and bacteriophages 5; viruses and / or bacteriophages and bacteria 57; whilst viruses only were isolated in 4 samples and bacteriophages only in 1 sample.

Microscopic nematodes were found in two samples from potable and closed sources.

Members of algae, group Bacillariophycophyta (the Diatoms) and Chlorophycophyta (the Green algae), were found in 16 samples of water. Members of Chlorophycophyta were present in all kinds of water while Bacillariophycophyta was found only in the open sources (Figure 13).

According to the routine method for examination of water recommended by law, water from 35.35% (35/99) of sources was found suitable for drinking. After using a broad spectrum of tests for detection of enteroviruses, bacteriophages, nematodes and algae, 10.10% (10/99) of samples were in compliance with the law. There was a significant difference between positive results, which were obtained using two different methods ($p<0.01$).

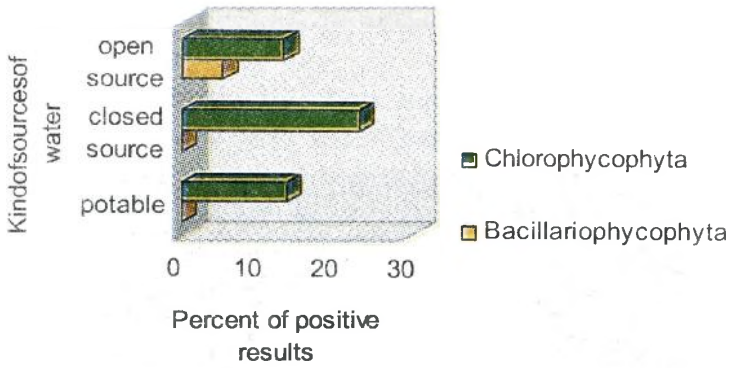


Figure 13: *The finding of algae in different sources of water*

Discussion and Conclusions

Urbanization and consequently the growing demand for water by communities has resulted in the establishing of different agencies in each country of the world who exercise jurisdiction over the many aspects of natural sources of water. The World Health Organisation has suggested physical and chemical water standards, which can be found in the W.H.O. Guidelines for drinking water quality. Protection from physical, chemical and biological contamination of water as well as monitoring of water quality is legally required in Bosnia and Herzegovina. Suggested standards of water quality and required tests can be found in "The rule book of hygienic quality of drinking water" Yugoslav Official Register 13/91 ("Pravilnik o higijenskoj ispravnosti vode za piće", Službeni list SFRJ 13/91).

Characteristics of good water quality as well as methods of examination are very similar all over the world. All guidelines for drinking water quality recommend the detection of coliform bacteria, Escherichia coli, Streptococcus faecalis as the most important sign of fresh faecal contamination of water and the detection of the presence of Pseudomonas acruginosa and sulphates-reducing bacteria as the sign of old faecal contamination (WHO 1981, WHO 1982, Poček B 1990, Yugoslav Official Register1992).

Following these guidelines for examination and estimation of water quality we found that 35.35% (35/99) of samples were in compliance with the law. From other samples (63/99) were isolated: coliform bacteria was 67 (E.coli 25,40%, Enterobacter/ Klebsiella 18.25%); Streptococcus faecalis (18); Pseudomonas aeuginosa (4); sulphate-reducing bacteria 4. Two, three or four kinds of bacteria were isolated from 35% of samples. The total number of coliform bacteria was quite high probably as a consequence of mixing different kinds of

water with drinking water. The positive total coliform bacteria test typically indicates surface intrusion in drinking water during the flood, but is not a specific indication of faecal contamination. It may serve as a screening test, because if total coliforms are absent, faecal bacteria are probably absent also.

Mycobacterium fortuitum and Mycobacterium gordone are prevalent in aquatic environments and they are usually saprophytic organisms but can be associated with a variety of infections in patients with immunodeficiency (Fujita Y at all. 2000). In this study they were found in 6.6% samples of water. Their being so prevalent in the potable water along with the increasing number of patients undergoing ambulatory dialysis in the area of Tuzla, infections from these organisms may emerge as a significant problem.

Until now, water has not been examined for mycobacterium in our country, but the finding of M. gordonae is common in some parts of the world and it is known as "tip water bacillus" (Koziumi T et al 2000).

Bacteriofages, coliphages and salmonella phages are bacterial in origin and could be used as an indicator of water polluted with Salmonella and Escherichia. Although Salmonella enteritidis was not detected in the water, its bacteriophages were found in 33/99 samples. These findings can explain the recent outbreak of Salmonella-infections. It was surprising that coliphages were detected in a lower percentage (19.19%) than Salmonella phage (33.33%). The frequency of bacteriophages in potable water was much higher (71,71%). than the finding of coliform bacteria (58,70%). The significance of coliform bacteria in detecting water pollution is known, but we consider it very important, as organisms indicative of pollution, to test for bacterophages and viruses. In a study, which took place in Israel and Spain in 1997, the frequency of isolation of bacteriophages (4.4% to 6.1%) was significantly higher than the frequency of isolation of coliform bacteria (1.9%) (Armon R at all1997). Samples of drinking water from different sources in Greater Cairo, Egypt, were positive for both total coliform and coliphage in 2.72%; 53% of samples were positive for coliphage but negative for total coliforms and faecal coliforms (el-Abagy et al 1990)).

Despite other intestinal micro-organisms, such as intestinal viruses (polio, coxackie, echo, hepatitis A, and rotaviruses), being potent pathogens which can cause serious diseases, we are not obliged by law to examine water for their presence. This study showed that virus pollution of water was very high in drinking water: potable water 63%; water from closed sources 53%; water from open sources 47%. Viral pollution of water is a very real problem in many countries. For example, over a period of two years, viruses were detected in 23% of 413 drinking water samples and 73% of 224 raw water samples in South

Africa. Enteroviruses were detected in 17% of drinking water samples (Grabow WO et al 2001).

Bacteria were associated with bacteriophages and viruses in 57 (58.16%) of samples whilst only viruses and bacteriophages were detected in 10 (10.2%) of samples. Coliforms concentrations could be correlated with enteric virus concentrations as well as with concentrations of coliphages but an absent of coliform bacteria does not exclude the present of viruses and bacteriophages.

Still other micro-organisms are regarded mainly as saprophytic, nuisance organisms. They create problems of odour, colour, and taste, or cause obstruction to the free flow of water. Among the most important nuisance organisms are slime-forming bacteria, iron bacteria, sulphur bacteria, algae and nematodes.

Members of the group Bacillariophycophyta and Chlorophycophyta are principally freshwater species. They are also found in moist soil and seawater. In this study algae (Bacillariophycophyta and Chlorophycophyta) were present in 16 samples of water. Their presence in drinking water in such high numbers is a sign a water inadequate chlorination or damage to a water-purification plant and/or supply system. Our results indicating high percentages of bacterial water pollution along with low levels or absence of chlorine serve to conform this statement.

Nematodes were found in two samples. There are thousands of different types of the microscopic worms on earth, many of them parasites of insects, plants or animals. Free-living species are abundant, including nematodes that feed on bacteria, fungi, and other nematodes. Their presence in drinking water is rare and is a sign of insufficient water purification and bacterial pollution.

Microbiological quality of water was absolutely insufficient in the area of Tuzla Canton in the specific time after the flood. Extremely high levels of bacterial pollution of drinking water were consistent with defects or breaks in the disinfections process.

Residual chlorine was found in sufficient concentration in 21.74%(10/48) of samples of water originating from municipal water supplies (potable water), while in other kinds of water, residual chlorine was not found. Different level of chlorination in different sources of water influenced the relationship between bacteria and viruses /bacteiophages. Numbers of isolated bacteria decreased and numbers of viruses increased in the water treated with an inadequate concentration of chlorine. This finding deserves further examination as it can be the consequence of the different methods that we have applied (using methods provided for the detection of live and dead viruses, we detected only live bacteria) and/or of liberation of viruses and bacteriophages from cells and their different biology.

In conclusion, after using a broad spectrum of tests for detection of bacteria, enteroviruses, bacteriophages, algae and nematodes, only 10% (10/98) of water samples were in compliance with the law. The routine method for examination of water recommended by law was not sufficient to detect serious contamination of water in 25.25% of cases. The difference is significant between positive results, which were obtained using two different methods ($p<0.01$). These results call for a revision of water quality guidelines based only on indicator organisms without also making reference to the absence of viruses.

We have pointed out the importance of all the parameters, which should be applied during emergencies such as the recent flooding. We also suggest that, along with routine examination of drinking water there should be periodically incorporated into the current protocol extra measures for detection of enteroviruses and bacteriophages.

Acknowledgements

We acknowledge Centro Regionale d' Intervento per la Cooperazione (C.R.I.C.) for funding the research and the authorities of Tuzla Canton for making available the resources for this work.

Special thanks due to:

All representatives from Health Centres from the area of Tuzla Canton who did the sampling of water in the area.

Mrs. Mubera Kutlovac for kindly giving so generously of her time to prepare some of the laboratory procedures.

Mrs. Patricia Prestidge for proof reading this document.

Apstrakt

Jedan od krucijalnih problema na podrucju Tuzlanskog kantona tokom prethodna dva desetoljeca je, sasvim izvjesno, deficit potrebnih kolicina vode, odgovarajuce kvalitete za humanu upotrebu. Nastao kao posljedica dugogodišnjeg slijeganja zemljišta usljed eksploatacije mineralnih sirovina, a potom oštećenja vodo-opskrbnih objekata usljed ratnih dejstava, te poplava koje su nedavno zahvatile ovo područje, uz neočekivano brzi porast broja stanovnika u toku ratnih i poratnih migracija, nedostatak vode pokazuje upozoravajuce tendencije daljeg rasta. Istovremeno, obilate oborine tokom ove godine, rezultirale su poplavama koje su, s obzirom na staru i oštećenu vodoopskrbnu infrastrukturu, dovele do zagadenja vode za piće. Bio je to razlog da pristupimo istraživanju kvalitete vode za piće, koristeći dvije

relevantne metode; standardnu metodu za pregled voda, uz dodatne posebne laboratorijske testove čiju korisnost potvrdjuju dobijeni rezultati.

Ciljevi mikrobiološke analize vode bili su:

a) ispitivanje vanjskog biološkog zagadenje, kao posljedice poplave u području Tuzlanskog Kantona u 2002.godini i

b)evaluacija propisanih laboratorijskih procedura u Bosni i Hercegovini za detekciju patogenih mikroorganizama u vodi za piće.

Ispitivanjem su obuhvaćena 99 uzorka vode: 48 uzoraka iz gradskih vodovoda, 13 iz zatvorenih izvorišta i 38 iz otvorenih izvorišta. Uzorci vode su testirani rutinskim bakteriološkim, parazitološkim i biološkim metodama, a reverzno transkripciona lančana reakcija sa polimerazom (reverse transcription –polymerase chain reaction / RT-PCR) je korištena za detekciju virusa.

Mikroorganizmi nisu nadeni u 4 (4.04%) od p99 uzoraka vode. Iz preostalih 95uzoraka vode izolovano je: 240 microorganizama i to: 114 sojeva bakterija, 56 virusa, 52 bakteriofaga (19 faga Escerichia coli, i 33 Salmonella enteritidisfaga), 2 nematode, 16 algi.

U skladu sa osnovnim mikrobiološkim pregledom vode za piće propisanim zakonom, voda iz 35,35% (35/99) izvorišta bila je odgovarajućeg kvaliteta, dok je nakon pregleda dodatnim testovima samo 10.10% (10/99) uzoraka bilo u skladu sa zakonskim propisima. Nadena razlika je visoko statistički značajna.

Ovi rezultati ukazuju na potrebu revizije osnovnog pregleda «Pravilnik o higijenskoj ispravnosti vode za piće», baziranog na detekciji indicatorskih mikroorganizama, bez pregleda na prisustvo virusa. Smatramo da je neophodno pratiti sve te parametre mikrobiološke ispravnosti vode u toku elementarnih nepogoda, kao periodično (mjesečno ili godišnje) u toku rutinskih pregleda.

References

Armon R, Kott Y. *A simple, rapid and sensitive presence /absence detection test for bacteriophage in drinking water.* J Appl Bacteriol 1993; 74 (4) : 490-496.

Armon R, Araujo R, Kott Y, Lucena F, Jofre J. *Bacteriophages of enteric bacteria in drinking water, compararison of their distribution in two countries.* J Appl. Microbiol 1997; 83 (5): 672-633

Brajković M. *Zoologija intervertebrata I dio, 1. izdanje.* Zavod za udžbenike i nastavna sredstva, Beograd 2001.

Cheesbrough M. *Medical laboratory manual for tropical countries, 1 st Edit.* Tropical health technology, Butterworth-Heinemann, Cambrige 1992.

el-Abaqu MM, Dutka BJ, Kamell M, el-Zanfaly HT. *Incidence of coliphage in potable water supplies.* App Environ Microbiol 1988; 54 (6): 1632-3.

Fujita Y at all. *Pulmonary infection caused by Mycobacterium gordone in imunocompetent patient.* Kekkaku 2000; 369-374.

Grabow WO, Taylor BM, de Villiers JC. *New methods for the detection of viruses: call for review of drinking water quality guidelines.* Water Sci Tehnol 2001; 43 (12): 1-8.

Hindak F. *Slatkovodne riasy.* Slovenske pedagogicke nakladatelstvo, Bratislava 1978.
Koziumi T at all. *Pulmonary Mycobacterium gordone infection treated with Clarthormycin.* Kekkaku 2000; 75 (12): 711-715.
Mandell GL, Bennett JE, Dolin R. *Principles and practice of infectious diseases.* 4th Edit. Churchill Livingston, New York 1995.
Nedachin AE, Dimitrieva RA, Doskina TV, Sidorenko SG, Rakhmanin IuA. *Coliphages as indicators of viral pollution of water supply.* Gig Sanit 1996; 5: 3-6
Poček B. *Metode za bakteriološki, virusološki I parazitološki pregled vode za piće.* (In): Đuričić M, Škunca-Milovanović S, Feliks R, Đurović B. Voda za piće – Standardne metode za ispitivanje higijenske ispravnosti. Beograd: Savezni zavod za zdravstvenu zaštitu NIP "Privredni pogled", 1990: 623-655.
World Health Organization. *Drinking-water and Sanitation 1981-1990.* A Wayo to Health, 1981. WHO, 1211 Geneva, 27-Switzerland.
World Health Organization. *WHO Guidelines for Drinking Water Quality,* Volumen I, EFP/82.39, 1982. WHO, 1211Geneva, 27-Switzerland.

ANU
BiH
АНУ
БиХ

PHARMACOLOGYCAL PROPHYLAXIS OF EARLY POSTTRAUMATIC EPILEPSY IN 314 CHILDREN AND ADOLESCENTES

Gavranović M.[1], *Konjhodžić F.*[1], *Zubčević S.*[1], *Ćatibušić F.*[1], *Zečić S.*[1]

Abstract

Introduction. Early posttraumatic epilepsies (EPTE) are epileptic attack that appear in first seven days after brain injury, with incidence of 3-5%.

Predictors for development of EPTE are: impressive skull fracture with rupture of dura, intracranial hemorrhage, neurogical deficit (brain contusion), posttraumatic amnesia longer than 24 hours. It is more common in children than in adolescents and adults. It carries four times increased risk for development of late posttraumatic epilepsy. Aspects of pharmacological prophylaxis was often considered, but scientifically neglected, without clear standings regarding controversial data in literature.

Methods. Patients with severe head injury, hospitalised at Neurosurgical Hospital, Clinical Center University of Sarajevo, in period from 6th of April 1992 til 1 th of Julz 1994, were included in studz. Prophzlaxis of EPTE was carried out with Phenobarbital (2 - 3 mg/kg) or Phenytoin (3mg/kg) parenteray. Decision was made upon clinical findings. CT scan was done in 13,5% patients and in 31,2% patients serum concentrations of antiepileptic drugs were monitored.

Results. 314 patients aged 0 – 20 years (106 patients 0-10years, and 209 patients 11-20 years) were investigated. Predictors of EPTE presented were posttraumatic amnesia longer than 24 hours in 90,4%, neurogical deficit in 86,6%, impressive skull fracture with rupture of dura in 81,5% and intracranial hemorrhage in 41, 1%. Only two boys developed EPTE in first 24 hours after injury.

Conclusion. This study has showd that use of antiepileptic drugs can decrease incidence of EPTE. However, problem remains, management of injured patients is still highly individualized, based on different experiences of doctors that treat patient and without clear guidelines.

Key words: eary posttraumatic epilepsy, prophylaxis.

[1] Clinical Center, University of Sarajevo, Bosnia and Herzegovina

Introduction

Craniocerebral injuries (CCI) have specific significance in everyday life of a modern man, due to their frequency, severity, neurological and psychological consequences. According to study of Konjhodžić (1995)[1] during the war in Bosnia and Herzegovina, in siege of Sarajevo, from 1992-1995, CCI were extremely severe. In etiology of epilepsies trauma participates in 23% of cases (Oliveros et al. 2002)[2]. Epileptic seizures can appear at different period after the trauma. Early posttraumatic epilepsy (EPTE) consists of epileptic attacks that appear in the first seven days after the head injury. Kuhl et al. (1990)[3] suggest that the incidence of EPTE is 3-6%. Lee et al. (1997)[4] investigated 3340 adults with severe closed head injury and found EPTE in 3,6% of traumatized patients. Predictors for development of EPTE are: impressive skull fracture with rupture of dura, intracranial hemorrhage, neurogical deficit (brain contusion), unconsciousness or posttraumatic amnesia longer than 24 hours[4,5,6,7,8]. EPTE usually presents in the form of partial motor seizures that are closely related to localization of lesion. About 33% of patients can have generalized tonic clonic seizures. Clinicians are still in doubt about pharmacological prophylaxis and use of antiepileptic drugs in patients with EPTE. There are no clear guidelines despite that the first clinical trials have had shown benefit of this therapy 50 years ago (Hoff and Hoff 1947[9]; Birkmayer 1951[10]).

Methods

Patients hospitalized at Neurosurgical department of Clinical Center, University in Sarajevo, between 6th of April 1992 and 1st of July 1994, with severe brain injury and pharmacological prophylaxis of EPTE, were included in study. During the war in Bosnia and Herzegovina we had only Phenobarbital and Phenytoin for parenteral use at disposition. During the studied period we run out of Phenytoin, and had to use only Phenobarbital. Parenteral treatment was started in admission room with Phenobarbital 2-3 mg/kg/day or Phenytoin 3 mg/kg/day. This treatment lasted for 7 days or shorter after the injury, and was followed by per oral administration after steady serum concentrations were achieved. Decision was made upon clinical findings in majority of cases.

Results

We investigated 314 patients aged 0-20 years (105 patients 0-10 years, and 209 patients 11-20 years). There were only 4 patients aged 18-20 years.

Presented predictors of EPTE were: posttraumatic amnesia longer than 24 hours in 284 patients (90,44%), neurogical deficit in 272 patients (86,62%), impressive skull fracture with rupture of dura in 256 patients (81,53%) and intracranial hemorrhage in 129 patients (41,08%).

Only in 42 patients it was possible to perform CT scan (13,38%).

In 98 patients we were able to assess serum concentrations of antiepileptic drugs (31,21%).

Out of 314 patients, 251 (79,94%) were receiving Phenobarbital, and 63 (20,06%) Phenytoin as a prophylaxis for EPTE.

Only two boys developed EPTE in the first 24 hours after injury. One was 6 and other 7 years of age. Both of them developed partial motor seizures, with secondary generalization. One had only one attack, and other had repeated seizure.

Discusion

It is very interesting that the aspect of pharmacological prophylaxis of EPTE was often discussed in clinical trials and basic studies, but scientifically it was neglected. There are still no clear recommendations with precise criteria for treatment, and the data in literature are controversial (Kuhl et al., 1990[3]; Segatore et al., 1993[11]; Kobayashi et al., 1997[12]). Why have we decided for pharmacological prophylaxis of EPTE with Phenobarbital or Phenytoin? There were several reasons. First, craniocerebral injuries were extremely severe, and more than 80% of traumatized patients did have three or more risk factors for development of EPTE. Second reason was that traumatized patients were children and adolescents, and in this age group patients have EPTE more frequently than others. This is in correlation with studies by other authors (Nakamura et al., 1997[13]; Asikainen et al., 1999[7]). Other, newer studies, are confirming that we made appropriate decision (Barlow et al., 2000[14]), and they are pointing that severity of brain lesion is dictating severity of EPTE and later neurological development. Besides that, EPTE carries 4 times greater risk for development of late posttraumatic epilepsy. American Academy of Physical Medicine and Rehabilitation (1998)[15] is also recommending prophylaxis of EPTE. Third, in our study from 1984 done by Konjhodžić et al.[16], with 1830 cases with closed and opened craniocerebral injuries, where the traumas were far less severe, and patients did not get prophylaxis of EPTE, incidence of EPTE was 2,40%.

This problem remains in focus of epileptologists through out the world. Studies from last several months are also recommending prophylaxis of EPTE, and are pointing out the reduction of incidence of

EPTE with appropriate treatment on time (Oliveros et al., 2002[2]; Brophy et al., 2002[17]).

In stead of conclusion we may with certainty say that this study has showed that use of antiepileptic drugs immediately after trauma can prevent EPTE, and probably decrease further invalidity of traumatized persons, as well as later development of late posttraumatic epilepsy.

However, problem remains, management of injured patients is still highly individualized, based on different experiences of doctors that treat patient, and without clear guidelines.

Apstrakt

Uvod. Rana posttraumatska epilepsija (RPTE) su epileptične atake koje se javljaju u prvih 7 dana nakon povrede, a incidenca se kreće od 3-5%. Prediktori za razvoj RPTE su: impresivna fraktura sa ruputurom dure, intrakranijalna krvavljenja, neurološki deficit (kontuzija mozga), posttraumatska amnezija (PTA) duža od 24 sata. Češća je kod djece nego kod adolescenata i odraslih, a značajna je jer nosi četiri puta veći rizik za kasnu posttraumatsku epilepsiju. Aspekt farmakološke prevencije je često razmatran, ali naučno zanemarivan i bez jasnih stavova, sa kontroverznim podacima u literaturi.

Metod. U studiju su uključeni pacijenti hospitalizirani u periodu od 06.04. 1992. do 01.07.1994. na Neurohirurškoj klinici Kliničkog centra Univerziteta u Sarajevu, koji su doživjeli tešku povredu mozga i kod kojih je provođena prevencija RPTE phenobarbitonom (2-3 mg/kg parenteralno) i phenytoinom (3mg/kg parenteralno). Odluka je donesena na osnovu kliničkog nalaza, a kod 13,4% pacijenata je učinjen CT mozga. Kod 31,2% pacijenata su određivani nivoi antiepileptika u serumu.

Rezultati: Ispitano je 314 pacijenata u životnoj dobi od 0-20 godina (105 od 0-10 godina i 209 od 11-20 godina), a prisutni predikatori za razvoj RPTE su bili: PTA duža od 24 h kod 90,4%; neurološki deficit kod 86,6%; impresivna fraktura sa rupturom dure kod 81,5%; intrakranijalno krvavljenje kod 41,1%. Samo dva dječaka su imala RPTE u prva 24h nakon povrede.

Zaključak. Ovo istraživanje je pokazalo da se primjenom antiepileptika može znatno smanjiti incidenca RPTE. Međutim, problem i dalje ostaje otvoren i briga za traumatiziranog je široko individualizirana u skladu sa iskustvima svakog pojedinog liječnika, jer nema jasnih stajališta.

Ključne riječi: *Rana posttraumatska epilepsija, prevencija*

Literature

Konjhodžić F. *Neurosurgical injuries in defensive war in Bosnia and Herzegovina.* Oko, Sarajevo; 1995.

Oliveros JA, Bertol V, Oliveros CA. *Preventive prophylactic treatment in posttraumatic epilepsy.* Rev Neurol 2002; 34(5): 448-459

Kuhl DA, Boucher BA, Muhlbauer MS. *Prophylaxis of posttraumatic seizures.* DICP 1990; 24(3): 277-285.

Lee ST, Lui TN, Wong CW, Yeh YS, Tzuan WC, Chen TY, Hung SY, Wu CT. *Early seizures after severe closed head injury.* Can J Neurol Sci 1997; 24(1): 40-43

Wilmore LJ. *Posttraumatic Seizures.* neurol Clin 1993; 4: 207-210

Kieslich M, Jacobi G. *Incidence and risk factors of posttraumatic epilepsy in childhood.* Lancet 1995; 345: 187

Asikainen I, Kaste M, Sarna S. *Early and late posttraumatic seizures in traumatic brain injury rehabilitation patients: brain injury factors causing late seizures and influence of seizures on long-term outcome.* Epilepsia 1999; 40(5): 584-589

Haltiner AM, Temkin NR, Winn HR, Dikmen SS. *The impact of posttraumatic seizures on 1-year neuropsychological and psychosocial outcome of head injury.* J Int Neuropsychol Soc 1996; 2(6): 494-504

Hoff H, und Hoff H. *Fortschritte in der Behandlung der Epilepsie.* Mschr. Psychiat. Neurol 1947; 114: 105-118.

Birkmayer W. *Die Behandlung der traumatischern Epilepsie.* Med. Wshr 1951; 63: 606-609.

Segatore M, Jacobs M. *Posttraumatic seizures: consensus and controversies.* Axone 1993; 15(2): 34-39

Kobayashi M, Ohira T, Ishihara M, Shiobara R, Kawase T, Toya S. *Cooperative multicentre study on posttraumatic epilepsy.* No To Shinkei 1997; 49(8): 723-727

Nakamura A, Ohira T, Ishihara M, Kobayashi M, Shiobara R, Toya S, Takakura K, Ohwada T, Murase I, Ichikizaki K, et al. Coop*erative multicentric study on posttraumatic epilepsy.* No To Shinkei 1995; 47(12): 1170-1176

Barlow KM, Spowart JJ, Minns R. *Early posttraumatic seizures in non-accidental head injury: relation to outcome.* Developmental Medicine & Child neurology 2000; 42: 591-594

Practice parameter: antiepileptic drug treatment of posttramatic seizures. Brain Injury Special Interest Group of the American Academy of Physical Medicine and Rehabilitation. Arch Phys Med Rehabil 1998; 79(5): 594-597

Gavranovic M. *Epilepsija kod kraniocerebralnih povreda.* U: Kraniocerebralne povrede (ur. Konjhodžić), Sarajevo, Veselin Masleša, 1984; 140-148.

Brophy GM, Tesoro EP, Schrote GL, Garnett WR. Pharmacist impact on posttraumatic on posttraumatic seizure prophylaxis in patients with head injury. Pharmacotherapy 2002; 22(2): 251-255.

ANU
BiH
АНУ
БиХ

UTILITY AND ALGORITHM OF IMAGING MODALITIES IN DIAGNOSIS AND STAGING OF BRONCHOGENIC CARCINOMA

Faruk Dalagija[1], Faris Gavrankapetanović[2], Šerif Bešlić[1], Amela Mornjaković[1]

Abstract

The utility and algorithm of diagnostic and particularly radiological conventional and digital methods in diagnosis and staging of central and peripheral, non-small cell and small cell bronchogenic carcinoma are presented in this paper.

Their sensitivity and specificity in the assessment of malignant mediastinal and hilar adenopathy, pleural and chest wall infiltration and tissue categorisation of the solitary pulmonary nodule are discussed.

It is concluded that digital methods - spiral CT, US and MRI, in spite of quoted limitations, have considerably improved the diagnosis and staging of bronchogenic carcinoma.

Although CT is superior over conventional radiological methods, it should be used in definitive staging complementary with the other imaging modalities, endoscopic and bioptic methods, as well as obligatory histological confirmation of the .diagnosis.

Key words: bronchogenic carcinoma, diagnosis, algorithm, imaging modalities.

Introduction

Although the first announcements regarding bronchogenic carcinoma (BC) have been seen in autopsy reports from 18th century, a hundred years ago BC compared to other types of carcinoma was rare.

Relation between BC and smoking is undisputable since long time ago.

The consumption of cigarettes in US have started to increase significantly around 1915, and twenty years later a parallel increase in number of diseased people and mortality caused by BC was apparent. In

[1] Institute of Radiology CCU Sarajevo
[2] Clinic for Orthopaedics and Traumatology of Sarajevo

1935, 4300 people died of it, and in 1957 more than 135 000 people died of the same cause (1).

According to the latest reports from World Health Organization the following has been concluded:

- In spite of thousands of studies which have shown that tobacco in all it's forms kills it's users and smoking of cigarettes kills a non-users as well, people continue to smoke, and the mortality caused by tobacco consumption is still increasing.
- Almost one billion people in the world smoke – about 35% in developed and 50% in developing countries. The trend in both countries shows that the level of smoking among men have reached it's peak and is slowly decreasing, having in mind that this is the habit of poor and less educated men.
- About 250 million women in the world smoke – about 22% in developed and 9% in developing countries. Smoking cigarettes among women is decreasing in many developed countries but not in all – in South, East and Central European countries is even increasing.
- In general, more and more people smoke and smokers consume more and more cigarettes. Tobacco production have reached the proportion of global epidemic. Tobacco industry around the world is producing almost one thousand cigarettes per each man, woman and child on this planet annually.
- The vast majority of smokers start using tobacco in their early youth. Among young people, almost one quarter of smokers start smoking before they reach 10 years-of-age. The sooner they start smoking the bigger the risk of acquiring diseases related to smoking (cancer, heart diseases).

A special emphasis is put on the danger from passive smoking:

- The risk of BC among non-smokers exposed to passive smoking have increased to 20-30%, and the risk of heart disease increased to 23%.
- 40% of world children is exposed to passive smoking in their homes.
- Smoking in pregnancy is dangerous both for the mother and for the fetus, and exposing of pregnant woman to passive smoking can damage fetus as well.

Further on, the following has been emphasized:

- Tobacco is the greatest killer, much greater than all other forms of pollution and greater than AIDS, drugs, car accidents, murders and suicides in total.

- Tobacco is a killer and should not be advertised or financially supported.
- Every cigarette shortens smoker's life for 7 minutes.
- Every 10 seconds in the world one person dies of a smoking-related disease.
- Children are most vulnerable. Girls and women throughout world are targets of expensive and attractive advertisements of smoking as a symbol of freedom, emancipation, enthusiasm and wealth.
- Tobacco companies are responsible for damaging consequences of smoking.
- Practically, all body part are exposed to damaging consequences of the tobacco use (hair, skin, limbs, bones, eyes, teeth, respiratory, cardiovascular, nervous, gastrointestinal, urogenital, haematopoetic and immune system, male and female reproduction etc).
- Smoking is responsible for: 90% of lung cancer, 75% of chronic bronchitis and emphysema, and 25% of ishaemic heart disease.
- Half of smokers die in their middle age – between 35 and 65 years-of-age.
- At the beginning of 21st century about one third of young people in the world, including the increasing number of women, use tobacco.

The results of the researches performed during last 5 years show more and more gloo - my picture caused by the danger of smoking:

- Annual mortality caused by smoking in industrial countries in 1950 was 0,3 million, in 1975 – 1,3 million, in 2002 – 2,1 million, and a prediction for the period between 2025 and 2030 is 3 million people.
- In developing countries annual mortality for 1950 is unknown, in 1975 was 0,2 million, in 2000 – 2,1 million, and a prediction for the period between 2025 and 2030 is 7 million people (2).

Regarding the therapy for BC, as we know, certain progress has been made in the last twenty years (introduction of new cytostatic drugs, their combination, synergism, reduced toxicity, it's combination with radiotherapy etc). But a significant increase in length of survival period have not been achieved yet, unfortunately.

In spite of the mentioned increase in the incidence and mortality from BC, the degree of resectability has not changed significantly, which emphasizes the fact that, still, there are no successful methods for

establishing an early diagnosis. That is why the fight against smoking in order to prevent and stop tobacco addiction, is the only safe way of reducing its incidence.

Diagnostic methods

Bronchogenic carcinoma is a mean disease because its symptoms may lack or are discrete and not typical, especially at the onset of the disease. That is the reason why its diagnosing is complex and difficult. The main goal during the evaluation of the patient with any neoplasm is to reach a correct patohistological diagnosis. The next step is to determine its degree of spread-classification or staging, in order to apply adequate (optimal) therapy as an important assumption for the extension of patient's survival (1,3).

Beside valuable clinical, laboratory and especially endoscopic and biopsy methods, there are digital radiological diagnostic methods with aspiration biopsies that are used in diagnosing BC.

When staging a non- small cell BC the aim is to determine its resectability in order to dissect a tumor with all involved mediastinal lymph nodes of the N_2 patient or in order to apply a pre-operative or post-operative chemotherapy and radiotherapy, which will help increase a 5-year survival of patients (4,5,6,7).

Although CT, MRI, scintigraphy and biopsy can discover occult extrathoracic lesions in brain, liver, suprarenal gland or bones in asymptomatic patients as well, these are not considered a routine methods and are only indicated on the basis of anamnesis, physical examination and laboratory results. There was a warning that there is an increase of incidence of adenocarcinoma with occult metastasis in brain in at least 20% of these patients and there is a possibility of resection of solitary brain metastasis (8).

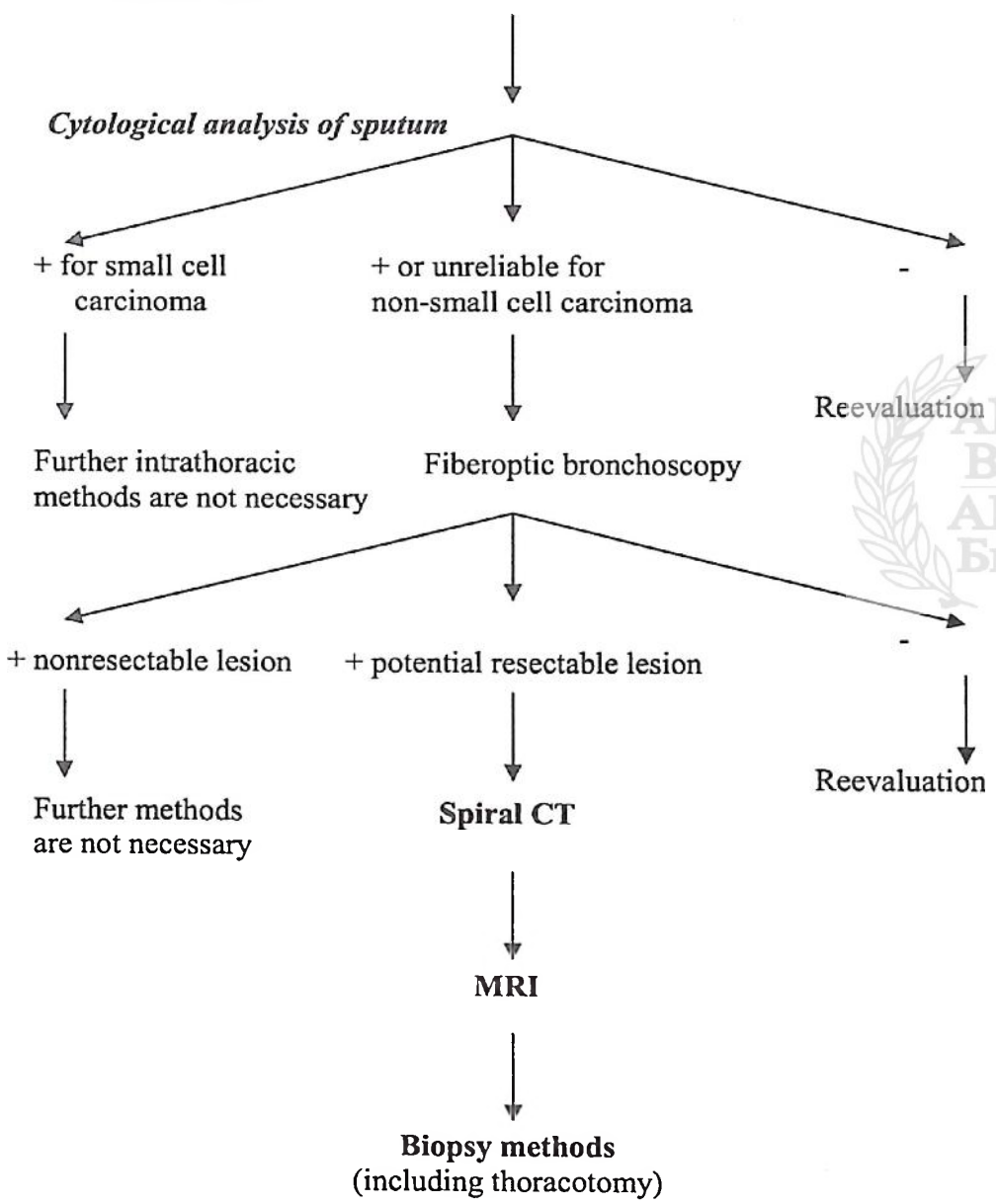
Staging of small cell BC is different compared to a non-small cell BC because of it's high tendency to metastasizing. According to results of huge studies, following percentages of these lesions were found: intracranial lesions – in 10-15%, liver lesions – in 28%, and bone lesions – in 29% of the patients (9). That is why CT, ultrasound, scintigraphy and biopsy were recommended as a routine methods in staging of patients with small cell BC. Unfortunately, when implemented for complete staging, about one third of patients will be in the stage of limited disease and two thirds in the stage of spread disease (1).

Algorithm and discussion

In algorithm of diagnostic methods in patients with central BC after performing a chest radiography of thoracic organs, cytological

analysis of sputum, bronchoscopy, CT and MRI, obligatory are biopsy methods (including thoracotomy) with a sequence interrupted in a case of small cell and nonresectable non-small cell carcinoma. (**Scheme 1.** adjusted to Robert D. Pugatch's Scheme, 1986.).

Scheme 1. *Algorithm of diagnostic methods*
- central bronchogenic carcinoma -



A standard radiography is considered useful in the initial detection and screening of patients with central BC.

False-negative results are seen in the initial phase of metastasizing into lymph nodes and small lesions in bronchial lumen with no obstruction, while false-positive results are seen when vascular structures or fat tissue collections are misdiagnosed as a tumor (10, 11).

Although a classic CT has been considered a standard method in detection of mediastinal abnormalities, it was possible to make an error in estimating invasion of mediastinal structures - pleura or thoracic wall (excluding bone structures), and spiral CT is considered superior in such cases (11, 12, 13, 14).

Earlier percentage of CT sensitivity regarding the involvement of lymph nodes following the criteria of its size was very high (up to 95%), while later it has decreased (down to cca 65%). False-negative results were seen in cases of positive lymph nodes without their enlargement, especially in adenocarcinomas (14, 15, 16, 17, 18).

Specificity, as well, was high (cca 80%) depending on the criteria of the size, and it has decreased (down to cca 65%). False-positive results were seen in cases of enlarged lymph nodes caused by something else (e.g. reactive hyperplasia), and biopsy is necessary (8, 14, 18, 19).

It is believed that MRI has advantages over CT in preoperative staging because it provides us with additional information; it easily differentiate lymph nodes from blood vessels; provides analysis of subcarinal and aortopulmonal region; easily differentiate the confinement of tumor to bronchus or its penetration to tracheal carina. MRI is a method of choice for apical tumors with the involvement of soft tissue structures of the neck (8, 14, 20, 21, 22, 23).

In the algorithm of diagnostic methods in cases of solitary pulmonary nodule (SPN) up to 3 cm in diameter, a chest radiography is performed and after that a CT, in order to determine the presence of calcifications. Nodules with low CT numbers undergo biopsy, and a positive ones or inconclusive ones are indication for the resection using thoracoscopy or thoracotomy (8, 24, 25) (**Scheme 2.** adjusted to Robert D. Pugatch's Scheme, 1986.)

The principle: " Watch and wait" in cases of SLN is not recommended, especially not in high-risk cases, and it is obligatory to reach a definite diagnosis in spite of the lesion's size (8).

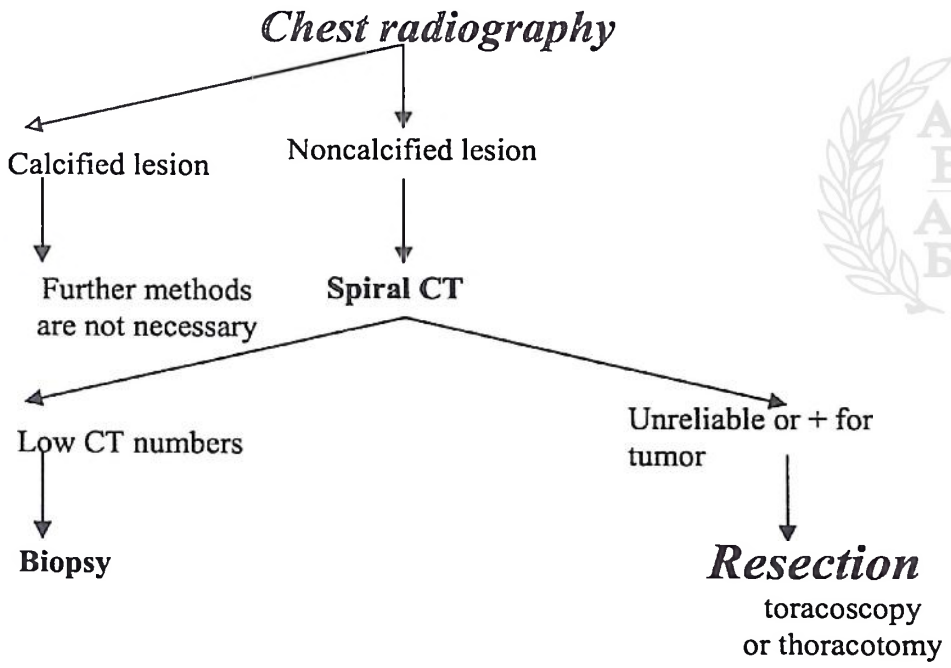
A chest radiography is considered an initial method in detection of all SPNs, and a threshold in detection is lesion's diameter of 1 cm. Lesions that have 1-2 cm in diameter can be overlooked because of the superposition of the bone and vascular structures, especially those that have less than 1 cm in diameter, which is confirmed by a comparison with previous x-rays (8).

The value of CT is in its possibility to confirm the presence of nodule; it is reliable in detecting and characterizing calcifications within a tumor, as well as in detecting enlarged lymph nodes and occult lesions on other sites (24, 25).

Sensitivity of classic CT in a detection of SPNs is high. False-negative results are seen in lesions whose diameter is below a threshold of resolution, in partial volume effect or moving of the lesion due to breathing (25, 26, 27).

Specificity of classic CT is very high in cases of high CT numbers and in cases when calcifications are present, and varies in cases of low CT numbers because such numbers are seen in 50% of benign and in majority of malignant lesions. False-positive results are seen in benign lesions without calcifications or not apparent ones, so the lesion is considered malignant (24, 25, 26, 27, 28).

Scheme 2. Algorithm of diagnostic methods
- peripheral bronchogenic carcinoma (SPN) -



BC show a benefit of using a low-dose CT in screening asymptomatic early BC (eight BCs were discovered in more than 700 heavy smokers; all eight of them were operable) (33).

CT fluoroscopy that provides a continuous CT image and easier visualization, is more and more used in interventional procedures (trans-bronchial biopsy). Its disadvantage is a high exposure to radiation (34).

It is also agreed that CT is useful in estimating a possibility of reaching a positive histological results in samples taken with fiberoptic bronchoscopic biopsy, by using numerous CT indicators (tumor's irregular form and blur contours; if the distance between a tumor and a starting point of the nearest lobar bronchus is less than 4 cm; endobronchial component in the tumor itself; segmental or some bigger air way that leads toward a tumor (35).

Because of a well known limitations (low proton density, artifacts of sensibility due to air – tissue interaction, loss of signal due to breathing and cardiovascular movements), lungs have been neglected in their imaging using MRI. But, thank to numerous technical improvements of the method and application of contrast media, its use has increased both in presentation of lungs as well as in performing lung angiography (characterization of lung nodules; estimation of stages of BC, especially in estimation of thoracic wall's involvement, estimation of inflammatory process in cases of interstitial disease of the lungs, acute lung embolism; chronic thromboembolic lung hyperten-sion; vascularization of malignant neoplasm; vascular abnormalities). Soon, a perfusion images (by using extracellular and intracellular contrast media) will be available, together with a ventilation images (by using inhaling hyperpolarizing gas, paramagnetic oxygen or contrast media in a form of aerosol). It is believed that combination of visualization, morphology and functional evaluation of ventilation and perfusion is better than any other technique (36).

It is also confirmed that ECG-triggered 3D contrast MR angiography has improved the quality of images and possibility of finding hilar and mediastinal invasion of BC (37).

Further on, it was found that a diagnostic potential of virtual bronchoscopy compared to bronchospcopy, axial CT sections, a multiplanar reconstruction (MPR) and minimal intensity projections (MIP), has advantages (passing by stenosis is possible only by using virtual endoscopy). That is why it is believed that virtual endoscopy is a non-invasive method of identification of endoluminal tumors and is comparable with real bronchoscopy (38).

Finally, PET scanning is considered superior to actual "gold standard" – CT scanning in clinical staging of mediastinum in cases of non-small cell BC (39).

Conclusions

Digital radiological diagnostic methods like CT, ultrasound, MRI and especially spiral CT have considerably improved diagnostics of BC. They are superior to classic radiological methods in estimating

its stage according to TNM classification and in planning optimal treatment.

Difficulties exist regarding its sensitivity and specificity in estimating malignant mediastinal and hilar adenopathy, invasion of mediastinal structures, pleura and thoracic wall, as well as in categorizing the nature of SPN.

Beside above mentioned difficulties, chest radiography and spiral CT are considered a valuable standard methods in diagnosis of local and regional spread of BC. But for definitive staging in algorithm of diagnostic methods, these should be used complementary to the other digital methods with obligatory endoscopic and biopsy methods that will provide us with patohistological confirmation of the diagnosis.

Apstrakt

Vrijednost i algoritam dijagnostičkih, a posebno radioloških konvencionalnih i digitalnih metoda u dijagnostici i steidžingu centralnog i perifernog, nemikrocelularnog i mikrocelularnog bronhogenog karcinoma, su prezentirani u ovom radu.

Njihova senzitivnost i specifičnost u procjeni maligne medijastinalne i hilarne adenopatije, infiltracije pleure i torakalnog zida, te karakterizacije prirode solitarnog plućnog nodula, su diskutovane.

Zaključeno je da su digitalne metode - CT, UZ i MRI, a posebno spiralni CT, i pored navedenih ograničenja, znatno unaprijedile dijagnostiku i steidžing bronhogenog karcinoma.

Mada je superioran nad konvencionalnim radiološkim dijagnostičkim metodama, CT u definitivnom staging-u treba koristiti komplementarno s ostalim imidžing, endoskopskim i bioptičkim metodama, uz obaveznu patohistološku potvrdu dijagnoze.

References

Bunn PAJr. *Lung cancer: current understanding biology, diagnosis, staging and treatment.* Bristol-Myers Company, 1988.

Mackay J, Eriksen M. *The Tobacco Atlas.* World Health Organization, 2002.

Nielsen ME, Heaston DK, Dunnick NR, Korobkin M. *Preoperative CT evaluation of adrenal glands in non-small cell bronchogenic carcinoma.* AJR 1982; 139: 317-20.

Gralla RJ. *Preoperative and adjuvant chemotherapy in non-small cell lung cancer.* Semin Oncol 1988; 15: 8.

Armstrong JG, Martini N, Kris MG. *Induction chemotherapy for non-small cell lung cancer with clinically evident mediastinal lymph node metastases: the role of postoperative radiotherapy.* Int J Radiat Oncol Biol Phys 1992; 23: 605.

Yashar J, Weitberg AB, Glicksman AS. *Preoperative chemotherapy and radiation therapy for stage III a carcinoma of the lung.* Radiology 1993; 186: 586.
Potepan P, Meroni E, Spagnoli I, et al. *Non-small cell lung cancer: detection of mediastinal lymph node metastases by endoscopic ultrasound and CT.* Eur Radiol 1996; 6: 19-24.
Pugatch RD. *Radiologic evaluation in chest malignancies. A review of imaging modalities.* Chest 1995; 107 (6 Suppl): 294 S-297 S.
Ihde DC, Hansen HH. *Staging procedures and prognostic factors in small cell carcinoma of the lung.* In Greco FA, Oldham RK, Bunn PAJr (eds): Small cell lung cancer. New York, Grune and Stratton, 1981: 261-83.
Faling LJ, Pugatch RD, Jung-Legg Y, et al. *Computed tomographic scanning of the mediastinum in the staging of bronchogenic carcinoma.* Am Rev Respir Dis 1981: 124-690.
Pugatch RD. *Bronchogenic carcinoma.* In Mc Neil BJ, Abrams HL (eds): Brigham and women's hospital handbook of diagnostic imaging. Little, Brown and Company,Boston-Toronto, 1986: 254-8.
Lewis JWJr, Pearlberg JL, Beute GH, et al. *Can computed tomography of the chest stage lung cancer? Yes and no.* Ann Thorac Surg 1990; 49: 591-596.
Primack SJ, Lee KS, Logan PM, et al. *Bronchogenic carcinoma: utility of CT on the evaluation of patients with suspected lesions.* Radiology 1994; 193: 795-800.
Hanson JA, Armstrong P. *Staging intrathoracic non-small-cell lung cancer.* Eur Radiol 1997; 7: 161-172.
Daly BDTJr, Faling LJ, Pugatch RD, et al. *Computed tomography: An effective technique for mediastinal staging in lung cancer.* J Thorac Cardiovasc Surg 1984; 88: 486.
Daly BDTJr, Faling LJ, Gunars Bite PAC, et al. *Mediastinal lymph node evaluation by computed tomography in lung cancer: an analysis of 345 patients grouped by TNM staging, tumour size and tumour location.* J Thorac Cardiovasc Surg 1987; 94: 664-672.
Webb WR, Gatsonis C, Zerhouni EA, et al. *CT and MR imaging in staging non-small cell bronhogenic carcinoma.* Radiology 1991; 178: 705-713.
Mc Loud TC, Bourgonin PM, Greenberg RW, et al. *Bronchogenic carcinoma: analysis of staging in the mediastinum with CT by correlative lymph node mapping and sampling.* Radiology 1992; 182: 318.
Glazer GM, Orringer MB, Gross BH, et al. *The mediastinum in non-small cell lung cancer: CT - surgical correlation.* AJR 1984; 142: 1101.
Shin MS, Anderson SD, Myers J, Ho KJ. *Pitfalls in CT evaluation of chest wall invasion by lung cancer.* J Comput Assist Tomogr 1986; 10 (1): 136-8.
Haggar AM, Pearlberg JL, Froelich JW, et al. *Chest wall invasion by carcinoma of the lung: Detection by MRI.* AJR 1987; 148: 1075-8.
Mc Loud TC, Filion RB, Edelman RR, Shepard JA. *MR imaging of superior sulcus carcinoma.* J Comput Assist Tomogr 1989; 13: 233-239.
Mountain CT, Libshitz HJ and Hermes KE. *Lung cancer. A Handbook for Staging, Imaging and Lymph Node Classification.* Charles P. Young Company, Houston, Texas, USA, 1999.
Godvin JD, Speckman JM, Fram EK, et al. *Distinguishing benign from malignant pulmonary nodules by CT.* Radiology 1982; 144: 349.

Pugatch RD. *Solitary pulmonary nodule.* In Mc Neil BJ, Abrams HL (eds): Little, Brown and Company, Boston-Toronto, 1986; 129-33.
Siegelman SS, Zerhouni EA, Leo FP, et al. *CT of the solitary pulmonary nodule.* AJR 1980; 135: 1.
Gurney JW. *Missed lung cancer at CT: imaging findings in nine patients.* Radiology 1996; 199 (1): 117-122.
Tarver RD, Holden RW, Ellis JH. *Experimental lung nodule model: CT numbers, nodule size, and actual calcium content.* J Comput Assist Tomogr 1983; 7(3): 402-6.
Seltzer SE, Judy PF, Adams DF, et al. *Spiral CT of the chest: comparison of cine and film-based viewing.* Radiology 1995; 197 (1): 73-78.
Kaneko M, Eguchi K, Ohmatsu H, et al. *Peripheral lung cancer: screening and detection with low-dose spiral CT versus radiography.* Radiology 1996; 201 (3): 798-802.
Mori K, Tominaga K, Hirose T, et al. *Utility of low-dose helical CT as a second step after plain chest radiography for mass screening for lung cancer.* J. Thorac Imaging 1997; 12 (3): 173-180.
Obuchi M, Shen Y, Nagashima J, et al. *Examination of scanning technique for lung cancer screening with helical CT.* Nipon Igaku Hoshasen Gakkai Zasshi 1997; 57 (1): 12-18.
Diederich S, Wormanns D, Leuzen H, et al. *Screening asymptomatic early bronchogenic carcinoma with low dose CT of the chest.* Cancer 2000; 89 (11 Suppl): 2483-4.
Kickuth R, Kirchner J, Laufer U, et al. *Better visualization of transbronchial biopsy using CT fluoroscopy.* Cardiovasc Intervent Radiol 2000; 23 (5): 347-50.
Bungay HK, Pal CR, Davies CW, et al. *An evaluation of computed tomography as an aid to diagnosis in patients undergoing bronchoscopy for suspected bronchial carcinoma.* Clin Radiol 2000; 55 (7): 554-60.
Kanczor HU, Kreitner KF. *Contrast enhanced MRI of the lung.* Eur J Radiol 2000; 34 (3): 196-207.
Ohno Y, Adachi S, Motoyama A, et al. *Multiphase ECG - triggered 3D contrast - enhanced MR - angiography: utility for evaluation of hilar and mediastinal invasion of bronchogenic carcinoma.* J Magn Reson Imaging 2001; 13 (2): 215- 24.
Rapp - Bernhardt U, Welte T, Doehring W, et al. *Diagnostic potential of virtual bronchoscopy: advantages in comparison with axial CT slices, MPR and MIP?* Eur Radiol 2000; 10 (6): 981-8.
Haag DW, Follette DM, Roberts PF, et al. *Advantages of positron emission tomography over computed tomography in mediastinal staging of non-small cell lung cancer.* J Surg Res 2002; 103 (2): 160-4.

ANU
BiH
АНУ
БиХ

OVERWIEV OF THE PULMONARY SARCOIDOSIS IN BOSNIA-HERZEGOWINA DURING FIVE YEARS

Zehra Dizdarevic[1], Hasan Zutic[1], Bakir Mehic[1], Kemal Dizdarevic[2]

Abstract

The aim of this study was to present relevant manifestations and clinical characteristics of the pulmonary sarcoidosis in Bosnia-Herzegowina during five year period.

The study was evaluated 446 patients with all forms of pulmonary sarcoidosis. The diagnostic work-up and treatment were carried out according the current standards. The analysis comprised: population statistics (F 63%, average age was 48); symptoms and signs of disease (female patients were often asymptomatic); biochemical findings and markers of activity (active alveolitis was found at 85%); bronchoscopy (abnormal in 88%) radiological (CT had an advantage to chest X-ray); BALF and pathohistological examination (76% positive findings); pulmonary functional tests; appraisal of extrathoracic sarcoidosis and season predilection (sarcoidosis frequently occurred between January-March).

Sarcoidosis in Bosnia&Herzegovina shows typical characteristics without significant differences compared to majority European countries.

Key words: *sarcoidosis – clinical charaseristics – five year period.*

Introduction

The aim of study was to monitor clinical characteristics of the pulmonary sarcoidosis in Bosnia&Herzegovina including patient's history of disease, gender, frequency of disease appearance by months, chest X-ray, CT scan, bronchoscope finding, broncho-alveolar lavage fluid examination (BALF), activity of sarcoidosis, and other complementary diagnostic investigations (1).

[1] Clinical Centre University of Sarajevo, Department of Pulmonary diseases and TB
[2] Clinical Centre University of Sarajevo, Department of Neurosurgery

Methods

The sample comprised 446 patients with all forms of sarcoidosis from all parts of Bosnia-Herzegovina with rather equal diagnostic methods during the five year period.

The diagnosis was established by complementary diagnostic methods according to usual diagnostic standards (2,3).

The analysis comprised:

- Population statistic: age, gender, season algorithm of disease appearance;
- Symptoms of disease: fever, joint aches, cough, chest pains, breathlessness, haemoptysis, or with no symptoms;
- Clinical signs: erythema nodosum, clubbing fingers, peripheral lymphadenopathy, and subcutaneous nodes;
- Biochemical findings and markers of sarcoidosis activity;
- Radiological examination: chest X-ray, CT scan with "window" for mediastinum and lung parenchyma, CT scan with contrast use;
- Bronchoscope finding: regular or irregular capillary net, wide carina, nodes and/or mucosal infiltration;
- BALF examination: cytology and immunology;
- Pathohistological examination: orderly non-caseous epitheloid granuloma of lung parenchyma, hystiocyte and lymphocyte mucosal infiltration;
- Results of pulmonary function tests;
- Parameters of sarcoid activity at relaps;
- Extrathoracic site of sarcoidosis;

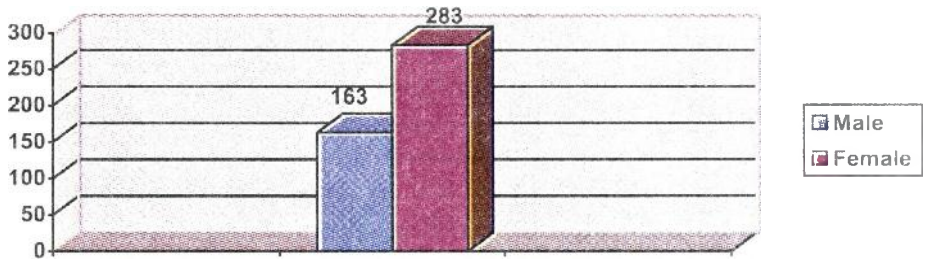
Results

Analysis comprises 446 subjects during the five-years period, 283 female and 163 male with average age of 48 years.

The results were presented on the next tables and figures:

Table 1: *Patients according to gender and age*

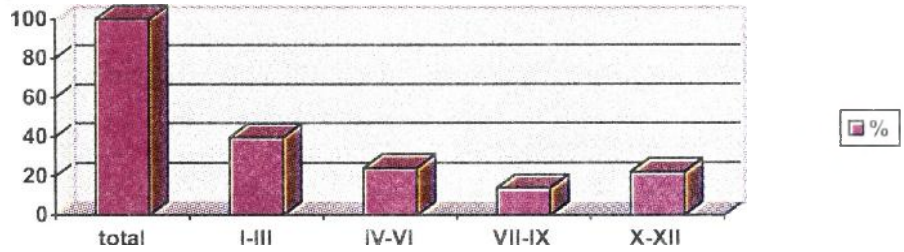
Gender	Total No of pts		Age of pts		Value of signiphicancy
	No	%	male	female	
Male	163	36.5	48.98		T = 1.04
Female	283	63.5		47.75	($p < 0.05$)
Total	446	100.0	48.20		NS
χ^2 test	$\chi^2 = 322.29$ ($p < 0.01$) HS				



The majority of patients were female (63.5%), with average age 47.75 year.

Table 2: *Season algorithm*

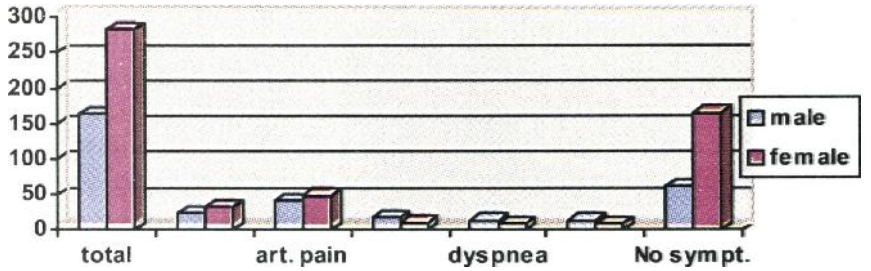
	Total pts	Period			
		Jan-March (I-III)	Apr.-Jun (IV-VI)	Jul-Sept. (VII-IX)	Oct.-Dec. (X-XII)
No	446	177	108	62	99
%	100.0	39.7	24.2	13.9	22.2
(I-III): (IV-VI)	$\chi^2 = 16.71$ ($p < 0.01$) HS				
(I-III): (VII-IX)	$\chi^2 = 53.33$ ($p < 0.01$) HS				
(I-III): (X-XII)	$\chi^2 = 22.04$ ($p < 0.01$) HS				
(IV-VI): (VII-IX)	$\chi^2 = 12.45$ ($p < 0.01$) HS				
(IV-VI): (X-XII)	$\chi^2 = 0.39$ ($p < 0.05$) HS				
(VII-IX : (X-XII)	$\chi^2 = 8.50$ ($p < 0.01$) HS				



Sarcoidosis was the most frequent in the period January-March.

Table 3: *Symptoms of disease*

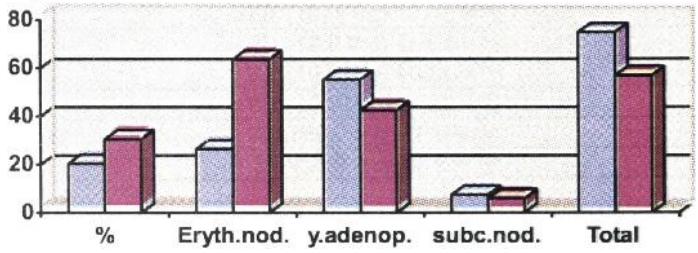
Gender	No %	symptoms					Without symptoms
		cough	Articular pains	Chest pains	dyspnea	weakness	
Male	163 14.1%	23 14.1%	41 25.1%	17 10.4%	13 8.0%	9 5.5%	60 36.8%
Female	283 63.5%	32 11.3%	48 17.0%	10 3.5%	9 3.2%	18 6.4%	165 58.3%



The most of patients had no symptoms, especially females. Artcular pain was the most frequent symptom.

Table 4: *Signs of disease*

Gender	No -%	Erythema nodosum	Peripheral lymphadeopathy	Subcutaneous nodes	Total
Male	163–26.5%	90–55.2%	12–7.4%	20–12.3%	122–74.8%
Fem.	283–63.5%	120–42.4%	17–6.0%	24–8.5%	161–56.9%



Erythema nodosum was the most frequent sign of disease.

Table 5: *Biochemical parameters (No = 446)*

ERS	252 (56%) increased
Serum proteins:	normal
Albumins and globulins	normal
Alpha1 globulin	normal
Alpha 2 globulin	Certain decreased values
Beta globulins: IgG, IgM, IgA, IgD, IgM	normal
Fibrinogen	95 (21%) decreased values
Serum Ly	285 (64%) decreased values
Calcium in urine	normal

ERS was increased in 56% of patients.

Table 6: *Advantage of CT scan comparing to chest X-ray (%)*

BHL	17.3
Symmetric hilar adenopathy	30.7
Total mediastinal adenopathy	51.9
Bilateral mediastinal adenopathy	75.0
Nodular lung opacifacations	26.9
Fibrosis	25.0

Chest CT had an advantage to chest X-ray, especially in evaluation of lymphadenopathy.

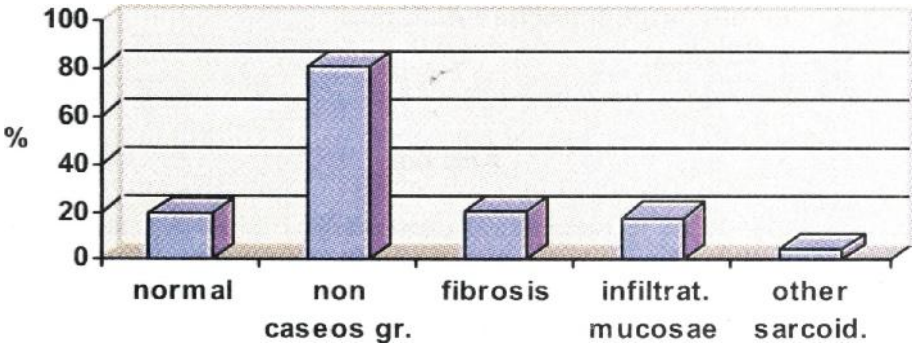
Table 7: *Lymphocyte subpopulations in BALF*

Alveolar Ly (%)	No of pts	Arithmetic mean and standard deviation		
		CD4	CD8	CD4/CD8
> 28%	186	59.97±17.26	26.83±21.21	4.87±5.34
15-28%	110	49.16±18.88	23.96±18.55	3.04±1.95
0-14%	50	45.95±18.95	30.96±20.55	1.95±1.04

Active alveolitis was found at 85% sarcoid patients.

Table 8: *PH finding of bioptic materials*

Total No %	Normal	Positive							Sarcoidosis of other organs
		total	Non caseous granuloma				Fibrosis and hyaline granuloma	Hystiocyte and Lymucosal infiltration	
			total	TBB	BB	NB			
418	81	337	179	142	22	15	84	74	19
100.0	19.4	80.6	42.8	34.0	5.2	3.6	20.1	17.7	4.5



The majority of patients had non-caseos granulomas at patho-histological examination.

Table 9: *Comparison of bronchoscope and PH findings*

Procedure	Total number of subjects		Finding		χ^2 test
			normal	positive	
Bronchoscopy	No	420	48	372	$\chi^2 = 9.56$ ($p < 0.01$) HS
	%	100.0	11.4	88.6	
Pathohistology	No	418	81	337	
	%	100.0	19.4	80.6	

Discussion

The results of this study shows similar results in frequency, gender distrubution, seasonal characteristics, clinical and diagnostic results like majority of Europiuan countries (1, 2,3).

Conclusions

- Sarcoidosis in Bosnia&Herzegovina appears at females in 63.4%; average age is 48 years
- The most frequent period is January-March (39.7).
- Asimptomatic form was seen at females in 58.3%, and in 36.8% at males.
- Biochemical parameters varied minimally in relation to normal finding.
- Pathohistology was positive in 80.6%.
- Active alveolitis was found at 85.5% subjects with high significance of CD4 in the group
- with alveolitis > 28% Ly ($t=8.3$, $p < 0.01$) and index of CD4/CD8 ($t=5.58$, $p < 0.01$).
- CT scan comparing with chest X-ray had significant advantage in precise radiological
- finding;

Aptrakt

Cilj studije je prezentiranje manifestacija i kliničkih karakteristika sarkoidoze pluća u Bosni i Hercegovini u periodu od pet godina.

Evaluirano je 446 bolesnika sa svim oblicima sarkoidoze pluća. Dijagnostički postupak i tretman su izvedeni po važećim standardima. Analiza je obuhvatila populacionu statistiku (Ž 63%, prosječna dob 48 godina); simptome i znakove bolesti (žene su bile češće asimptomatične); biohemijaske analize i markere aktivnosti (aktivni alveolitis u 85%); bronhoskopiju (abnormalna u 88%), radiološki nalaz (CT je precizniji u odnosu na klasičnu radiografiju); BAL i patohistološki pregled tkiva (76% pozitivnih nalaza);

funkcionalno ispitivanje pluća; postojanje ekstratorakalne sarkoidoze i sezonski karakter bolesti (sarkoidoza je bila češća u periodu januar-mart).

Sarkoidoza u Bosni i Hercegovini pokazuje tipične karakteristike bez značajnijih odstupanja u poređenju sa većinom evropskih zemalja.

Ključne riječi: *sarkoidoza – kliničke karakteristike – petogodišnji period*

References

Casa Editrice Mattioli. *Sarcoidosis, vasculitis and diffuse lung diseases,* 2000.

James DG. *Sarcoidosis and other Granulomatous Disorders.* Marcel Dekker. Newyork 1994.

Costabel U, Bross KJ, Guzman J, Nilles A, Ruhle KH, Matthys H. *Predictive value of bronchoalveolar T cell subsets for the course of pulomonary sarcoidosis.* Ann NY Acad Sci 1986, 465:418-426.

ANU
BiH
АНУ
БиХ

MALIGNANT LYMPHOMAS IN SARAJEVO REGION ACCORDING TO W.H.O. CLASSIFICATION OF LYMPHOID NEOPLASMS

Periods 1989-1991 (before the agression), 1992-1995 and 1997-1999 (after the aggresion)

Ivan Selak[1], Nurija Bilalović[1], Semir Vranić[2], Edin Šabanović[3]

Abstract

Our comparative study concerning malignant lymphoma in Sarajevo Region according to W.H.O. Classification from 1997 in periods 1989-1991 (before the aggresion) and 1997-1999 (after the aggresion). We expected increased incidence as well as better patient's survival after the war due to better diagnostic and therapeutic procedures.

The study included reclassification of all cases in period 1989-1991 and their immunohistochemical study. We compared results and determined distribution and relative frequency of each subtype. The prognostic indices and parameters are also included in our study.

Keywords: *malignant lymhoma, Hodgkin's disease (HD), Non-Hodgkin lym-phoma (NHL), W.H.O. Classification, Sarajevo Region.*

Introduction

WHO classification of hematological neoplasms is represented by a list of disease entities and its variants, opened for inclusion of new entities, updating of diagnostic criteria and changes of nomenclature. Clinical relevance of the proposed entities has been evaluated by the clinicians, members of Clinical Advisory Committee. The presentation of W.H.O. Classification in our review is limited to lymphoid neoplasms.

In Federation of Bosnia and Herzegovina lymphoid and myeloid neoplasms are fifth leading malignancy. (6.1% of all malignant neoplasms in 2001).

[1] Medical Faculty, Institute of Pathology, University of Sarajevo, Bosnia and Herzegovina
[2] Medical Faculty, University of Sarajevo, Bosnia and Herzegovina
[3] Federal Institute of Statistics, Sarajevo, Bosnia and Herzegovina.

Hypothesis

We expected increased incidence of malignant lymphoma after the war and longer patient's survival due to diagnostic and therapeutic improvements.

Aims

Reclassification of all malignant lymphoma cases in period 1989-1991 according to W.H.O. Classification of Lymphoid Neoplasms from 1997

Compare results in these two periods (1989-1991 and 1997-1999)

Determine the distribution and relative frequency of each subtype of malignant lymphoma

Make prognostic indices (sex and age distribution, sites of their presentation)

Make prognostic parameters (survival index, Ann Arbor staging, number and sites of extranodal localization and high diameter of tumor)

Materials and methods

Material

The pathology files of Sarajevo Clinical and University Center consist of 178 cases of paraffin tissue and clinical findings for all cases; There were 68 cases for period 1989-1991 and 104 for period 1997-1999. Six (6) cases were not malignant lymphoma. The specimens from all the cases were fixed in 10% unbuffered formalin, processed by routine methods, and embedded in paraffin. Sections of 4-μm thickness were used for hematoxylin and eosin, histochemical, immunohistochemical stains and in situ hybridization. Reticulin staining was performed in cases with histologic and immunologic features of angioimmunoblastic T-cell lymphoma.

Methods

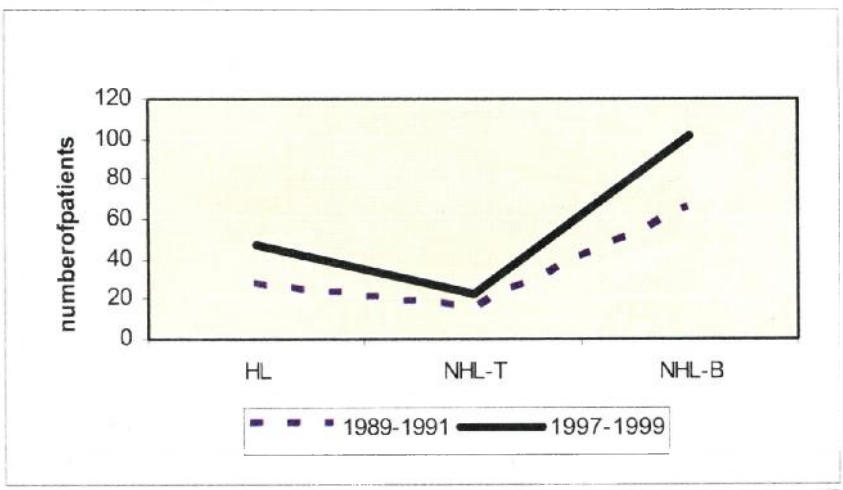
Light microscopy mmunohistochemistry.

Immunohistochemical study was performed using the labeled streptavidin-biotin peroxidase method (LSAB kit; Dako Corporation, Carpinteria, CA) and an antigen retrieval technique was applied when needed for each individual antibody. The antibodies we used were bcl-2, CD3, CD5, CD20 (L26), CD68 (PG-M1), wide-spectrum cytokeratin, kappa and lambda light chains, myeloperoxidase, epithelial membrane

antigen (EMA) (Dako Corporation), CD10, CD16, CD56, CD57, cyclin D1 (Novocastra, New Castleupon-Tyne, UK), CD43, CD45, CD45RO (UCHL-1), granzyme B (Monosan, Monosan/Caltag, San Francisco, CA) and T-cell intracellular antigen-1 (TIA-1) (Immunotech, Coulter/ Immunotech, Westbrook, ME). All non-B-cell lineage specimens of NHL were stained further with CD30 to exclude anaplastic large cell lymphoma. All T-cell lineage specimens of NHL were stained with CD56 to exclude T-/NK cell lymphoma.

Results

A total of 244 cases were found in computer database at Clinic for Hematology. We analyzed 172 cases. Thirteen cases (5.33%) were not malignant lymphomas than reactive and inflammatory conditions. Twenty three (9.43%) of all were treated out of Sarajevo region. Thirty six (14%) paraffin tissues were destroyed during the war. There were 104 cases for period 1997-1999 and 68 for period 1989-1991. (*See graph 2*). In period 1989-1991 among 68 cases 21 (30.9%) was HL and 47 (69.1%) were NHL. Among the NHL cases, 64 (62%) were of B-cell lineage and 9 (13%) were of T-cell lineage. In period 1997-1999 among 104 cases HL were 26 (25%) and NHL 78 (75%). (*See graph 1*). Among the 78 NHL cases, 38 (56%) were of B-cell lineage and 9 (13%) were of T-cell lineage. The ratio between NHL and HL in period 1997-1999 were 4:1 and NHL-B:NHL-T=4:1. These results confirm statistical data in literature. In period 1989-1991 the ratios were NHL:HL=2:1 and NHL-B:NHL-T=4:1.



Graph 1: *Ratio between NHL (including NHL-B cell and NHL-T cell) and HL in periods 1989-1991 and 1997-1999*

Linear correlation demonstrated no significant difference in distribution of malignant lymphoma in pre- and postwar period related to date in literature.

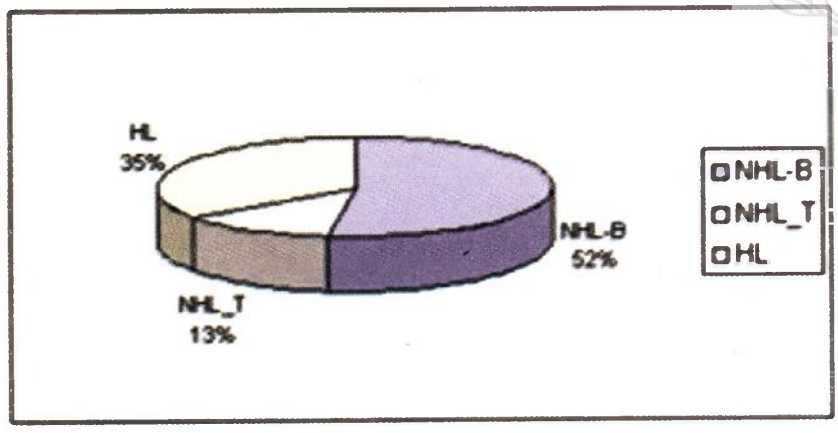
Table 1: *Linear correlation between NHL (including NHL-B cell and NHL-T cell) and HL in periods 1989-1991 and 1997-1999*

Periods	1989-1991	1997-1999	Data in literature
Linear correlation (r)	0.799	0.759	0.767

According to t – test, there is no statistical evidence that histological subtypes differed significantly in two periods (1989-1991, 1997-1999 respectively) except NHL-B lymphoma ($t = 1.966$; $p < 0.05$). The same test was performed for malignant lymphoma classified according to clinical features. It showed only significant difference for indolent lymphoma ($t = 1.946$; $p < 0.10$).

Features of malignant lymphomas in period 1989-1991
Prognostic parameters

Of the 68 cases, 21 (30.8%) were HL, 47 (69.1%) were NHL. NHL-B cell were 38 (56%) and NHL-T cell were 9 (13%) cases. These results are not fully relevant due to missing of paraffin tissue. The ratio between NHL:HL was 2:1 and NHL-B cell : NHL-T cell was 4:1. (*See graph 2*)



Graph 2. *Distribution of malignant lymphomas for period 1989-1991*

Table 2. *Prognostic parameters for period 1989-1991*

DIAGNOSIS	No	%	M	F	Age, mean (yrs)	Average of survival
B-cell chronic lymphocytic leukemia	8	11,7	6	2	61	41
MALT- extranodal B cell lymphoma	6	8,8	2	4	54	46
Follicular lymphoma	4	5,8	3	1	54	48
Mantle cell lymphoma	4	5,8	2	2	55	52
Diffuse large B-cell lymphoma	13	19,1	8	5	54	66
Precursor T-lymphoblastic lymphoma/ leukemia	1	1,5	1	0	55	12
Enteropathy-type T-cell lymphoma	1	1,5	0	1	58	56
Peripheral T-cell lymphoma, NOS	4	5,9	3	1	55	39
Anaplastic large cell lymphoma	3	4,4	2	1	24	114
Nodular lymphocyte predominance/Hodgkin lymphoma	2	2,9	1	1	37	137
Nodular sclerosis/Hodgkin lymphoma	10	13,2	6	4	42	112
Mixed cellularity/Hodgkin lymphoma	9	13,2	6	3	40	104
Nodal marginal zone B cell lymphoma	3	4,4	2	1	64	32
Total	68		42	26		

The most common NHL subtypes in period 1989-1991 include Diffuse Large B-cell lymphoma 13 (19.1%), chronic lymphocytic leukemia/small lymphocytic lymphoma 8 (11.8%), MALT-extranodal B cell lymphoma 6 (8.8%) and Peripheral T-cell lymphoma 4 (5.9%). Among HL subtypes the most common are: Nodular sclerosis 10 (13.2%) and mixed cellularity 9 (13.2%). The uncommon types include Precursor T-cell lymphocyte leukemia and Enteropathy-type T lymphoma 1 (1.5%). (*See table 2*)

The best prognosis with the mean survival 52-114 months include: DLBCL, MALT and ALCL. The worst prognosis was linced with following diagnosis: Precursor T lymphoblastic lymphoma, PTCL and NMZBCL (the mean survival < 40 months).

In period 1989-1991 men form 42 (62%) of all cases while women form 26 (38%) of all cases. Age distributions for HL show

bimodal curve with two peaks, first in period 31 to 40 year, second from 51 to 60 years with levels till 20 years. We noticed increased NHL in older population. (*See table 2*)

The most common NHL was presented by enlargement of the following lymph node: neck 44 (63%), sternoclavicular 24 (34%), axillary 1 (27%), mediastinal 10 (14%), retroperitoneal 12 (17%) and hilar 7 (10%). Other symptoms include: B symptoms 25 (36%), hepatomegaly 22 (31%) and splenomegaly 17 (24%).

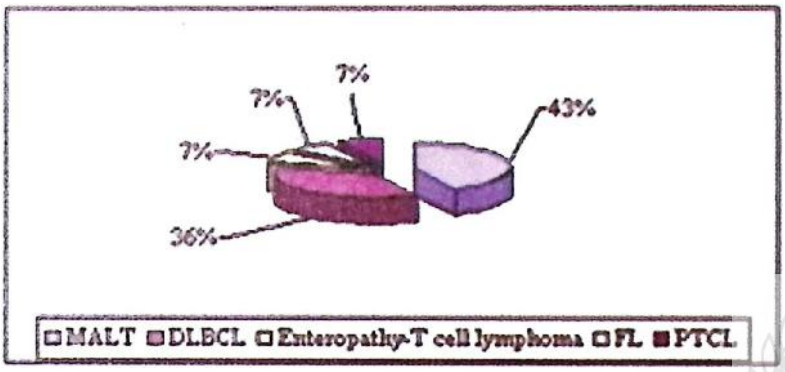
The most common HL presentation was presented by enlargeent of the following lymph nodes: neck 18 (86%), supraclavicular 9 (42%), mediastinal and axillary 4 (19%), retroperitoneal 3 (14%) and hilar 2 (9.5%). Other symptoms include: B-symptoms 11 (52%), hepatomegaly 7 (30%) and splenomegaly 5 (23%).

Table 3. *Relative frequency, staging, number and size of nodal involvement of NHL and HL according W.H.O. classification for period 1989-1991*

Malignant lymphomas for period 1989-1991				
Diagnosis	**Count**	**Stage**	**No of nodal sites**	**Size**
B-cell chronic lymphocytic leukemia	8	IIa, IIIa (2), IIIb (3), Ivb	5(1), 3(2), 3(2), 4(3)	7.8
MALT- extranodal B cell lym-phoma	6	Ia (4), Ie, Iia, IVa,	1(5), 2(1), 3(1)	6.3
Follicular lymphoma	4	Ia, Iia, IIIa, IIIb	1(1), 2(2), 5(1)	5
Mantle cell lymphoma	4	IIa, IIIa, Iva (2)	2(2), 5(2)	3.3
Diffuse large B-cell lymphoma	13	Ia (3), Id, Ie, Iia, IIb (3), IIIa, IIIb (2)	1(7), 2(3), 3(2), 5(1)	5.1
Precursor T-lymphoblastic lymphoma/leukemia	1	IIIa	3(1)	4
Enteropathy-type T-cell ly-mphoma	1	IIIa	5	6
Peripheral T-cell lymphoma, NOS	4	Ia, Iib, IIIa, IIIb	1(1), 4(2), 5(1)	5.5
Anaplastic large cell lymphoma T/null cell, primary systemic type	3	Ia, Ib, Iib	1(2), 2(1)	3.7
Nodular lymphocyte predomi-nance Hodgkin lymphoma	2	Iib, IIIb	2(1), 3(1)	4
Nodular sclerosis Hodgkin lymphomasis (Grades 1 and 2)	10	Iia (4), Iib (3), IVa, Ivb (2)	2(4), 3(3), 4(2), 5(1)	5.2
Mixed cellularity Hodgkin lymphoma	9	Iia (3), Iib(2), IIIa (3), IIIb	2(3), 3(2), 4(4)	3. 7
Nodal marginal zone B cell lymphoma	3	IIb, IIIb, Ie	1(1), 3(2), 4(2)	3
Total	**68**			

Distribution between extranodal and nodal lymphomas for period 1989-1991

There are 14 (20.6%) extranodal lymphoma in this period. The ratio between nodal and extranodal lymphoma was 4:1 (80%:20%). The extranodal lymphoma include the following subtypes of malignant lymphomas: MALT- extranodal B cell lymphoma (±monocytoid B cells) 6 (43%), diffuse large B-cell lymphoma 5 (36%), follicular lymphoma 1 (7%), peripheral T-cell lymphoma 1 (7%), enteropathy T-cell lymphoma 1 (7%). (*See graphs 3 and table 4.*)



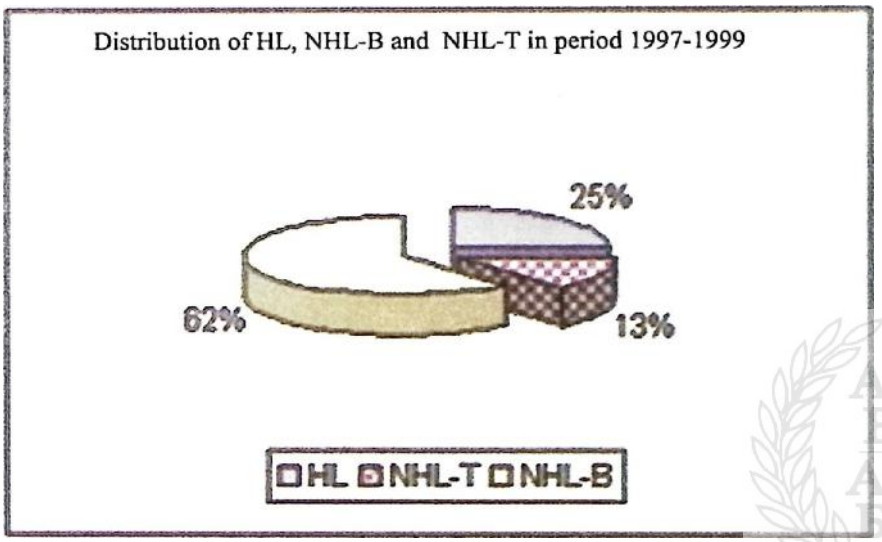
Graph 3: *Frequency of extranodal lymphomas in period 1989-1991*

Table 4. *Extranodal presentation of NHL in period 1989-1991*

Malignant lymphoma	Thyroid Gland	Stomach	Skin	Colon	Small Intestine	Eye	Naso-pharynx	Extranodal
MALT- extranodal B cell lymphoma		6						6
Diffuse large B-cell lymphoma			2	1		1	1	5
Enteropathy-T cell lymphoma					1			1
Follicular lymphoma					1			1
Peripheral T cell lymphoma					1			1
Total		**6**	**2**	**1**	**3**	**1**	**1**	**14**

Features of malignant lymphomas in period 1997-1999
Prognostic parameters

In period 1997-1999 among 104 cases, HL was 26 (25%) and NHL 78 (75%). (*See tables 5 and graph 4*). Among the 78 NHL cases, 38 (56%) were of B-cell lineage and 9 (13%) were of T-cell lineage (*Graph 4*). The ratio between NHL and HL in period 1997-1999 was 4:1 and NHL-B:NHL-T=4:1. These results confirm statistical data in literature.



Graph 4: *The ratio between HL, NHL-B and NHL-T cell lymphoma in period 1997-1999*

The most common NHL types in postwar period include diffuse large B-cell lymphoma 24 (23.1%), Extranodal Marginal B-cell Lymphoma 9 (8.6%) and Mantle cell Lymphoma 8 (7.7%). The most common subtypes of Hodgkin lymphoma include Nodular sclerosis 12 (11.5%) and Mixed cellularity 12 (11.5%). The most uncommon NHL types include: Lymphoplasmacytic lymphoma, Hairy cell leukemia, Nodal marginal zone B cell lymphoma, Anaplastic large cell lymphoma, T/null cell, primary cutaneous type; Anaplastic large cell lymphoma, T/null cell, primary systemic type and Nodular lymphocyte predominance Hodgkin lymphoma 1 (0.96%). (*See table 5*)

Table 5: *Prognostic parameters for period 1997-1999*

Malignant lymphomas for period 1997-1999						
Diagnosis	**No**	**%**	**M**	**F**	**Age, mean (yrs)**	**Average of survival***
Precursor B-lymphoblastic leukemia/lymphoma)	2	1,92	2	0	11	27
B-cell chronic lymphocytic leukemia/small lymphocytic leukemia	3	2,9	1	2	57	14
Lymphoplasmacytic lymphoma	1	0,96	0	1	63	18
MALT- extranodal B cell lymphoma	9	8,6	3	6	62	38
Hairy cell leukemia	1	0,9	0	1	43	30
Follicular lymphoma	4	3,8	2	2	55	30
Mantle cell lymphoma	8	7,7	3	5	57	37
Diffuse large B-cell lymphoma	24	23,1	10	14	49	33
Burkitt lymphoma/Burkitt cell leukemia	5	4,8	4	1	40	29
Precursor T-lymphoblastic lymphoma/ leukemia	4	3,8	3	1	49	6
T-cell prolymphocytic leukemia	2	1,9	2	0	75	8
Anaplastic large cell lymphoma, T/null cell, primary cutaneous type	1	0,9	1	0	66	8
Peripheral T-cell lymphoma, NOS	4	3,8	2	2	41	11
Angioimmunoblastic T-cell lymphoma	2	1,9	0	2	48	18
Anaplastic large cell lymphoma, T/null cell, primary systemic type	1	0,9	0	1	27	4
Nodular lymphocyte predominance/Hodgkin lymphoma	2	1,9	1	1	29	2
Nodular sclerosis/Hodgkin lymphoma (Grades 1 and 2)	12	11,5	5	7	34	42
Mixed cellularity/Hodgkin lymphoma	12	11,5	9	3	31	40
TCRBCL - T cell rich B cell lymphoma	6	5,8	3	3	39	18
Nodal marginal zone B cell lymphoma	1	0,9	1	0	45	36
Total	**104**		**52**	**52**		

* For some subtypes it is still too early to bring any conclusions regarding survival in period 1997-1999.

There is approximately same sex distribution of malignant lymphomas for period 1997-1999.

The most common NHL subtypes were presented by enlargement of the following lymph nodes: neck 59 (57%), sternoclavicular 30 (29%), mediastinal and retroperitoneal (16%), hilar 16 (15%), axillary 11 (10%). Other symptoms include: B-symptoms 45 (43%), hepatomegaly 19 (18%) and splenomegaly 26 (25%).

The most common HL presentation were presented by enlargement of the following lymph nodes: neck 18 (69%), supraclavicular 13 (50%), hilar 9 (34.6%), axillary, mediastinal and retroperitoneal 5 (19.2%). Other symptoms include: B-symptoms 13 (50%), hepatomegaly 7 (27%) and splenomegaly 11 (42.3%).

Table 6: *Relative frequency, staging, number and size of nodal involvement of NHL and HL according W.H.O. classification for period 1997-1999*

Malignant lymphomas for period 1997-1999				
Diagnosis	**No**	**Stage**	**No of nodal sites**	**Size**
Precursor B-lymphoblastic leukemia/ lymphoma	2	IVb	5(2)	3
B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma	3	IIIa, IVa, IVb	3(1), 1(1), 7-1	3
Lymphoplasmacytic lymphoma	1	IVb	1	
MALT- extranodal B cell lymphoma	9	Ia(4), Id, IIa(2), IVa	1(6), 2(2)	4.75
Hairy cell leukemia	1	IVb	1(1)	4
Follicular lymphoma	4	IIa(2), IIIa, IIIb	1(3), 2(1)	3
Mantle cell lymphoma	8	Ia(3), Ib; IIb, IIIa, IIIb, IIa	1(7), 2(1)	3.8
Diffuse large B-cell lymphoma	24	I(3), Ia(2), IIa(6), IVa(2), IVb, IVd, IIIb (5); IIb(2), Ib	2(5), 3(9), 1(10)	4.7
Burkitt lymphoma/Burkitt cell leukemia	5	IIa(2), IIIa(2), IVb	1(4), 7(1)	4.3
Precursor T-lymphoblastic lymphoma/ leukemia	4	IIb(2), IIIb, IVb,	1(1), 2(2), 6(1)	4.7
T-cell prolymphocytic leukemia	2	IIIb, IVa	1(1), 5(1)	15
Anaplastic large cell lymphoma, T/null cell, primary cutaneous type	1	Ivb	7(1)	3
Peripheral T-cell lymphoma, NOS	4	Ia(2), IIa, IIIb, IVb,	1(1), 2(3), 6(1)	7
Angioimmunoblastic T-cell lymphoma	2	IIIb(2)	1(1), 2(1)	2
Anaplastic large cell lymphoma, T/null cell, primary systemic type	1	Ivb	7(1)	7
Nodular lymphocyte predominance/ Hodgkin lymphoma	2	Ivb	1(1)	2
Nodular sclerosis/Hodgkin lymphoma (Grades 1 and 2)	12	Ia(2), IIa(3), IIb(3), IIIa, IIIb, Ivb(2),	2(8), 3(2), 4-1, 5(1)	3.5
Mixed cellularity/Hodgkin lymphoma	12	Ia(2), IIa(2), IIb(2), IIIa, IIIb, IVa, IVb(4)	1(4), 2(4), 6-2	2.4
TCRBCL - T cell rich B cell lymphoma	6	Ib, IIb(2), IIIb(2), IVb	1(6)	4
Nodal marginal zone B cell lymphoma	1	Ivb	6	3
Total	104			

Distribution between extranodal and nodal lymphomas for period 1997-1999

The ratio between nodal and extranodal lymphomas for period 1997-1999: 19 (18.3%) patients with extranodal and 85 (81.7%) with nodal lymphomas. The extranodal presentation includes following subtypes of malignant lymphomas: MALT-extranodal B cell lymphoma (±monocytoid B cells) 9 (47%), diffuse large B-cell lymphoma 5 (26%), Burkitt lymphoma/Burkitt-cell leukemia 2 (11%), precursor B-lymphoblastic leukemia/lymphoma (precursor B-cell acute lymphoblastic leukemia) 1 (5%), T-cell prolymphocytic leukemia 2 (11%).

Graph 5: *Frequency of extranodal lymphomas in period 1997-1999*

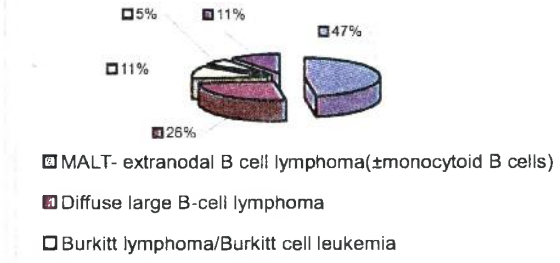


Table 7: *Extranodal presentation of NHL in period 1997-1999*

Malignant lymphoma	Thyroid gland	Stomach	Skin	Parathyroid gland	Colon	Small Intestine	Extranodal
MALT-extranodal B cell lymphoma	1	5		1	1	1	9
Diffuse large B-cell lymphoma	2	1	1			1	5
Burkitt lymphoma			1		1		2
Precursor B-lymphoblastic leukemia			1				1
T-cell prolymphocytic leukemia		1			1		2
Total	**3**	**7**	**3**	**1**	**3**	**2**	**19**

Conclusions

- All malignant lymphomas from period 1989-1991 were reclassified according to W.H.O. Classification of Lymphoid Neoplasms from 1997.
- There is ono significant difference in frequency of HL, NHL-B and NHL-T lymphoma in both periods.
- There is no significant difference in diagnosis frequency in both periods compared with data in literature.
- Immunohistochemical study was performed for all cases from period 1989-1991.
- The ratio between NHL and HL was 2:1 and 4:1 (1989-1991 and 1997-1999 periods, respectively). The ratio between NHL-B and NHL-T was 4:1 in both periods.
- According to t–test, there is no statistical evidence that histological subtypes differed significantly in two periods (1989-1991 and 1997-1999) except NHL-B lymphoma ($t=1.966$; $p< 0.05$). The same test was performed for malignant lymphoma classified according to their clinical features. It showed only significant difference for indolent lymphoma ($t=1.946$; $p< 0.10$).
- The most common NHL lymphomas in period 1989-1991 include: Diffuse Large B-cell Lymphoma 13 (19%), chronic lymphocytic leukemia/small lymphocytic lymphoma 8 (11.8%) and MALT-extranodal B-cell Lymphoma 8 (8.8%). The most common NHL lymphomas in period 1997-1999 include Diffuse Large B-cell Lymphoma 24 (23%), MALT-extranodal B-cell Lymphoma 9 (8.6%) and Mantle cell Lymphoma 8 (7.7%). Diffuse Large B-cell Lymphoma is the most common type in both periods with higher ratio in period 1997-1999 (23%).
- Nodular sclerosis and mixed cellularity were the most common subtypes of HL in both periods (13% and 11.5%, respectively).
- Decreased number of B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma was found in period 1997-1999 (11.7% and 2.8%, respectively).
- Increased number of Burkitt lymphoma/Burkitt cell leukemia was found in postwar period (5 cases, 4.8% of all malignant lymphomas).
- A higher frequency of nodal lymphoma in both periods (80-82%). The ratios between nodal and extranodal lymphoma were 1:4.8 and 1:4 for periods 1989-1991 and 1997-1999, respectively. According to data in literature the frequency of extranodal lymphoma should be at least 40%. (Significance $t = 4.588$ for period 1997 – 1999 and $t` = 3.841$ for period 1989-1991).

- The most common sites of primary extranodal NHL include gastrointestinal tract (stomach particularly) and skin in both periods. The high seropositive rate for *Helicobacter pylori* may account for the high incidence rate of MALT-extranodal B-cell lymphoma (8.8% and 8.6% respectively).
- A higher ratio of malignant lymphoma was found in women in period 1997-1999 (50%) related to 38% in period 1989-1991. (Chi-square test did not show any significance between genders in both periods).

Apstrakt

Naša studija zasniva se na komparativnoj analizi malignih limfoma u sarajevskoj regiji u vremenskom intervalu od šest godina (tri godine prije agresije [1989-1991] i tri godine poslije agresije [1997-1999]). Polazna teza je uključivala povećanu incidencu malignih limfoma u poslijeratnom periodu ali i bolje preživljavanje pacijenata prevashodno zbog poboljšanih dijagnostičkih i terapijskih procedura.

Svi su limfomi klasificirani prema Klasifikaciji hematoloških neoplazija datoj od strane Svjetske zdravstvene organizacije iz 1997. godine, odnosno izvršena je reklasifikacija malignih limfoma iz prijeratnoga perioda kao i njihova imunohistohemijska analiza. Također je određena distribucija i učestalost svakoga podtipa limfoma, te su izrađeni prognostički indeksi i parametri za svaki podtip.

Ključne riječi: *maligni limfomi, Hodgkinova bolest, ne-Hodgkinovi limfomi, Klasifikacija hematoloških neoplazija Svjetske zdravstvene organizacije, Sarajevo.*

Literature

A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. *Blood* 1997; 89:3909-3918.

Cecyn KZ, Oliveira JS, Alves AC, Silva MR, Kerbauy J. *Prognostic factors in non-Hodgkin lymphomas.* Sao Paulo Med J 2000;118(1):7-12.

Elgin J, Phillips JG, Reddy VV, Gibbs PO, Listinsky CM. *Hodgkin's and non-Hodgkin's lymphoma: spectrum of morphologic and immunophenotypic overlap.* Ann Diagn Pathol 1999;3(5):263-75.

Frizzera G, Wu DC, Inghirami G. *The Usefullness of Immunophenotypic and Genotypic Studies in the Diagnosis and Classification of Hematopoietic and Lymphoid Neoplasms.* Am J Clin Pathol 1999;111(Suppl 1):S13-S39.

Habermann TM. *Non-Hodgkin's lymphoma: present status and future prospects.* Hosp Pract (Off Ed) 1999;34(13):81-4, 87-8.

Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, Lister TA, Bloomfield CD. *The World Health Organization classification of Neoplastic diseases of the hematopoietic and lymphoid tissues. Report of the Clinical Advisory Committee meeting, Airlie House, Virginia, November, 1997.* Ann Oncol 1999;10(12):1419-32.

Harris NL, Jaffe ES, Stein H, Banks PM, Chan JKC, Cleary ML, Delsol G, De Wolf-Peeters C, Falini B, Gatter KC, Grogan TM, Isaacson PG, Knowles DM, Mason DY, Muller-Hermelink H-K, Pileri SA, Piris MA, Ralfkær E, Warnke RA. *A Revised European-American Classification of Lymphoid Neoplasms: A Proposal from the International Lymphoma Study Group.* Blood 1994;84:1361-1392.

Harris NL. *Hodgkin's disease: classification and differential diagnosis.* Mod Pathol 1999; 12(2): 159-75.

Institute for Public Health of Federation Bosnia and Herzegovina. Vital Statistics for 2001.

Jaffe ES. *Introduction: Hodgkin's Lymphoma - Pathology, Pathogenesis, and Treatment.* Sem Hematol 1999;36:217-219.

Kitamura Y, Nanba E, Inui S, Tanigawa T, Ichihara K. *Diagnosis of lymphoma in paraffin wax sections by nested PCR and immunohistochemistry.* J Clin Pathol 1996;49(4):333-7.

Mason D, Gater K. *Lymphoma classification.* The pocket guide. Distributors Marston Book Services. Registered at the United Kingdom 1998.

PCR-RFLP DETECTION AND TYPISATION OF HUMAN PAPILLOMAVIRUSES (HPVs)

Đemo Subašić[1], Muhamed Čurić[1], Irma Salimović[1], Kemal Šerić[1]

Abstract

Human papillomaviruses (HPV) are the members of the family *Papovaviridae*. Viral particles are spherical, with icosahedral symetry, 55 nm in diameter, containing double strand molecule of DNA (mol. weight $8x10^3$). HPV cause benign and malignant lesions of epthelial and mucous tissue. On the basis of epidemiological and experimental data it is known that HPV is the main etiological causative agent of cervix cancer which can be transmited sexual contact. This virus has very unstable genome which mutates very often. At present, there are more than 70 different HPV types divided into three following groups:
HPVs of low oncogenic risk (types 6 and 7);
HPVs of intermediate oncogenic risk (types 31, 35 and 45);
HPVs of high oncogenic risk (types 16, 18 and 33).

It is very important in therapeutical and preventive sense early detection of HPV and corresponding tzpisation. Now it is possible to achieve this by combination of two molecular methods such as PCR (*Polymerase Chain Reaction*) and RFLP (*Restriction Fragment Length Polymorphism*) which are adapted for analysing of L1-region HPV-DNA (*1, 2, 3*).

We analysed total 123 specimens by HPV-DNA-PCR (L1-ORF) method (41 cervix specimens, 41 urine-supernatant and 41 urine-pellet specimens). Samples detected as positive (4, 5, 28, 33, 38, 39 and 40) in all PCR analysis are typed by RFLP.

Out of 16 HPV-DNA-PCR positive specimens analysed by RFLP detected HPV types were: 6, 2a, 16, 33 and 10a.

The percentage of successful typisation of HPVs by RFLP was 40%. For five specimens after RFLP analysis, electrophoretic patterns were unclear. These PCR products and other which showed undefined restriction fragments must be controled and confirmed by sequencing method.

Key words: *HPVs, PCR, RFLP*

[1] Institute for Microbiology, Immunology and Parasitology, Clinical Centre University of Sarajevo

Introduction

Till today it has been identified over 70 types of Human papillomaviruses (HPVs) according to their ability to induce formation of lesions in mucous tissue and derm. 25 types infect anogenital tract of men and women. This type differentiation is also done according to the type of clinical lesions. So, anogenital HPVs a divided into the three groups:

HPVs of low oncogenic risk (types **6** and **7**);

HPVs of intermediate oncogenic risk (types **31, 35** and **45**) usually cause genital warts and rarely CIN (*cervical intraepithelial neoplasia*) or cancer;

HPVs of high oncogenic risk (types **16, 18** and **33**) often cause CIN lesions and invasive cervix cancer (*4, 5, 6, 7, 8*).

The papillomaviruses are currently classified as a genus within the *Papovaviridae* family. Viruses are non-enveloped, small, icosahedral, with double-stranded DNA genome and infect mainly mammals. There are genus *Papillomavirus* and *Polyomavirus* within *Papovaviridae* family, differing in size and characteristics of genome, genus-specific antigens and biological properties.

Papillosis and papillomatosis are benign tumours (skin, mucous membranes, epithelial tissue) caused by HPV infection. Different types of HPV cause morphologically different papillosis among animal hosts. Some of the HPV types can be the major etiologic factor for malignancy develop. HPV infection alone is not sufficient for malignant transformation. The mechanisms of transformation are not clear at all but it is known that HPV-DNA integrates into the host genome (integrated form of HPV-16 DNA was often found in malignant tumors of human genital tract). HPV-DNA has 7.904 nucleotides. Period from 1985. till 1996. is specific because of acumulation of datas supporting the hypothesis that HPV infection is one of the major factor of pathogenesis some of the human epithelial benign and malignant neoplasia (*9*).

Very important cytologic parameters such as abnormal mitosis and nucleus atypia show to precursor states before forming of cancer. Specific lesions named as cervical intraepithelial neoplasia- CIN are caused by HPV and divided into 4 grades (*10*).

Early diagnosis of HPV is of great terapeutic and prophylactic importance before histological and clinical signs. Extremly sensitive and specific HPV-DNA PCR in combination with RFLP is adapted for this purpose analysing L1 ORF (*open reading frame)* of HPV genome using MY11/MY09 and GP5/GP6 primers specifically amplifying L1 region (position 6772-7170, **figure 1**). L1 HPV-DNA region is useful for detection of HPV types **11, 16** i **18,** however E5 HPV-DNA region

is often analysed by specific detection primers for determination of HPV type 6. Molecular method such as PCR and RFLP helping us to research mentioned sequences which are specific for the most important types of HPV (*11*).

In 1989. the first identification of HPV-DNA was done by PCR technology (*1*) and this technology found application in epidemiology research of genital HPV infections. PCR technology in combination with next two methods were used for HPV-DNA research:

1. ELISA- (*Enzyme-Linked Immunosorbent Assay*)
2. RFLP- (*Restriction Fragment Length Polymorphism)*

PCR-RFLP analysis of L1-ORF HPV in different clinical specimens is fast and sensitive method for detection and typisation of HPV-DNA and for determining of oncogenic potential, as well. For typisation of HPV by ELISA high specific biotin labelled non-radioactive hybridization probes are used for types 6, 11, 16, 18 and 33. RFLP analysis understands usiness of restriction endonucleases for amplificated HPV-DNA digestion (*12,13*).

On the basis of available references there are no similar research for HPV in BiH region that has been done by PCR-RFLP for parallel collected specimens of the cervix and urine.

Material and methods

Cervical and urine specimens were selected and taken in the period of 1997-1999. Cervical specimens (in 2 ml PBS) and urine (20 ml) were treated after delivery.

Treatment of specimens (extraction and incubations DNA-HPV)

Cervical specimen was transferred into the 1.5 ml tube and after centrifugation at 13.000 rpm, 3 min. pellet was resuspend in 200 µl of cetus buffer, treated with Proteinase K (12 µl Proteinase K and 200 µl of specimen was incubated 1 hr at 55°C), incubated 10 min. at 96°C, transferred on ice for 2 min. and left on -20°C.

Urine specimen was at first centifugated 10 min. at 3.500 rpm. Then the 500 µl of supernatant was treated with 30µl of Proteinase K Digestion was done at 55°C for 1 hr. After 10 min. incubation at 95°C specimen was transferred on ice shortly and left on -20°C.

Two round amplification detection of HPV using specific primers, agarose gel electrophoresis with PCR markers. PERKIN ELMER thermocycler (GENE AMP PCR SYSTEM 2400).

Positive control for PCR were CaSki cells (*Carcinoma Skin Cells*), 600 copies of HPV-DNA (dilutions 10^{-4}, 10^{-5} and 10^{-6}). As a negative control we used Rnase free destiled water.

RFLP (Restriction Fragment Length Polymorphism) procedure

For HPV typisation by detection method named restriction fragment length polymorphism of DNA (RFLP) were used PCR positive cervix and urine specimens after 1st round amplification. Positive PCR products were digested by these restriction endonuclease: *Rsa* I, *Hae* III and *Pst* I. Those enzymes cut phosphodiesteric bonds of nucliotide DNA strand at the specific sites. *Hae* III endonuclease isolated from *Haemophilus aegyptius* cuts the DNA at GG↓CC. *Pst* I endonuclease is isolated from bacteria *Providencia stuartii*. Restriction sites of this enzyme is CTGCA↓G. *Rsa* I endonuclease isolated from bacteria *Rhodobacter sphaeroides* recognises palindromic restriction site GT↓AC (*14*).

RFLP markers

Plasmid pBR 322 DNA digested by *Hae* III endonuclease gave the fragments of this size: 587, 540, 504, 458, 434, 267, 234, 213, 192, 184, 124, 123, 104, 89, 80, 64, 57, 51, 21, 18, 11 and 8. 1 kb leader DNA had lineary differing fragments in 1018 bp (from 12.216-1.018 bp) used for RFLP typisation analysis and also this system contained vector's DNA fragments of size: 506, 396, 344, 298, 220, 201, 154, 134 and 75 bp.

RFLP- digestion by restriction endonuclease

1st round PCR amplification products that were HPV-DNA positive after 2nd round PCR amplification and detection were typed by RFLP. 30-40 µl of PCR I product were added to 22 µl mix of endonucleases *Rsa* I, *Hae* III and *Pst* I (1:1:2). So, to 18 µl of 1xL buffer (*Rsa* I buffer) was pipetted 1 µl of each, *Rsa* I and *Hae* III, and 2 µl of *Pst* I were added. Samples were 1 hr incubated at 37°C, heated 5 min. at 95°C in a dry heating block and than put on ice for 2 min.

Separation of digested HPV-DNA by electrophoresis

Separation of digested HPV-DNA PCR products was done by 3% agarose gel electrophoresis in 1x TBE buffer in the absence of ethidium bromide (EtBr). 20 µl of each RFLP product was added to 10 µl of sample buffer (*Orange G*) and 10 µl of 1xTE buffer. The first and the last sample well of agarose gel was reserved for markers (35 µl).

Electrophoresis was performed in Midi gel apparatus (LKB Pharmacia) in 1xTBE buffer for 2.5-3 hrs at 100 V (voltage). Gel was stained in 1xTBE 45 min. which contained EtBr (10 mg/ml) 100 µl na 100 ml 1xTBEbuffer. Gel was analysed by UV transilluminator and photographied by polaroid camera. Determination of HPV-types was done on the basis of well known values of HPV-DNA digestion fragments for determined HPV types found in restriction maps. Standard HPV-DNA restriction fragments or as a control group for comparation of our experimental datas and restriction maps as well are showed in the table 2. Precise computer determination of HPV-DNA fragments after restriction endonuclease digestion (*Hae* III, *Pst* I, *Rsa* I) was done according known lengths of RFLP markers (pBr 322, *Hae* III DNA and 1 kb DNA *leader*).

Results

PCR-HPV analysis

It was analysed 41 cervical and 82 urine specimens in parallel (41 supernatant and 41 pellet samples) by HPV-PCR. At each round of amplification the positive result had sample no. 40. Samples 28, 33, 38 and 39 were HPV-DNA negative in urine supernatant. Sample no. 4 was negative in urine supernatant (first amplification round) and pellet (first amplification round), sample no. 5 was negative in urine pellet (first amplification round).

Samples no. 4 (cervix, urine-pellet), 5 (cervix, urine-supernatant), 28 (cervix, urine-pellet), 33 (cervix, urine-pellet), 38 (cervix, urine-pellet), 39 (cervix, urine-pellet) and 40 (cervix, urine-supernatant and pellet) were HPV-DNA-PCR positive and proceeded to RFLP analysis for typisation, respectively, determination of their oncogenic potential.

RFLP analysis

Typisation of HPV-DNA positive specimens (cervix, urine) was done according the polymorphism of HPV-DNA restriction fragments

after digestion by restriction endonucleases *Hae* III, *Pst* I and *Rsa* I. Fragment separation was done in 3% agarose gel..

Order of analysed fragments is shown in figure 1 (A and B) and represents electrophoregram of HPV-DNA restriction fragments from positive PCR samples of cervix and urine:

Paths: 1, 2 - sample no. 4 (cervix, urine-pellet)
3, 4 - sample no. 5 (cervix, urine- supernatant)
5, 6 - sample no. 28 (cervix, urine-pellet)
7, 8 - sample no. 33 (cervix, urine-pellet)
9, 10 - sample no. 38 (cervix, urine-pellet)
11 - control (dH_2O- Rnase free water)
12, 13 - sample no. 39 (cervix, urine-pellet)
14 - sample no. 40 (cervix)
15, 16 - sample no. 40 (urine- supernatant and pellet)

Label M in each figure of electrophoregram means RFLP markers.

According the known size of RFLP marker fragments the size of HPV-DNA restriction fragments separated in agarose gel was calculated. Table 1 (A, C) shows the size of of those fragments labeled as Mol.Wt. for markers and and (QUERIES) for samples in figure 1 (A, B).

Compairing the known and experimental size of HPV-DNA fragments for individual types of HPVs we determined the presence of the next HPV types in analysed specimens: HPV-6, HPV-16, HPV-2a, HPV-33 and HPV-10a. For the rest of the samples with unclear HPV type it is necessary to perform DNA sequencing (table 1. P).

Figure 1.A. *RFLP electrophoregram. 1, 2-sample no.4; 3, 4-sample no.5; 5, 6-sample no.28; 7, 8- sample no.33; 9, 10- sample no.38; 11-negative control; 12, 13- sample no.39; 14, 15, 16- sample no.40. M-RFLP markers.*

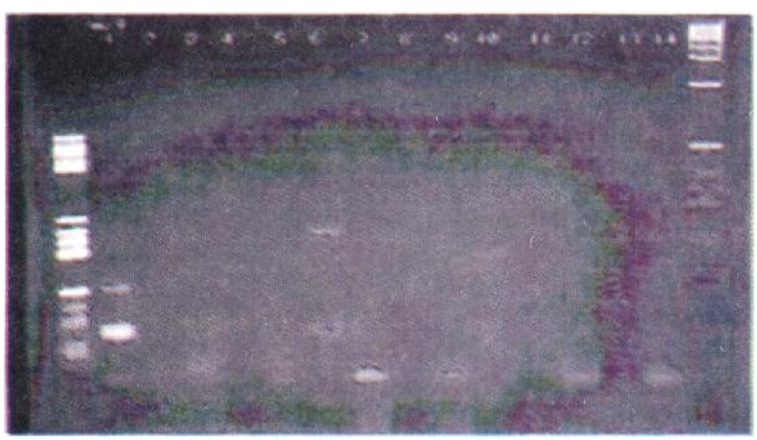


Figure 1.B. *Computer analysis of RFLP electrophoregram (figure 1A) (ISI 1000 Digital Imagining System). M- RFLP markers.*

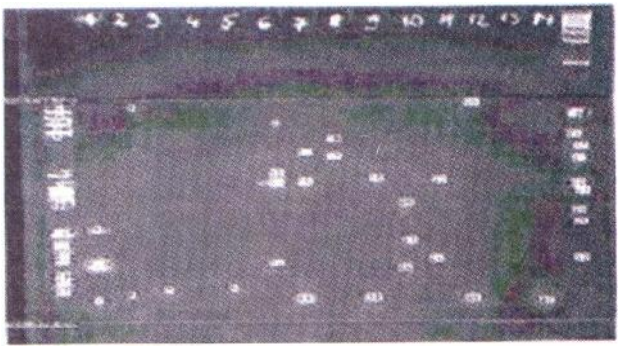


Figure 3. *RFLP electrophoregram sample no.40 (lines 15 and 16). M-RFLP markers.*

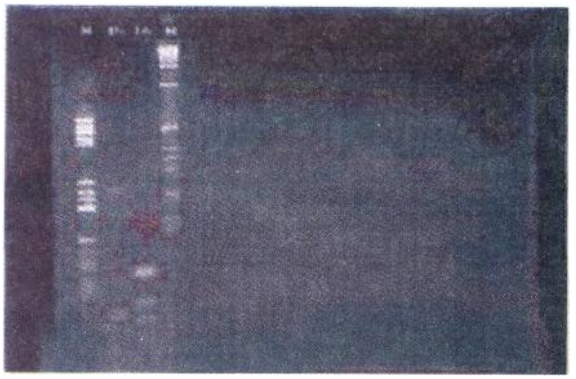


Figure 4. *Computer analysis of RFLP electrophoregram (figure 3) (ISI 1000 Digital Imaging System) M-RFLP markers.*

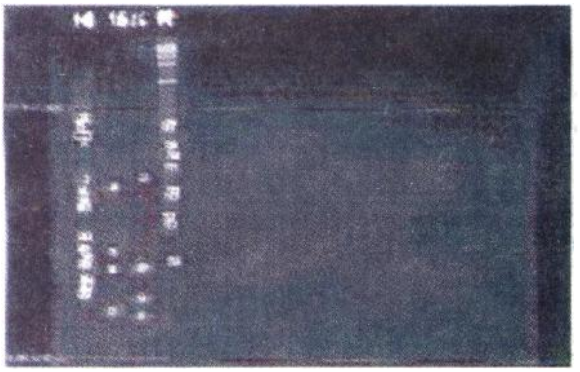
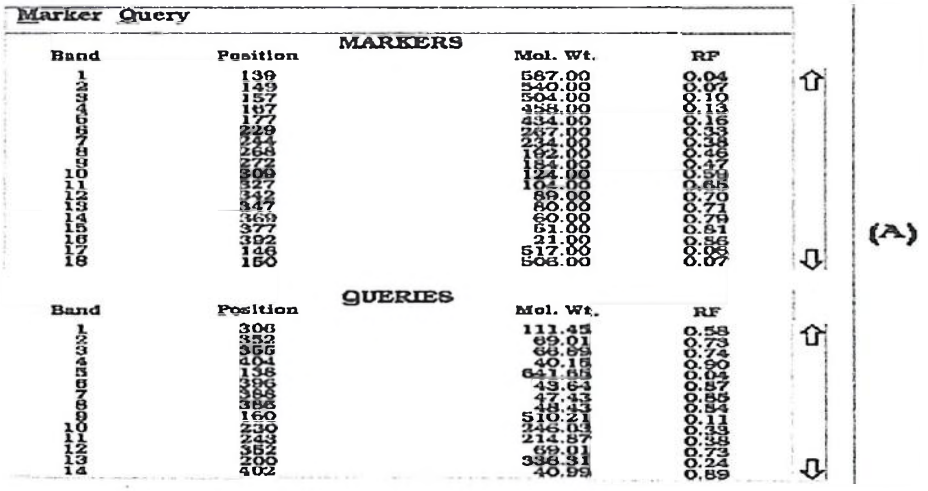


Table 1-A. *Restriction fragment size (Mol. Wt.) of RFLP markers (MARKERS) and analysed samples (QUERIES).*



Marker Query

MARKERS

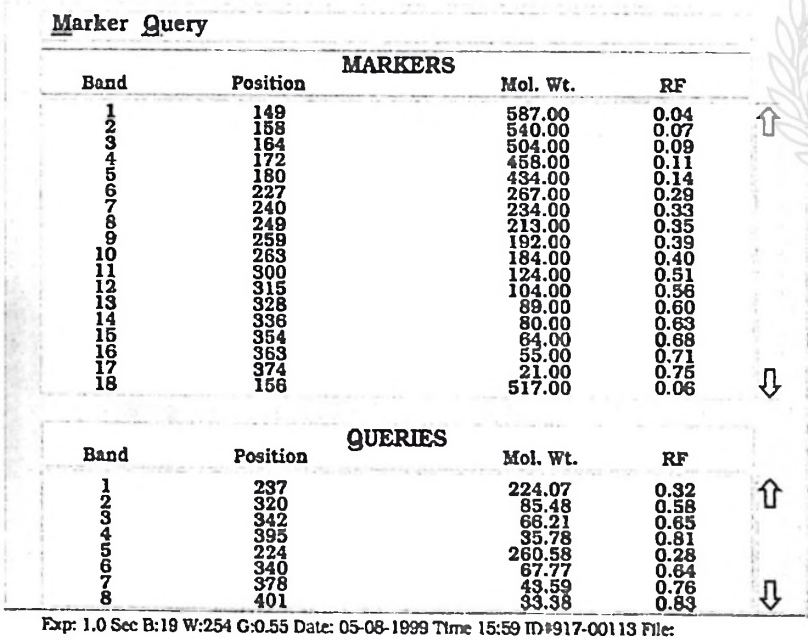
Band	Position	Mol. Wt.	RF
1	139	567.00	0.04
2	149	540.00	0.07
3	157	504.00	0.10
4	167	458.00	0.13
5	177	434.00	0.16
6	229	267.00	0.33
7	244	234.00	0.38
8	268	192.00	0.46
9	272	184.00	0.47
10	309	124.00	0.59
11	327	104.00	0.65
12	342	89.00	0.70
13	347	80.00	0.71
14	369	60.00	0.79
15	377	51.00	0.81
16	392	21.00	0.86
17	146	517.00	0.08
18	150	506.00	0.07

QUERIES

Band	Position	Mol. Wt.	RF
1	306	111.45	0.58
2	352	69.01	0.73
3	366	66.99	0.74
4	404	40.15	0.90
5	138	641.85	0.04
6	396	43.63	0.87
7	368	47.45	0.85
8	386	48.44	0.84
9	160	510.21	0.11
10	230	246.03	0.38
11	248	214.87	0.35
12	352	69.01	0.73
13	200	398.31	0.24
14	402	40.99	0.89

(A)

Table 1-C. *Restriction fragment size (Mol. Wt) of RFLP markers (MARKERS) and analysed samples (QUERIES).*



Marker Query

MARKERS

Band	Position	Mol. Wt.	RF
1	149	587.00	0.04
2	158	540.00	0.07
3	164	504.00	0.09
4	172	458.00	0.11
5	180	434.00	0.14
6	227	267.00	0.29
7	240	234.00	0.33
8	249	213.00	0.35
9	259	192.00	0.39
10	263	184.00	0.40
11	300	124.00	0.51
12	315	104.00	0.56
13	328	89.00	0.60
14	336	80.00	0.63
15	354	64.00	0.68
16	363	55.00	0.71
17	374	21.00	0.75
18	156	517.00	0.06

QUERIES

Band	Position	Mol. Wt.	RF
1	237	224.07	0.32
2	320	85.48	0.58
3	342	66.21	0.65
4	395	35.78	0.81
5	224	260.58	0.28
6	340	67.77	0.64
7	378	43.59	0.76
8	401	33.38	0.83

Exp: 1.0 Sec B:19 W:254 G:0.55 Date: 05-08-1999 Time 15:59 ID:917-00113 File:

(C)

Table 1-P. *Size of experimental HPV-DNA restriction fragments after agarose gel electrophoresis was compaired with the size of known fragments for individual HPV types.*

Fragment length	HPV type	Length of known fragments for individual types
Path 1: 40 bp, 66 bp, 89 bp, 111 bp	6	37, 67, 89, 111
Path 2: 43 bp, 641 bp	?	-
Path 3: 47 bp	?	-
Path 4: had not intensive DNA bends	-	-
Path 5: 40 bp	?	-
Path 6: 69 bp, 214 bp, 246 bp, 510 bp	16	66, 68, 70, 218
Path 7: 40 bp, 217 bp, 336 bp	2a	40, 190, 225
Path 8: 315 bp, 405 bp	?	-
Path 9: 41 bp, 231 bp	2a	40, 190, 225
Path 10: 69 bp, 94 bp, 126 bp	33	38, 67, 90, 137
Path 11: control (RNase free dH_2O)	-	-
Path 12: 40 bp, 668 bp	?	-
Path 13: had not intensive DNA bends	-	-
Path 14: 39 bp	?	-
Path 15: 36 bp, 66 bp, 85 bp, 260 bp	10a	29, 67, 110, 229
Path 16: 33 bp, 43 bp, 67 bp, 260 bp	10a ?	29, 67, 110, 229

Discusion and conclusion

In these papers are shown the results of high sensitive and specific PCR analysis after the screening programme for the cervical and urineclinical specimens using specific detection primers for the L1-HPV-DNA region.

Till today, research of L1- ORF- HPV- DNA showed that the amplicons of this part of nucleotide sequence can be typed according to their restriction fragment size after digestion by endonucleases *Psa* I, *Pst* I and *Hae* III, and comparative study with standards in considered HPV-DNA restriction maps (*8*). For all HPV types with known DNA sequence the length of their restriction fragments after digestion by mentioned restriction enzymes are known and are used in HPV-DNA typisation in this work, as well. Results of our HPV-DNA-PCR-RFLP

research in comparation with the standard restriction map for HPV are shown in the table 2.

Table 2. *Results of HPV-DNA-PCR-RFLP analysis (A) in comparation with results of colleges from Cambridge (C) and standard lengths of HPV-DNA restriction fragments.*

Electrophoretic path	HPV-DNA restriction fragment length (bp) (A)	HPV type	Standard HPV-DNA restriction fragment length (*8*) (B)	Results of HPV-DNA PCR analysis in Cambridge (*11*) (C)
7/9	40, 217, 336/41, 231	2a		225, 190, 40
		3		220, 70
		5	464	377, 99
1	40, 66, 89, 111	6	122, 78, 73, 71, 67, 37	113, 68, 64, 34
		7		460
		8	288, 176	300, 184
15/16	33, 16, 85, 224/33, 43, 67, 260	10a		229, 110, 67, 29
		11	135, 124, 73, 71, 26, 10	140, 124, 75, 20
		14		303, 121, 40, 8
6	69, 214, 246, 510	16	216, 71, 70, 68, 25	218, 70, 68, 66
		17		330, 130
		18	134, 107, 85, 73, 38, 18	132, 110, 85, 70
		20		350, 130
		31	138, 118, 98, 73, 25	131, 124, 90, 80
10	69, 94, 126	33	140, 101, 96, 73, 39	137, 90, 67, 38
		49		200, 56, 10
		50		135, 110, 81, 67,10
		57	297, 153	303, 146, 10

According to reference datas (*8*) and compairing with standard restriction map, variability in fragment size is very often for HPV-DNA analysed by PCR-RFLP. We also confirmed that fact (table 2). It can be explained with the presence of partial digestion products which are made as a result of new restriction HPV-DNA sites that arise by point mutatuions in specific sites of nuclieotide HPV-DNA sequence.

Obtained results unidentical at all with standard HPV-DNA restriction maps could arise as a consequence of dual infection. In these cases regularly is detected larger number of restriction fragments than it is in standard restriction map. Among all reference datas there is only

one research that can be compaired with this work and was done in Cambridge (England) where were used the identical restriction enzymes and analysed identical clinical specimens (cervix, urine). The same amplification and detection primers (MY09/MY11 and GP5/GP6) were also used for L1-ORF HPV-DNA analysis.

Analysis that was done by research team from Cambridge was checked by sequencing so the HPV types were determined with great insurancy. They determined the presence of these HPV types (nucleotide sequence of HPV-DNA had more than 10% homology in consideration of nucleotide sequence of all known HPV: 2a, 3, 5, 6, 7, 8, 10a, 11, 14, 16, 17, 18, 20, 31, 33, 49, 50 and 57.

Our PCR-RFLP analysis showed the presence of these HPV types: 2a, 6, 10a, 16 and 33. For six samples we could not determine HPV type definitely but the large probability is that it was HPV type 10a in one of those samples (table 2.). In consideration of the fact that this work is the first for our territory and determines HPV types it would be interesting to investigate the sequence of HPV types 2a, 6, 10a, 16 and 33 and to consolidate the homology among their nucleotide sequence with HPV types from Camridge.

Investigation realized by several research centres showed the prevalence of these HPV-DNA types using PCR-RFLP typisation in clinical specimens of cervix: HPV-16 (48%) (*15*); HPV-16 (42%); HPV-18 (39%); HPV-6 (26%); HPV-11 (15%); HPV-45 (10%); HPV-52 (3%); HPV-31 (1.5%); HPV-68 (1.5%); HPV-33 (1.5%) (*16*); HPV-16 (10%); HPV-18 (10%); HPV-6 (2.2%); HPV-11 (2.2%) (*17*); HPV-16 (50%); HPV-18 (14%); HPV-45 (8%); HPV-31 (5%) (*18*). Predominant type in Indonesia is HPV-16, in Western Africa HPV-45, in Central and souh Africa HPV-39 and HPV-59 (*19, 20, 15*).

From large number of PCR positive samples those which were positive in cervix even in urine samples (pellet and supernatant) were further analysed for their oncogenic potential and typed by RFLP. Those were: sample no. 4 (cervix, urine-pellet), sample no. 5 (cervix, urine-supernatant), sample no. 28 (cervix, urine-pellet), sample no. 33 (cervix, urine-pellet), sample no. 38 (cervix, urine-pellet), sample no. 40 (cervix, urine- supernatant and pellet), sample no. 4 (cervix)- HPV- type 6, sample no. 28 (urine-pellet)- HPV- type 16, sample no. 33 (cervix)- HPV- type 2a, sample no. 38 (cervix)-HPV- type 2a, sample no. 38 (urine- pellet)_ HPV- type 33, sample no. 40 (urine- supernatant)- HPV- type 10a, sample no. 40 (urine-pellet)- HPV- type 10a.

Determined HPV types ordered according the oncogenic potential:

HPV- types of low oncogenic potential (HPV 6);

HPV- types of intermediate oncogenic potential (HPV 33);

HPV- types of high oncogenic potential (HPV 16);

Currently, oncogenic potential of HPV types 2a, 10 and 10a is not clear at all. RFLP analysis showed the presence of HPV type 6 in sample no. 4 (cervix) which is the major etiologic agent of lesions and rarely give arise the progressive malignancy. HPV-33 was identified in sample no. 38 (urine-pellet). It belongs to a group of typical HPV representative that infect anogenital tract. Very often is identified in cervical malignant lesions and according its oncogenic potential is classified within a group of HPV- types with intermediate oncogenic potential. HPV-16 was identified in sample no. 28 (urine-pellet). This type is classified within the group of HPV- types with high oncogenic potential and is often detected in cervical malign lesions. For all these cases, we got a precise informations about HPV types that supplement diagnosis and help clinics to evaluate a risk of epithelial cervical cells malignancy development.

Experimental datas show that all samples with unclear results of RFLP analysis are checked by sequencing because that is the best way to determine and confirm mutations in HPV-DNA nucleotide sequence (*14*). Besides the practical importance of introducing the sequencing for the final results it is necessary to emphasis the sequencing as a prognostic factor of determining the integrated forms of HPV-DNA types with high oncogenic risk, especially for types 16 and 18 in consideration of episomal forms of HPV-DNA (*21,22*). Using these methods, PCR and sequencing, for differentation of integrated and episomal forms of HPV-DNA, needed conditions for HPV detection and its research will be realized in the purpose of effective preventive and therapy of patients.

Results of our research represent original contribution to knowledge of HPV in Bosnia and Herzegovina. It is sure that sequencing method and quantitative PCR would improve the quality of detection and research on the molecular level and increase the percentage of HPV typisation, what would have a great importance for definitive diagnostics and molecular-epidemiological research of HPVs in Bosnia and Herzegovina.

Apstrakt

Humani papilomavirusi (HPVs) pripadaju familiji papovaviridae. Virusne partikule su sferičnog oblika, ikosaedralne simetrije promjera 55 nm, a sadrže dvolančanu DNA (mol. mase $8x10^3$). Uzrokuju benigne i maligne leyije epitelijalnog i mukoynog tkiva. Na osnovu epidemioloških i eksperimentalnih podataka sasvim se pouzdano zna da je HPV glavni etiološki uzročnik kancera cerviksa a prenosi se seksualnim putem. Ovaj virus ima nestabilan genom koji vrlo često mutira, tako da je do danas utvrđeno preko 70 tipova ovog virusa koji su podijeljeni na tipove:

HPV tipovi niskog onkogenog rizika (tipovi 6 i 7);
HPV tipovi srednjeg onkogenog rizika (tipovi 31, 35 i 45);
HPV tipovi visokog onkogenog rizika (tipovi 16, 18 i 33).

Od izuzetnog značaja je u terapeutskom i preventivnom smislu rana detekcija HPV određivanja onkogenog potencijala (tipizacija). To se danas u svijetu postiže kombinacijom PCR (*Polymerase Chain Reaction*) i RFLP (*Restriction Fragment Length Polymorphism*) prilagođenim za analiziranje L1 regiona HPV-DNA (*1,2,3*).

Metodom HPV-DNA-PCR analizirano je ukupno 123 uzorka (41 uzorak cerviksa, 41 uzorak urina-supernatant i 41 uzorak urina-talog). Uzorci koji su bili pozitivni (broj 4, 5, 28, 33, 38, 39 i 40) bili su podvrgnuti procesu tipizacije pomoću RFLP metode (Polimorfizam restrikcionih fragmenata DNA).

Od analiziranih 16 HPV-DNA pozitivnih uzoraka sa RFLP metodom, utvrđeni su slijedeći HPV tipovi: 6, 2a, 16, 33 i 10a.

Procenat uspješne tipizacije HPV sa RFLP metodom je 40%. Za pet uzoraka, poslije RFLP analiza, elektroforeske trake su bile nejasne. Ovi PCR produkti i drugi koji pokazuju nedefinisane restrikcione fragmente moraju biti kontrolirani i potvrđeni metodom sekvenciranja.

Ključne riječi: *HPVs, PCR, RFLP*

References

Manos, M.M. Ting, Y., Wright, D.K. Lewis, A.J., Broker, T.R., Walinsky, S.M., 1989: *Use of polymerase chain reaction amplification for the detection of genital human papillomaviruses.* Cancer Cells, 7, 209 – 214.

Forslund, O., Hansson, B.G., Rymark, P. and Bjerre, B., 1993: *Human papillomavirus DNA in urine samples compared to that in simultaneously collected urethra and cervix samples.* J. clin. Microbiol.31, 1975 – 1979.

Cartwright, N.C., Sant Cassia, L.J., Easton, A.J., and Morris, A.G., 1996: *Detection of human papillomavirus in vulal carcinoma using seminested PCR and restriction enzyme typing: a rapid and sensitive technique.* J.Clin.Pathol: Mol.Pathol.49, 236 – 239.

Gissman, L., Wolnik, H., Ikenberg, H., Koldovsky, V., Schnurch, G.H. and Zur Hausen, H., 1983: *Human papillomavirus types 6 and 11 DNA sequnces in genital and laryngeal papillomas and in some cervical cancers.* Proc. Natl. Acad. Sci. SA 80, 560 – 563.

Boshart, M., Gissman, L., Ikenberg, H., Kleinheinz, A., Scheurlen, W., Zur Hausen. H., 1984: *A new type of papillomavirus DNA its presence in genital cancer diopsies and in cell lines derived from cervical cancer.* EMBO J., 3, 1151 – 1157.

Koutsky, L.D., Galloway, D.A., and Holmes, K.K., 1988: *Epidemiology of genital human papillomavirus infection.* Epidemiologic Reviews 10, 123 – 163.

Van der Brule, A.J.C., Walboomers, J.M.M., du Maine, M., Kenemans, P., and Meijer, C.J.L.M., 1991: *Differnce in prevalence of human papillomavirus genotypes in cytomorphologically normal cervical smears is asociated with a history of cervical intraepithelial neoplasia.* Int. J. Cancer. 48, 404-408.

Lungu, O., Sun, X.W., Felix, J., Richart, R.M., Silvestern, S. And Wright, T.C., 1992: *Relationship of human papillomavirus type to grade of cervical intraepithelial neoplasia.* Int. J. Cancer. 48, 404-408.

Singleton, P. and Sainbury, D., 1993: *Dictionary of microbiology and molecular biology. Second Edition.* A Wiley Interscience Publication-Wiley and Sons.

Gissman, L. and Zur Hausen, H., 1980: *Partial characterization of viral DNA from genital warts (Condyloma accuminatum).* International Journal of Cancer. 25, 605 – 609.

Brooks, F.G., Butel, V.S. and Morse, S. A., 1998.; *Medical microbiology. Appleton and Longe England.* 326 – 546.

Crum, C.P.,Mitao, M., Levine, R.V. and Silverstein, S., 1985: *Cervical papillomaviruses segregate within morphologically distinc precancerous lesions.* Journal of virology 54, 675 – 681.

Gray, J., Strauss, S. and Desselberger, V., 1997: *Human papillomavirus PHLS Reference Laboratory.* First interim report. Cambridge, England.

Ting, Y. And Manos, M.M., 1990: *Detection and typing of genital human papillomaviruses.* In PCR protocols: A guide to methods and applications (Gelfand, M.m Sninsky, J. and White T. Eds) 356-367. San Diego, CA-Academic Press Inc.

Lungu, O., Wright, T.C. and Silvestern, S., 1992: *Typing human papillomaviruses by polymerase chain reaction amplification with L1 consensus primers and RFLP analysis.* Mol. Cell. Probes 6, 145 – 152.

Stewart, M., Gravitt, E., Cheng, S. And Whecler, M., 1996: *Generation of entire human papillomavirus genome by Long PCR Frequency of errors producted during amplification.* Genome research. Volume 5. Number 1. 79-87.

Hernandez-Avila, M., Lazcano-Ponce, E.C., Berumen-Campos, J., Cruz-Valdez, A., Alonso de Ruiz, P.P., Gonzalez-Lira, G., 1997: *Human papillomavirus 16-18 infection and cervical cancer in Mexico: a case-control study.* Arch. Med. Res. 28 (2): 256-71.

Chabaud, M., Le Cann, P., Mayelo, V., Loboulleux, D., Diallo, A.S., Enogat, N., Afoutou, J.M., Anthonioz, P., Coll-Seck, A.M., Coursaget, P., 1996: *Detection by PCR of human papillomavirus genotypes in cervical lesions of Senegalese women.* J. Med. Virol. 49 (4): 259-63.

Glooen, K., Plsen, A.O., Magnus, P., Grinde, B., Sauer, T., Orstavik, I., 1996: *Prevalence of human papillomavirus in cervical scrapes, as analyzed by PCR, in a population-based sample of women with and without cervical dysplasia.* APMIS 104 (1): 68-74.

Yadav, M., Nurhayati, Z.A., Padmanathan, A., Abdul Aziz, Y., Norhanom, A.W., 1995: *Polymerase chain reaction detection and restriction enzyme typing of human papillomavirus in cervical carcinoma.* Med. J. Malaysia 50 (1): 64-71.

Bosch, F.X., Manos, M.M., Munoz, N., Sherman, M., Jansen, A.M., Schiffman, M.H., Moreno, V., Kurman, R., Shah, K.V., 1995: *Prevalence of human papillomavirus in cervical cancer: a world-wide perspective. International biological study on cervical cancer (IBSCC) Study Group.* J. Natl. Cancer Inst. 87 (11): 796-802.

Karaloglu, D., Yazici, H., Alatli, C., Yaman, F., Aslay, I., Onat, H., Dalay, H., 1996: *Detection of HPV 16 and HPV 18 infection in patients with cervical neoplasia.* Eur. J. Gynaecol. Oncol. 17 (4): 296-8.

Chen, C.M., Shyu, M.P., Au, L.C., Chu, H.W., Cheng, W.T., Choo, K.B., 1994: *Analysis of deletion of the integrated human papillomavirus 16 sequence in cervical cancer: a rapid multiplex polymerase chain reaction approach.* J. Med. Virol. 44 (2): 206-11.

Donaldson, Y.K., Arends, M.J., Duvall, E., Bird, C.C., 1993: *A PCR approach to discriminate between intergrated and episomal HPV DNA in small clinical specimens.* Mol. Cell. Probes 7 (4): 285 – 92.

ANU
BiH
АНУ
БиХ

CLINICAL EVALUATION OF CONGENITAL HEART DISEASE IN CHILDREN IN TUZLA CANTON AREA

Begić Hidajeta, Tahirović F. Husref[1]

Abstract

The purpose of this study has been evaluation our possibilities of diagnostic and treatment children with congenital heart disease. We were analyzing medical documentation from 352 children with discovered congenital heart disease in period of six years in Tuzla Canton area. disease. The average age at diagnosis was 2,15±2,28 years. During first cardiac examination 51,98% children presented symptoms of cardiac disease. Growth retardation in postnatal period is noticed in 13,35%. Electrocardiographic changes are registered in 47,76%, while changed chest radiogram in 53,85% of patients. Medicament treatment was needed in 20,74% and cardiovascular surgery procedure in 40,60%. Urgent surgery treatment was needed in 62 patients with critical heart disease. Cardiovascular surgery was made in 23,29% of patients, and average age was 4,81±3,23 years. Mortality of children with congenital heart disease is relatively high and it is 19,60%, the biggest number of them (95,65%) died before and only 4,35% after cardiosurgical treatment. Average age of children who died was 0,51±0,59 year. Considering clinical indicators of congenital heart disease in Tuzla Canton area, it is evidenced that this children health problem, which due difficult clinical picture, big involvement in infant mortality, growth retardation with progression in chronic cardiopathy, require urgent measures with main goal on advanced organizing of it's diagnostic and treatment.

Key words: *congenital heart disease, children, Tuzla Canton area.*

[1] Pediatric Clinic, University Clinical Center, Tuzla, Bosnia and Herzegovina

Introduction

Congenital heart diseases (CHD) present heterogeneous group of morphological and functional changes of heart and big blood vessels. With its high prevalence, from 5 to 10 cases in 1000 live newborns children, they are on the second place among all congenital anomalies. Clinical demonstration of CHD depends on morphological complexity of disease, or on level of hemodynamical disorders and age of children, as well as combination with other diseases and disorders. For adequate medical treatment of CHD is necessary timely diagnostic, or early definition of morphological and hemodynamical disorders on heart. According to information from Program of Pediatric cardiology from New England (1), excluding premature with open patent ductus arteriosus in 10 children with CHD, three are requesting cardio surgical treatment in its early childhood, and five are requesting some of therapeutic procedures during childhood.

Prevalence of CHD in Tuzla Canton area is 6,12 in 1000 of live born, and 1,46 in 1000 live born have critical disease of heart, or anomaly which vitally endangers its health during first days of life (2).

Course and prognosis of diseases depend on time of diagnosing, type of anomaly, degree of hemodynamical disorders and development level of pediatric cardiology and cardiosurgery. Considering variations of clinical picture, course of disease could be followed in wide diapason, from heavy anomaly with fatal end in first hours or days of life up to those, which have spontaneous recovery. According to Brodeu (3) (1989) 25% of not treated children with heart disease die in first month of their life, and 25% until the end of first year of life, and 12% until age of 15.

Mortality rate is significantly different in relation to type of anomaly and level of socio-economical development of the region in which anomaly has been discovered. In our country due impossibility to offer early cardiac surgical therapy, mortality is almost hundred percentage in children with transposition of great artery, hypoplastic heart left syndrome, atresion of the tricuspid or pulmonary valve and other complex anomaly.

With long-term following it has been registered that children with CHD in Tuzla Canton area present significant part of pathology of children, and that its treatment is not in accordance to recent modern accomplishments in field of pediatric cardiology and cardiac surgery, and in accordance to above mentioned this problem could be considered as open one. That is a reason why this research has as a main goal analyzing of diagnostic and treatment possibilities of these anomalies and gained results are used as basic for resolving of above mentioned problem.

Material and methodes

Source of information was register at Department of cardiology at Pediatric Clinic in Tuzla, which is in advanced conceived to collect relevant information on CHD. By research has been included medical documentation of 352 patients both sex, age up to 14 years, in which have been discovered congenital heart disease in period from 1.1. 1994. to 31.12. 1999. In research were included all patients in which have been determined CHD with echocardiography, catheterization or autopsy. In research were not included children with bicuspidal aortal valve without aortal stenosis, premature children with patent ductus arteriosus which closed until third months of life, as well as children with mitral valve prolapse which is not part of Marfan syndrome.

The method of work was retrospective analyze of medical documentation from Department of Cardiology at the Pediatric Clinic in Tuzla. There were analyzed anamnesis, clinical manifestation, diagnostically procedures (electrocardiography, chest radiografy and echocardiography), usage of therapeutically measures and results of disease in children with proved CHD. In patients who died due CHD it was analyzed autopsy findings if was done.

Results

Age when CHD was diagnosed is shown in Table 1. CHD in fetal age has not been discovered, but there were 188 diagnosed cases in newborn and infant age, from which 61 or 32,45% were in year of 1999. Average value of life age in which CHD was diagnostic was 2,15 ±2,28 year.

Table 1. *Age when congenital heart disease was diagnosed*

Year	CHD (n)	Age when congenital heart disease was diagnosed					
		Praenatal	Newborn	Infant	2-7 year	Scool age	X*
1994	48	-	7	10	29	2	2.10
1995	54	-	8	22	20	4	2.32
1996	54	-	13	23	13	5	1.94
1997	53	-	5	24	17	7	2.68
1998	58	-	8	17	21	12	3.22
1999	85	-	21	40	22	2	0.99
Total	352	-	62	136	122	32	2.15

*Average age when CHD was discovered.

By analyzing reason for cardiac examination, we have found that from total 352 children with congenital heart disease, 225 or

63,92% were referral on examination due changed auscultatory findings on heart, and 110 or 31,25% due occurrence of cardiovas-cular disorders. Reason for cardiac examination in 10 or 2,84% of patients was some of risk factor in anamnesis, in 4 or 1,14% changed chest radiograph, and in 3 or 0,85% pathological electrocardiographically finding.

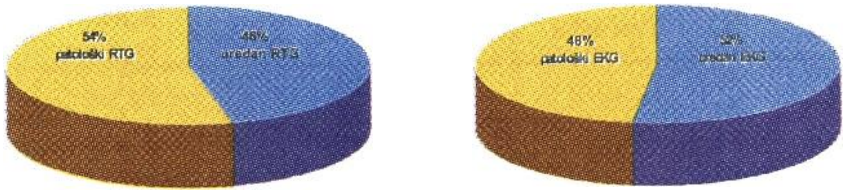
According to analyze of history of disease, we have found cardiovascular disorders in 183 or 51,987% of patients. In more than half or 98 patients, it was about multiply symptomatology. The most frequent symptoms were fatigue (47 or 25,68%) and cyanosis (39 or 21,30%). Information on frequent respiratory infections was found in 19, and growth retardation in 17 patients.

Analyzing anthropometrics parameters during first cardiac examination it has been found that from totally 352 children with CHD, 47 of them or 13,35% have had values of body weight bellow third per-centile for age, and in 28 or 7,95% value of body height were below third percentile for age.

Arterial blood pressure during first cardiac examination has been measured in 288 patients. From that in 11 patients (3,82%) values were according to nomogram for blood pressure (4) above 90th percen-tile. In 6 patients from those 11, ere noticed coarctation of the aorta.

Chest radiograph has been done in 325 patients. Finding was regular in 150 examiners or (46,15%) and pathological in 175 or 53,85% of patients. Electrocardiogram has been done in 335 patients in which 175 or 52,24% was regular, and in 160 or 47,76% pathological (figure 1).

Figure 1. *Chest radiography and electrocardiography in children with congenital heart disease*



Final estimation of morphological and hemodynamical changes is made on basis of echocardiographical examination, by which is, using sequenced segmental analyses in 5 examiners was registered situs inver-sus, in one right atrial isomerism and in the others 364 situs solitus. In

level of artioventricular connection, by echocardiographcal examination in all examiners was found biventricular harmonious connection. By analyze of ventricul-arterial connection in 13 patients was registered discordant, in 3 patients double outlet by right ventricle and in others concordant connection.

While estimating about needs for further treatment during first examination we have found that 73 (20,74%) patients have needed medicament therapy. Cardiotonic and diuretics were for 41 patients, only diuretics for 3 patients, propranolol for 11 and prostaglandin E11 for 18 patients. From totally 352 patients with CHD, 188 requested only control examination, 61 invasive diagnostic and 157 or 44,60% cardiosurgical treatment. From those patients, 62 were critical CHD with heavy hemodynamical disorders, which requested urgent cardiosurgical intervention. Among them 41 (66,13%) patients were in infant age.

Table 2. *Course of disease in children with congenital heart disease*

Congenital heart disease	Course				
	n	Spontaneous recovery	Need operation	Done operation	Died
Ventrikular septal defect	140	13	5	20	20
Atrijal septal defect	53	-	8	18	3
Patent ductus arteriosus	26	7	4	6	-
Pulmonary stenosis	24	-	1	3	-
Atrioventricular canal	23	-	7	3	13
Tetralogia of Fallot	23	-	1	16	6
Aortic stenosis	22	-	2	6	3
Transposition of great artery	12	-	1	3	8
Coarctatio of the aorta	9	-	-	4	1
Tricuspid atresia	7	-	-	2	7
Hypoplasion of the left ventricle	4	-	-	-	4
Total anomalous pulmonary vein returns	2	-	-	-	2
Fibroelastosis	2	-	-	-	2
Truncus arteriosus	1	-	-	1	-
Epstain' s disease	1	-	-	-	-
Other congenital heart disease	3	-	-	-	-
Total		20	29	82	69

By analyzing implementation of requested therapy we have found that medicament treatment was implemented in accordance to recommendation, except in cases of prostaglandin E1 which was needed in 18 patients but was used only in one patient. Catheterisation of heart

as a diagnostic procedure, independently on surgical intervention, has been done only in 5 examiners, and in preoperative preparation in 68 examiners.

Course of disease according to type of anomaly is shown in table 2. It is noticeable that from 20 patients in which were registe-red spontaneous recovery, 13 have had ventricular septal defect (10 muscular and 2 perimembranous defect). In two patients after complete invasive diagnostic surgical treatment was contraindica-ting. In group of patients who are waiting for surgical treatment, in two of them (one with atrioventricular septal defect and one with transposition of great artery) have been done palliative operation.

Cardiosurgical procedure has been done in 82 patients. From they 15 patients have been operated in Sarajevo, and the others were operated in cardiosurgical centers outside of Bosnia and Herzegovina. Two children died in early postoperative period, and one six months after the surgery. In 12 examiners were registered post operative complications: in five temporary disorder of heart rhythm, in four postpericardiotomy pericardial effusion and in three disorder in using type complete artioventricular block. In patients with complete atrioventricular block, in the same cardio surgical centers where have been done correction CHD it has been done implantation of pace-maker as well.

There were no surgical procedures in infant age among patients. The highest number of patients (50 or 60,97%) had surgical procedure in preschool age, and less number in school age (20 or 24,90%) and infant age (12 or 14,63). The youngest patient had surgical procedure in age of 0,17 and the oldest in age of 15,9 years. The average age in which children have had surgical procedure was 4,81±3,23 years with standard deviation 3,95.

From total 352 patients with CHD 69 or 19,6% died. Those 66 died earlier, and 3 after surgical procedure. In neonatal age 32 (8 in first day of life) patients, 27 infants, and 10 died until age of 5 year. Average age of children who died was 0,51±0,59. Compara-tive review of patients who had surgical procedure and those who died in period of examination is shown in figure 2. Number of died was from 5 in 1995 to 17 in 1996, even although in the last three years was equal. Number of those with surgical procedure had shown increase from 4 in 1994 to 27 in 1999.

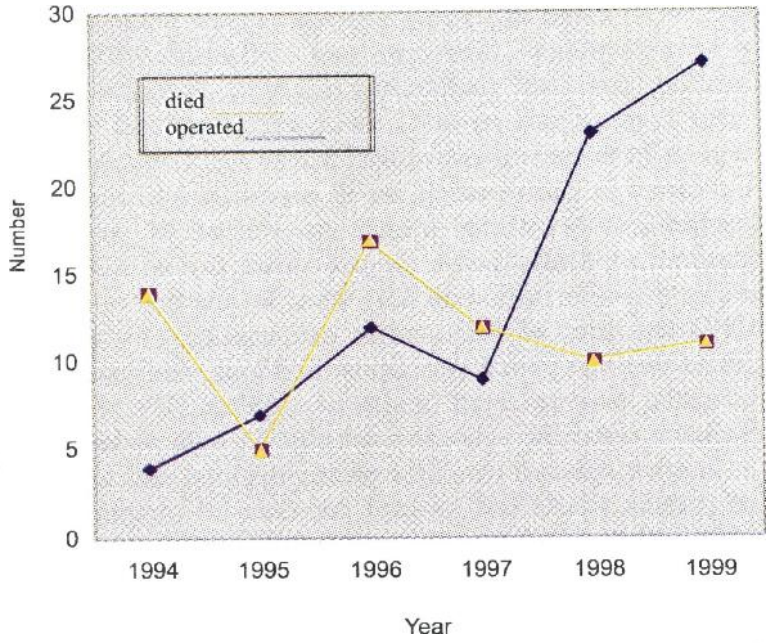


Figure 2. *Comparative review of patients who had surgical procedure and those who died*

Discussion

Diagnostic possibilities for discovering of CHD in Tuzla Canton area until 1992 were very limited, thus children with suspicious on this disease were refereed to different centers out Bosnia and Herzegovina, in order to do diagnostic and eventual treatment. In such conditions great number of CHD, especially those critical, stayed unknown. All these as result had impossibility of its precise registration, and that is a reason that information about epidemiological and clinical indicators practically never existed.

When conditions for non invasive diagnostic of CHD at the Pediatric Clinic in Tuzla and organizing of health care for children in Tuzla Canton area were provided in 1994, it was possible existing of an diagnostic center for this pathology. This has opened possibilities for further continuing determination and following of patients with CHD. Results of diagnostic center during its first six years of existing we have evaluated in our research, what is also presents first step in forming register of CHD in this region.

According to our results there were no CHD diagnosed in fetal age, even there are many studies, which point on success of fetal echocardiography. Andersen and colleagues (5), by applying this method, from total number of CHD have determined 5,5% intrauterine, what from aspect of early discovering presents significant success, especially when is about critical anomalies. Even although, some of age indicators in our research are in accordance to research in developed countries, still results, which are related to preschool and school children evidently point on late discovery of CHD. By analyze of anamnesis information on time when for the first time was suspected on CHD and time of first cardiac examination of our patients, we have found that that period was unjustified long, and mostly as result of non educated parents about health of children. By introducing fetal and neonatal screening echocardiography it would make this period shorter, or in other words it would be mostly avoided, and with health education of parents would lead toward decrease of complication of CHD and neonatal mortality.

Reason for first cardiac examination we have analyzed from anamnesis survey, and gave answer on question: "Why this child was send for cardiac examination?" Received results points that the most frequent reason for cardiac examination were deranged auscultation finding on heart and presence of cardiovascular disorders, while less seldom reason were presence of some potential risk factor and changed electrocardiographically finding. Great frequency of changed auscultatory findings on heart, as one of the reason for cardiac examination is expected since, that is consequence of hemodynamical disorders, presented in all types of CHD. Results on higher frequency of cardiac vascular disorders, as reason for cardiac examination in our research, shows that diagnosis of CHD was relatively late.

Frequency of growth retardation in our patients, which is defined by anthropometrics measurement of body mass and weight, has tendency of increasing in relation on fetal growth. Results point that stoppage in body mass is bigger in relation to stoppage in body height, what is in accordance to results from literature (6,7). Those mentioned authors have proved that level of stoppage in growing depends on type of anomaly, and they are more expressed in category of cyanotic anomaly. Some results (6,8) point that the main cause of stoppage in growing of children with CHD is hypoxemia, which occurred as consequence of hemodynamical disorders. Namely, they have proved that first step are control of growing and development of children with CHD, what would be basis for further studies.

Values of arterial blood pressure were increased in, 82% of patients. All examiners with coarctation of the aorta have had higher blood pressure, what indicates that routine measure of arterial blood

pressure in primary health care is successful method for detection of coarctation of the aorta.

Electrocardiogram (ECG) was changed in almost half of patients. Swenson and colleagues (9) found changes on ECG in 8% patients, to who after auscultation was excluded possibility of existing of CHD. To those patients have been done echocardiography and has been determined atrial septal defect. Those results point that even with advanced and modern and powerful diagnostic procedures, ECG still remains unchangeable diagnostic method in evaluation of children with CHD.

Changed chest radiogram we have found in almost half of patients, what is significantly higher in relation to 28% what is found in literature (9). Chest radiogram still remains as important tool in evaluation and control of children with CHD. Opinions about its routine usage are divided. Namely, from aspect of expenses, chest radiogram is recommended in routine use for all patients, but in trying to avoid eventual harmful effect of radiation, it has been recommended only while indicating cases, or there where is expected its help in determining correct diagnosis.

In estimation of needs for further treatment during first examination we have found that 20,74% of patients have requested medicament therapy, from what 24,66% cases needed usage of prostaglandin E1. Indication for cardiosurgical procedure during first examination was determined in 44,6% patients, what is in accordance to data from literature (3). Because critical CHD urgent surgical intervention was needed in 17,89% of patients what is less in relation to data from literature (10,11). Less number of children with CHD does not mean that they are seldom in our region. We could explain that as possibility that certain number of children with critical CHD died immediately after birth, before cardiac test was performed and which parents never approved autopsy.

By analyzing recommended therapy we have found that prostaglandin E1 was used only in one case from those 18 that were necessary. Similar information we have not found in literature, what was expecting, because it is about medicament palliative treatment, what is part of routine therapy, especially in centers such as ours, without possibilities for urgent catheterisation and cardiosurgical therapy. Results Dinarević and colleagues (12) point on successful usage of prostaglandin in neonatal cardiology in our country.

Spontaneous recovery was registered in 14,29% of our patients with ventricular septal defect. According to literature (1) it was registered in 15% to 50% of children with ventricular septal defect. Such great variation depends mostly on diagnostically possibilities. In region where is possible detection of small muscular ventricular septal

defect, frequency of spontaneous recovery is higher, and there where CHD was diagnosed in older age there is higher number of children with this anomaly will stayed as unknown.

According to our results, it is necessary to upgrade early diagnostic of CHD in our country. Lateness in diagnostic is expressed with information that in our 2 patients with CHD was discovered late, when is already developed heavy lung hypertension where surgical intervention was contraindicated.

From those 150 patients who needed cardiac surgical intervention, the same was done only in 51,57%. With critical anomaly were 63 patients, and surgical intervention have been done in only 3 or 4,76% cases. Fact that there were no surgeries in newborn age is very disturbing. According to results of Bosi and colleagues (13) in newborn age have been 12% of surgical interventions among all diagnosed CHD, from what only in one third part have been done palliative procedure.

Our research which is related on treatment of patients with CHD, started from usage of prostaglandin E1, need for urgent or diagnostic catheterisation, early palliative procedure and finally corrective cardiac surgical procedure point on its insufficiency. Justifying with high price of treatment is also unsaid, when we have fact that many other undeveloped countries, by investing money in development of cardiac surgery more and more reaching world standards for advanced treatment of CHD. Also, it is necessary to make relation between cost of treatment and advantages which will reach in that case as: decrease of mortality of children, better quality of life, and less number of chronic ill persons in older age. Garson and colleagues (14) analyzing cost of treatment of CHD from birth to age of 21 in the USA have found that cost is in average 59,877US$, what makes it on 5th place in relation to some other chronic diseases (hemophilia, cystic fibrosis, malignant diseases, muscular dystrophy). All this point on necessity of producing systematic and unique program of medical treatment of children with CHD, starting with control of epidemiological parameters, permanent monitoring, diagnostically possibilities up to providing necessary therapeutic measures. By individual consideration of justification and cost it is evident that many needed measures could fit into current financial situation in our country.

Results on mortality of patients with CHD show that the biggest number of them died in age of newborn, what is in accordance to other studies (13,15). Comparative review of performed cardiac surgical procedures and mortality in accordance to age, point that the highest mortality is in age group for which cardiac surgery in our region is the least available.

Considering epidemiological and clinical indicators of CHD in Tuzla Canton area it is evident that this is a public health problem,

whose resolving requests coordinated work of all segment of public health care of children as well as engage of broader social community. Our research, which presents beginning of total following and control of children with congenital heart disease, gave great benefit on focusing problem related to its diagnostic and treatment in our region.

Apstrakt

Cilj istraživanja bio je procjena mogućnosti dijagnostike i liječenja urođenih anomalija srca (UAS) u našim uslovima. Analizirana je medicinska dokumetacija od 352 djece sa ovim oboljenjem u šestogodišnjem periodu na području Tuzlanskog kantona. Srednja vrijednost životne dobi, u kojoj je UAS dijagnostikovana, bila je 2,15±2,28 godina. Tokom prvog kardiološkog pregleda, 51,98% djece je imalo prisutne simptome bolesti srca. Zastoj u tjelesnoj masi i tjelesnom rastu u postnatalnom dobu nađen je u 13,35%. Elektrokardiografske promjene su registrovane u 47,76% a izmijenjen radiogram srca i pluća u 53,85% ispitanika. Medikamentozno liječenje je bilo potrebno u 20,74%, a kardiohirurški zahvat u 40,60% ispitanika. Hitno kardiohirurško zbrinjavanje je bilo neophodno u 62 ispitanika sa kritičnom UAS. Kardiohirurški zahvat je urađen u 23,29% ispitanika, a prosječna dob u kojoj je urađen iznosila je 4,81±3,23. Smrtnost djece sa UAS je relativno visoka i iznosi 19,60%. Najveći broj njih (95,65%) umrlo je prije, a samo 4,35% nakon kardiohirurškog zahvata. Prosječna dob u kojoj su djeca umirala iznosila je 0,51±0,59 godina. Analiza kliničkih pokazatelja UAS u djece na području Tuzlanskog kantona ukazuje na to da se radi o zdravstvenoj problematici djece, koja zbog teške kliničke slike, velikog učešća u perinatalnoj i dojenačkoj smrtnosti, usporenja rasta i razvoja s progresijom u hroničnu kardiopatiju, zahtijeva hitne mjere savremenog organizovanja njihove dijagnostike i liječenja.

Ključne riječi: *urođene anomalije srca, djeca, Tuzlanski kanton.*

Literature

Fyler DC (1980) *Part of the New England regional infant cardiac program.* Pediatrics 65:375-461

Begić H (2001) *Epidemiološko-kliničke karakteristike urođenih anomalija srca u djece na području Tuzlanskog kantona.* (magistarski rad)

Brode P (1989) *Učestalost, raspodjela i spontani tok prirodjenih srčanih grešaka.* U: Keck EW i suradnici ur. Pedijatrijska kardiologija, Zagreb: Školska knjiga, 1-3

Anonymus (1987) *Report of the Second Task Force on Blood Pressure in Children.* Pediatrics 79:1-25

Andersen S, Vik T, Linker DT (1994) *Congenital heart disease in Sor-Tronbelag. Incidence, diagnosis, soutse and treatment.* Tidsskr Nor Leageforen 29-32

Jacobs EG, Leung MP, Karlberg JP (2000) *Postnatal growth in southern Chinese children with symptomatic congenital heart disease.* J Pediatr Endocrinol Metab 13: 387-401

Tambić-Bukovac L, Malčić I (1993) *Growth and development in children with congenital heart defects.* Lijec Vijesn 115 (3-4):79-84

Schuurmans FM, Pulles-Heintzberger CF, Gerver WJ, Kester AD, Forget PP (1998) *Long-term growth of children with congenital heart disease: a retrospective study.* Acta Pediatri 87: 1250-1255

Swenson JM, Fischer DR, Miller SA, Boyle GJ, Ettedgui JA, Beerman LB (1997) *Are Chest Radiographs and Electrocardiograms Still Valuable in Evaluating New pediatric Patients With heart Murmurs or Chest Pain?* Pediatrics 99:1-3

Šamanek M, Voriškova M (1987) *Infants with critical heart disease in territory with centralized care.* International Journal of Cardiology, 16: 75-91

Vukmanović V, Papić R, Košutić J, Mogić M, Janković B, Cvetanović G (1999) *Kritične urođene anomalije srca.* Problemi u pedijatriji 98, 19: 91-111

Dinarević S, Kurtagić S, Maksić H. (2000) *Upotreba prostaglandina u neonatalnoj kardiologiji.* Med Arh 54(5-6):279-82

Bosi G, Scorrano M, Tosato G, Forini E, Chakrokh R and the Working Party of The Italian Societi of Pediatric Cardiology (1999) *The Italian Multicentric Study on Epidemiology of Congenital Heart Disease: first step of the analysis.* Cardiol Young 9: 291-299

Garson A, Alen HD, Gersony WM, Gillette PC, Hohn AR, Pinsky WW, Mikhail O (1994) *The Cost of Congenital Heart disease in Children and Adults.* Arch Pediatr Adolesc Med, 148: 1039-1045

Šik T (1976) *Važnost rane dijagnostike i intervencije prirođenih grešaka srca za smanjenje mortaliteta novorođenčadi i dojenčadi.* Referat. Jugoslovenski simpozij: Invazivna dijagnostika u kardiologiji i kardiološka konfrontacija. Beograd

MULTISLICE SPIRAL CT IN DIAGNOSTICS OF CORONARY ARTERY DISEASE

Šerif Bešlić[1], *Faruk Dalagija*[1]

Abstract

The aim of this study is to rewiev the contribution of multislice computed tomography (MSCT) as a screening tool for coronary artery disease by measuring quantity of coronary calcium. During the period of one year, 59 patients with clinical symptoms of cardiac disease were examined in our Institute of Radiology.

The average age of the examined patients was 62 years. The youngest patient was 18 and the oldest 82 years old. 41 patients (69.5%) were male with average age of 62.7 years and 18 patients (30.5%) female with average age of 63 years.

The CT examinations were performed with ECG gating MSCT "Somatom Volume Zoom" Siemens native and with intravenous contrast medium administration (140 ml Omnipaque with 50 ml physiological solution) and with 3 mm slice thicknes.Special attention was paid to the coronary vessel calcification according to Agatston's total calcium score(TCS) for account coronary calcification.

Calcifications of coronary arteries were found in 52 patients and TCS was made according to Agatston's method. Among these patients were12 (20,3%) with mild coronary atherosclerosis (total calcium scor -TCS 10-100), 11 (18,6%) with moderate coronary atherosclerosis (TCS 100-400), and 29 (49,1%) with severe coronary atherosclerosis (TCS > 400).

42 (71,2%) of these patients were smokers, 22 (37,3%) had hypertension, 9 (15,2%) diabetes, 6 (10,2%) hypercholesterolemia and 5 (6.8%) triglyceridemia, everybody with coronary calcifications.

Only 24(40,7%) of these patients had symptoms of angina pectoris.

Infarctus myocardii was found in 7 (11.9%) patients, among them 6(85,7%) with severe coronary atherosclerosis (TCS > 400).

Calcifications of the coronary arteries are always connected with atherosclerosis.

[1] Institute of Radiology CCU Sarajevo

For this reason an early detection and quantification of coronary calcifications play an important role in the diagnosis and prognosis in patients with coronary artery disease.

MSCT has shown to be the most sensitive modality for this purpose and enables, safe and non-invasive screening investigation of coronary arteries.

Key words: *Multislice CT (MSCT), coronary atherosclerosis (CA),total calcium score(TCS)*

Introduction

Coronary atherosclerosis is a progressive pathological process, which can stay non-symptomatic for many years or even for whole life. In the first stage of coronary artery disease it could be spread over the large number of the population. It can be compared with ice-berg. Angina pectoris can develop in these patients or sudden coronary attack can happen.

That is the main cause of death in the west countries, and includes 5-10% male population (1). About 30% heart attacks appear in people who have not usual risk factors (increased cholesterol level, high blood pressure or diabetes) (2). 60% men and 42% women who come with acute myocardial infarct or sudden death didn't have disease in anamnesis before (3).

Every year over 300 persons per 100.000 inhabitants get myocardial infarct (IM), often as the first symptom of their cardiovascular disease. Patient dies in over 35% cases (4). There were 500.000 persons in USA in 1956 and 600.000 ones in Europe in 1998 who died for these reasons (5,6).

Coronary disease is manifestation of atherosclerosis in coronary arteries. It is a multifactor process, which leads to myocardial ischemia and can be showed as angina pectoris, myocardial infarct, and heart disrhythmia, congestive heart disease or sudden death. Half of the patients with ventricular dysfunction are aware of their condition or they have no adequate conditions for medical treatment, while only half of them with recognised symptoms get appropriate treatment. This statistical set is called "rule of the half" (7).

Great efforts have been put into early identification of persons with cardiovascular risk factors.Any reliable remedy wasn't found for examination of atherosclerosis progress or retreating.

In this moment, there are different ways of heart visualization: radiography, fluoroscopy, echocardiography, Doppler, Multislice CT (MSCT), Electron Beam CT (EBCT), A single photon emission tomography (SPECT), Proton emission tomography (PET), Magnetic resonance imaging (MRI) and intravascular ultrasound (US). Diagnostic tests, which were used, for screening of normal population for detecting

of obstructive lesions that disturb the coronary circulation, are treadmill test of loading, echo stress test, talium scintigraphy and so on. But, all these tests can not discover non-obstructive coronary plaques, which carry rupture risk and can provoke unstable angina, acute myocardial infarcts or sudden death without any warning signals. Angiography is gold standard for all these methods, while PET is that for quantitative measuring of myocardial blood circulation and getting metabolism information (8).

More than a million angiographies have been done in the USA every year, but the method is invasive and expensive and requires short hospitalisation at least (6). Heart catheterisation with diagnostic complex costs between 1500 – 3000$ in Europe. The cost prise in acute myocardial infarct if a patient survived is 3800$, but if he died 1770$ (7). Because of that it's desirable to replace angiography with non-invasive screening investigation which can enable to see marshy part of the berg of coronary artery disease before appearance of fatal result.

Research have established close link between stratums of coronary artery calcifications and atherosclerosis plaque in post-mortem studies of coronary victims, such as tight correlation between coronary artery calcifications and coronary artery stenosis (1).

From there is an idea that calcium deposits can serve as markers of early coronary artery disease. Detecting of coronary calcifications shows early atherosclerosis forms before clinical symptoms. Because of that estimation of coronary calcium has been suggested for detecting coronary artery disease in patients with pain in their chests. Severe calcifications of coronary arteries are very suggestive on significant stenosis of coronary arteries. It was suggested that coronary vessel calcification in patients over 50 years old can refer to bad prognosis. The patients with severe coronary vessel calcifications had on increase of myocardial infarct frequency during autopsy (5). Radiography and fluoroscopy can detect coronary calcifications, but these methods are of small sensitivity, especially for mild forms of calcifications.In 1982. it was detected that CT is very sensitive method for detecting coronary calcifications, and a little bit later thanks to development of fast CTs, Agatston and all. developed quantification system of calcium contents in coronary arteries known as Agatston's method (fig.1). Identification and quantification of coronary calcifications are improved by development of ultra fast scanner types EBCT and MSCT (fig.2).

Quantitative determination of coronary calcifications point to presence of coronary atherosclerosis as first indicator of heart disease. This research can be positive when all other non-invasive screening methods are negative. But number of the false negative tests is not known yet.

Whatsoever, coronary calcifications of atherosclerosis plaques happen in early stage of disease. MSCT makes possible non-invasive detection of atherosclerosis plaque (10). Reliability of coronary calcium quantification, especially for soft plaques has been improved by using retrospective ECG "gating" at MSCT, which make possible to predict if changes in coronary artery calcifications have predictive value for future coronary strokes.

Investigations have shown that the absence of coronary calcium in old people highly correlated with normal coronary arteries, so that MSCT can eliminate need for coronary angiography. MSCT can be used not only in detecting of coronary calcifications as marker for total coronary atherosclerosis, but also for estimation of preventive therapy effects. In that way modern screening techniques made possible heart to be returned in Radiology.

The aim of this investigation is a review of the contribution of MSCT as screening method in detecting coronary artery disease on the base of coronary calcium quantification.

Figure 1. *"Calcium Window" for identification of calcified plaques*

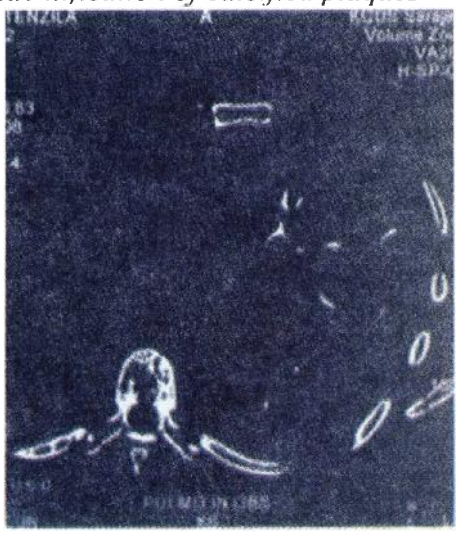


Figure 2. *Calcification of left coronary artery*

Material and method

During the period of one year, 59 patients with clinical presentation of cardiac disease were examined in our Institute and they were kept under different diagnosis, from angina pectoris, hypertension, different kinds of heart ischemia and disrhythmia, to myocardial infarct.

The average age of the examined patients was 62 years. The youngest patient was 18 and the oldest one, 82 years old.

Among those patients 41 (69,5%) were male, with average age of 62, 7 years, and 18 (30,5%) female, with average age of 63 years.

The CT examination was performed with multisection CT device "Somatom Volume Zoom" SIEMENS, with special program for heart examination, 4x2,5mm colimation, 500msec. rotation time, pitch 1,5 and FOV 200. The examination was performed first native, than with contrast bolus, 140ml non-ionic contrast medium administration (370mg/1ml) which was given in anthecubital vein with flow rate 3-4ml/sec, followed by 50ml physiological solution. Retrospective gating, 35-450msec before next R wave has done the reconstruction of the image.

Special attention was paid to detecting of coronary artery calcifications as sign of coronary atherosclerosis (CS), in keeping with Agatston's method where the product of total zone of calcified plaque for each stratum was determined, and counted so called total calcium score (TSC), for each patient. The patients were divided in three groups, one with soft (TSC 10-100), mild (TSC 100-400) and one with severe coronary calcifications (TSC>400). At the end correlation between clinical findings and real condition shawn through TSC Agatston. The attention was paid to establishing frequencies of coronary calcification in smokers.

Results

Calcifications in coronary arteries as a sign of coronary atherosclerosis were detected at 52 (88,1%) of the patients. Among these patients, there were 38 (73,1%) male (M) and 14 (26,9%) female (F). Relation between male and female patients was 2,7:1 in male benefit.

Although they had some symptoms of heart disease, there were 7 (11,9%) patients without severe calcifications in coronary arteries, 3 (5,1%) male in the average age of 45 years and 4 (6,8%) female in the average age of 48,3 years.

Table 1. *Frequency of coronary sclerosis (CS) according to clinical diagnosis, risk factors and smoking*

Clinical diagnosis	Soft CS	Mild CS	Severe CS
Angina pectoris 24 (40,7%)	5 patients	4 patients	15 patients
Myocardial infarct 7(11,9%)	1	0	6
Hypertension 22(37,3%)	5	8	9
Diabetes 9(15,2%)	2	2	5
Hypercholesterolemia 6(10,2%)	2	1	3
Tryglyceridemia 5(6,8%)	3	0	2
Smokers 42(71,2%)	8	7	24

Table 2. *Frequency of coronary sclerosis (SC) at patients according to total calcium score (TSC) and age and sex.*

Level of CS	Number of patients	Sex	Age	Sex	Age	TSC
Soft CS	12(20,3%)	8(13,6%)M	64,5 years	4(6,8%)F	49 years	10-100
Mild CS	11(18,6%)	7(11,9%)M	69,6	4(6,8%)F	66,3	100-400
Severe CS	29(49,1%)	23(39%)M	64,2	6(10,1%)F	71,8	>400

Table 3. *Frequency of coronary sclerosis (CS) at myocardial infarct, TCS and smoking*

Diagnosis	Number of patients	Sex	Age	Smoking	TSC
M. infarct	7(11,9%)	6(85,7%)M	69,2	(85,7%)smokers >30 years	>400
		1(14,3%)F	71		>400
Recent MI		M	48	Non-smoker	10 (soft CS)

A patient with recent myocardial infarct had diabetes and hypertension with positive family history and raised tryglicerides.

Discussion

The aim of this and quoted studies in introduction part is reducing of disease and mortality at coronary disease caused by atherosclerosis process. Identification of coronary artery calcifications and calcium score can be useful in detecting of early stage of coronary disease at asymptotic patients, if coronary calcification is found. The reports show that TCS could be radiology equivalent to Babinski in clinical neurology. Coronary calcium score shows close link between coronary calcifications and cardiovascular disease and tells us about difficulty of atherosclerosis process.

According to our research 88,1% of the patients with symptoms of cardiovascular disease have had coronary calcifications, and 49,1% of them refer to severe coronary calcifications with TCS>400 (table 1). Percentage of severe coronary calcifications in patients with myocardial infarct was 85,7%, with TCS>400 (table 3). Our information is in accordance with those in literature, where it is quoted that the patients with calcium score over 2000 had 8 times better chance for heart attack compering with patients whose calcium score was under 100 (11). Also, information from literature tells us that 90% hearts with myocardial infarct have at least one calcification in coronary arteries (7).

Most investigations show that quantity of calcifications found at CT has correlation with seriousness of coronary sclerosis, although absence of calcifications on specific place doesn't exclude possibility of atherosclerosis plaque in that zone. This could have significant results for primary prevention of coronary artery disease, because coronary calcium points to developed stages of atherosclerosis.

Estimation of calcium can be useful in two cases: in non-symptomatic patients with high level of risk from coronary artery disease and at patients with non-typical chest pain (12). Calcium presence suggests higher risk for appearance of infarct and sudden death. So, detecting and quantification of coronary calcium play important role in diagnostics and prognosis in patients with and without coronary disease. CT is the most sensitive method for this purpose and enables fast, safe and non-invasive screening (13). Coronary artery calcium score according to Agatston's method can be effective weapon in estimation of therapy efficiency and can play a role in planning therapy technique (1,14).

High TCS can be important in making decision about more aggressive treatment of risk factors in non-symptomatic patients and can influence the selection of treatment procedure, like angioplastic, stenting, endarterectomy or rotating ablation.

Most of our patients who were analysed and who had at least one of risk factors (hypertension, diabetes, hypercholesterolemia, trygliceridemia or were smokers), have had calcifications in coronary arteries and often high TCS. Only 49,1% of these patients had signs of angina pectoris as warning symptom. Smoking, hypertension, male sex and older age in our small series have most often been united with coronary calcifications and high TCS. 42 (71,2%) of our patients were smokers (table 1,2). Relation between male and female patients was 2,7:1. This confirms previous knowledge about larger endanger of male from artery disease.

Development of new CT devices with scanning speed under 500msec like MSCT is powerful weapon for analysing coronary artery disease. That is simple non-invasive method to examin of the patients with non-typical chest pain. It is valuable method of examination, which can become an alternative to heart catheterisation or intravascular US (2).

Our first experiences with series of 59 patients with signs of heart disease confirmed existing of coronary artery calcifications in 88,1% cardiac patients and showed high sensitivity of this non-invasive way of examination.

According to information from literature MSCT enables virtual angioscopy of coronary arteries with sensitivity of 88% and specificity of 93% for detecting chemodinamic significant stenosis in proximal

segments of coronary arteries (2,6,15). In those case it can give important information in non-invasive way, not only about luminary condition of coronary artery, but also about artery wall, and evolution of plaque after therapy can be followed (2,10).

When calcifications in coronary arteries are detected as a warning sign, the risk can be reduced by suggesting patients to change the way of living, drugs for reducing cholesterol and so on (5,7). Contribution of MSCT is simultaneous visualization of aorta ascendens and lungs, which can sometimes be priceless and is not to be done with other methods.

But, there are some investigations, which show that 20% of patients under 50, who got acute myocardial infarct, have no calcium in coronary arteries (1). The similar case was with our 48 years old patient, non-smoker man, with recent myocardial infarct, whose TCS were 10 and who had risky factors: diabetes, hypertension, tryglicerides and positive family history (table 3).

For that some authors think that calcium score 0 does not exclude presence of atherosclerosis in patients under 50, more exactly that screening of coronary calcium should not be used to exclude acute coronary attack in young people (1,5). It is shown that small stained classifying lesions mostly dominate in patients with first myocardial infarct (16).

There are some opinions that sclerosis of arteries is not something to be less afraid of than of some fatty inflamed plaque and its rupture risk and making clot (10,11). All of these point out that risk of fatal heart attack is more complex than we thought before. Because of that, we need more studies.

New variants of MSCT show that they could become non-invasive device detecting soft plaques, and make possible identification of these patients in early stage (2). For now, it gives us a chance for easy non-invasive visualisation of calcified atherosclerosis in coronary artery wall and its measuring (TCS) to determine coronary risky factor. In that way necessary coronary angiography could be avoided (7). All these have the aim to improve non-invasive diagnostic procedure, intensify understanding of heart disease and reduce getting sick and patient mortality (3,7). Tight co-operation between radiologists and cardiologists is necessary.

Conclusion

Calcifications of coronary arteries are always in close connection with coronary atherosclerosis, and with heart diseases also, especially myocardi. Because of that early detecting and quantification of coronary calcifications play an important role in diagnostics and

prognosis in patients with coronary arteries disease. According to this investigation and research of other authors MSCT has been shown as very sensitive way for this purpose, because it make possible fast, safe and non-invasive screening examination of coronary arteries. It could be used as a way of selecting patients for coronarography or more intensive conservative treatment. It is relative cheep and easy accessible method for everyone to check coronary arteries, in non-symptomatic patients or those with inexplicable chest pain.

Apstrakt

Cilj ove studije je prikaz doprinosa višeslojne kompjuterske tomografije (MSCT) kao sredstva za screening koronarne arterijske bolesti, pomoću kvantifikacije koronarnog kalcija.

U periodu od jedne godine pregledali smo na našem Institutu 59 pacijenata sa kliničkim simptomima bolesti srca. Najmladi pacijent je imao 18 godina a najstariji 82 godine. Medu ovim pacijentima 41 (69,5%) su bili muškarci, prosječne starosti 62,7 godina., a 18 (30,5%) žene, prosiječne starosti 63 godine.

CT pregled je bio učinjen, sa retrospektivnim ECG "gating"-om na višeslojnom CT-u "Somatome Volume Zoom"SIEMENS, nativno i sa intravenski datim kontrastnim sredstvom (140 ml Omnipaque sa 50ml fiziološkog rastvora) i 3mm debljinom sloja. Posebna pažnja je bila usmjerena na kalcifikacije koronarnih sudova u skladu sa Agatston-ovim ukupnim kalcijum skorom (TCS) za proračun koronarnih kalcifikacija.

Kalcifikacije koronarnih arterija nadene su kod 52 (88,1%) pacijenata, a ukupni kalcijum skor je učinjen prema metodi Agatston-a. Medu ovim pacijentima je bilo 12 (20,3%) sa blagom koronarnom aterosklerozom (ukupni kalcijum skor 10-100), 11 818,6%) sa umjerenom koronarnom aterosklerozom (TCS 100-400), i 29 (49,1%) sa ozbiljnom koronarnom arteriosklerozom (TCS>400).

42 (71,2%) od ovih pacijenata su bili pušači, 22 (37,3%) su imali hipertenziju, 9 (15,2%) diabetes, 6 (10,2%) hiperholesterolemiju i 5 (6,8%) trigliceridemiju, svi sa koronarnim kalcifikacijama. Od ovih pacijenata samo 24 (40,7%) je imalo simptome angine pektoris. Infarkt miokarda je naden kod 7 (11,9%) pacijenata, od kojih 6 (85,7%) sa teškom koronarnom aterosklerozom (TCS>400).

Kalcifikacije koronarnih arterija su uvijek u vezi sa aterosklerozom. Iz tog razloga otkrivanje i kvantifikacija koronarnih kalcifikacija igra važnu ulogu u dijagnostici i prognozi kod pacijenata sa koronarnom arterijskom bolesti.

MSCT se pokazao da je najosjetljiviji način sa ovom namjenom i mogućnosti brzog, sigurnog i neinvazivnog screening ispitivanja koronarnih arterija.

Ključne riječi: *Višeslojni CT (MSCT), koronarna ateroskleroza (CS), ukupni kalcijm skor (TCS).*

References

Shemesh J. *Spiral methods quantify coronary calcification.* Diagnostic imaging Supplement; 1994; 11:30-32.
Hudon R. *Heart scan defects soft plaque in coronary arteries.* Diagnostic imaging 2001; 23 (2): 66.
Reinmüller R. *Non-invasive Coronary artery imaging.* ECR NEWS LETTER, State-of -the-art symposia/spe focus sessions. 2001; 11: 8.
Schoept UJ; Becher Ch. R, Rust. F, Ohuesorge B, Schaller S, Reiser M. F. *Multislice CT as screening Tool colon cancer, lung cancer and coronary artery disease.* Medical Imaging International. 2001; 11 (2): 8-11.
Reddy GP, Higgins Ch. B. *EBCT aids diagnosis of coronary artery disease.* Diagnostic Imaging Europe. 1999; 1 (2): 43-50.
Copp A, Schroeder S, Claussen C. *CT promises revolution in coronary diagnosis.* Diagnostic Imaging Europe. 2000; 11; 36-39.
Reinmüller R, Reiter U, Schrottner B. *Collaboration proves vital for cardiac success.* Diagnostic Imaging Europe. 2001; 17 (1): 14-31.
Schemesh J,Shreter U. *Coronaryartery calcification scoring with CT-T win.* Elscint Report.1996; 8 (1):11.
Agatston AS, Janowitz WR, Hildner FJ, et al. *Quantification of coronary artery calcium using ultrafast computor tomography.* J. Am. Coll Cardiol 1990; 15: 827-832.
Carrington C. *Multislice CT excels at spotting arterial plaque.* Diagnostic Imaging Europe. 2000; 16 (4): 7.
Brice J. *MSCT shows its worth in coronary artery screening.* Diagnostic Imaging Supplement. 2002; 1: 21-2.
Becker Ch, Knez A, Ohnesorge B. *High Grade Post Stent Stenosis Dected By Retrospectively ECG gating.* Multislice CT. Somatom Sessions "Siemens". 1998; 1: 4-5.
Becker Ch. *Coronary screening. Somatom Sessions "Siemens".* 1998; 5:4-5.
Brice J. *CT faces opstacles for testing coronary arteries.* Diagnostic Imaging Europe. 2000; 16 (1): 14.
Carrington C. *Multislice CT makes strides in coronary angiography.* Diagnostic Imaging Europe. 2001; 17 (3): 14-16.
Shemesh J. Motro M. *Progression of Coronary Artery disease: Comparison of imaging Modalities.* Med Imaging International. 2001; 11 (5): 6-10.

THE INFLUENCE OF CO-FACTORS AND HPV INFECTION ON THE APPEARANCE OF CERVICAL LESION

Ermina Iljazović[1]

Abstract

The cervical cancer (CC) is in the second position in the world, by incidence and mortality, and in the first place in the developing countries. According to the information from literature different risk factors are more or less significant for the development of CC. One of the most important factor for the development of premalignant and malignant cervical lesion is infection with human papillomaviru (HPV). The strongest epidemic corelation was found in connection to the age at the time of first sexual intercourse, especially prior to the age of 18, and 21. The objective of this study is to determine the influence of etiological factors (smoking, age at the time of first sexual intercourse, number of intercourses, number of partners and status of circumcision) on the occurrence of pre-malignant and malignant cervical lesions among patients with persistent HPV infection.
Key words: *cervical lesion, HPV infection, cofactors*

The cervical cancer (CC), is in the second position in the world, by incidence and mortality (Anonymus-NIH Consensus Development Conference, 1997), and in the first place in the developing countries. After 1947, its incidence and the rate of mortality dropped by 50 to 70% in the developed countries, despite the sex revolution, numerous migrations and an increased prevalence of infection by the human papiloma virus (HPV). This fact is mainly explained by the results achieved in the implementation of cancer screaning (William, 1999). However, in some countries of the third world, CC is still the leading malignome and the cause of death among female population (William, 1999). This points to the necessity to apply screaning for cervical lesion already in the late teenage years, when this disease's rates are beginning to increase (Zarcone, 1998).

According to the information from literature (Smith et al., 1993; Haverkos et al., 2000) different risk factors are more or less significant for the development of CC. Ever since 1842 it has been recorded that

[1] Department of Pathology, University Clinical Center Tuzla, BiH

the uterus, cervical cancer is much more frequent among married than among single women and that it is very rare among nuns. (Griffiths, 1991). Studies which were carried out at the beginning of the 20th century indicate that, in the epidemics card of this disease, an early marriage and low socio-economic status of women have an important role (Terris, 1980), with certain components related to a man. The strongest epidemic corelation was found in connection to the age at the time of first sexual intercourse, especially prior to the age of 18, and 21 (Koutsky, 1992). Visits to prostitutes, circumcision status, sexually transmitted diseases (STD) are considered to be important predicotrs of risk for cervical cancer to occur, linked primarily to the insufficient genital hygiene (Adam et al., 2000). Certain authors are also emphasising, within the combination of the said risk factors, the importance of over-consumption of alcohol, while smoking, according to the same authors, has a protective role towards persistent HPV infections (Ho et al.,1998).

The objective of this study is to determine the influence of etiological factors (smoking, age at the time of first sexual intercourse, number of intercourses, number of partners and status of circumcision) on the occurrence of pre-malignant and malignant cervical lesions among patients with persistent HPV infection.

Patients, material and methods

Patients

The survey included 101 patients of the Gineacological-obstetrician Clinic of the University Clinical Centre and the Institute for Health Care of Women within the Tuzla Health Care Centre. In the case of these women, detection and identification of HPV has been indicated because of a suspicious citological result. Each patient with an identified high risk lesion –HGSIL (CIN II, CIN III, CIS, SCC) has been recommended to get a histological confirmation of the change that is present. Surveyed women were at the ages between 20 to 52. The control group was made of 100 surveyed women with a good result of citological examination (examination results were within the normal limits or benignant celular change) and without a verified HPV infection.

Material and methods

The applied work method was that of a prospective study, carried out in the period since January 1999 until January 2000. Information about a patient's occupation, age, age at first sexual intercourse, number of partners, number of intercourses per week, and

circumcision of partners, has been collected by using a specially designed form at the time of first examination of a surveyed woman.

Samples of the cervical smear for the HPV analysis were being taken during routine gineacological examinations, by using sticks with cotton, taken from the Digene Specimen Collection Kit, from the whole surface of a portion, and by mild rotating moves from the outer cervical entrance.

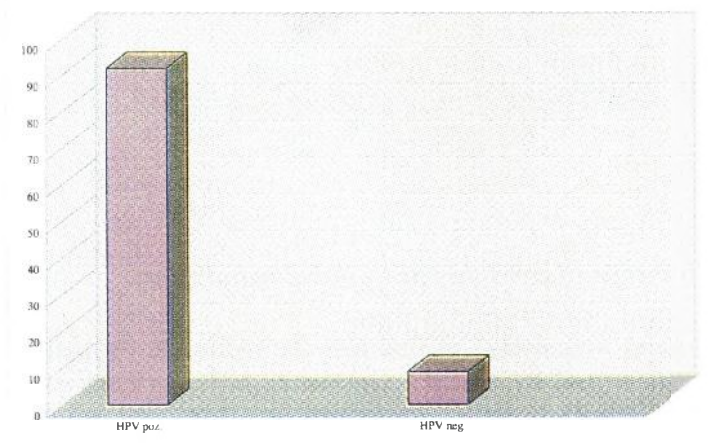
Detection of the presence of HPV in the cervical smear was done by the Digene HPV Test-Hybride Capture II (Hagenesee, 1999). The morphological changes found by using light-microscope method were classified according to the WHO classification of cervical lesion (XX).

For all the citological examination results, suspicious for either the presence of koilocytes in the specimen as a cito-morphologic characteristic of HPV infection, or for the weight of the citologically found lesia, a histologic verification of changes was carried out. All the morphological changes found by using light and microscope have been classified according to the WHO classification of the cervical lesion. Bioptical material phornaline fixed, paraphine embeded, has been cut into standard 5μ cuts and coloured by applying the standard procedure for HE (hematoxsiline eozin) colouring, and then mounted by using Canada balsam.

Statistical processing

The obtained indicators were processed at the level of counting the percentages of occurrence and numerical values, which then represented their co-relation.

Results

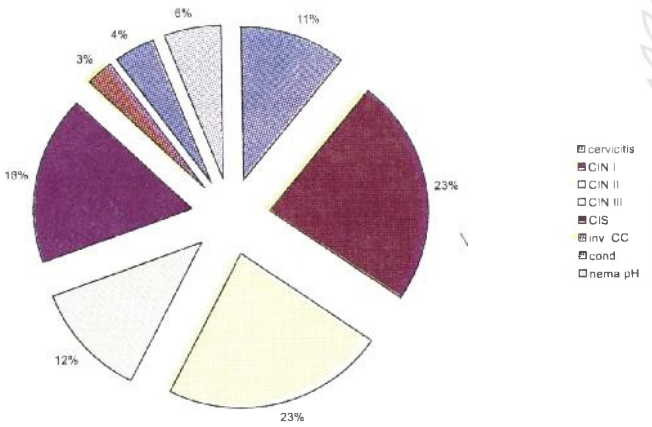


Picture 1. *The ratio of HPV positive and negative patients*

Picture 1 presents the ratio between HPV positive and negative patients. Out of 101 patients 92 (91,08%) were HPV positive, while 9 (8,91%) were HPV negative.

Histological verification of changes has been indicated for all cytological examination results, suspicious either because of the presence of koilocytes in the specimen as cito-morphologic characteristic of HPV infection, or because of the weight of a citologically found lesion. Histological verification was not done for 6 out of 92 HPV positive patients, or it was done after the study period for the examined group. Cytological examination of these 6 patients in 4 cases pointed to High Grade Squamous Intraepithelial Lesion (HGSIL), and in 2 cases to Low Grade Squamous Intraepithelial Lesion (LGSIL).

Distribution of pato-histologically verified lesias of cervix, irrespective of the age and presence of HPV infection, is presented in Graph 2. In the samples which were pato-histologically analysed, 10,89% chronic inflammations of cervix were verified, 3,96% condyloma, 23,76% mild displasias – CIN I, 22,77% moderate dysplasia-CIN II, 17, 82% Carcinoma in Situ – CIS, 11,88% severe dysplasias – CIN III, and 2,97% invasive cancers of the surface epithels of the cervix.



Picture 2. *Presence of cervical intra-epithel neoplasias*

The most frequently verified was the moderate dysplasia – CIN II with 25%, then mild dysplasia – CIN sa 22,82%, carcinoma in situ – CIS sa 16,30%, while invasive carcinoma at 2,17% and condyloma at 4,34% are less frequent. 4 out of 6 patients without pato-histologically verified lesion have had a HGSIL citological examination result, 2 of

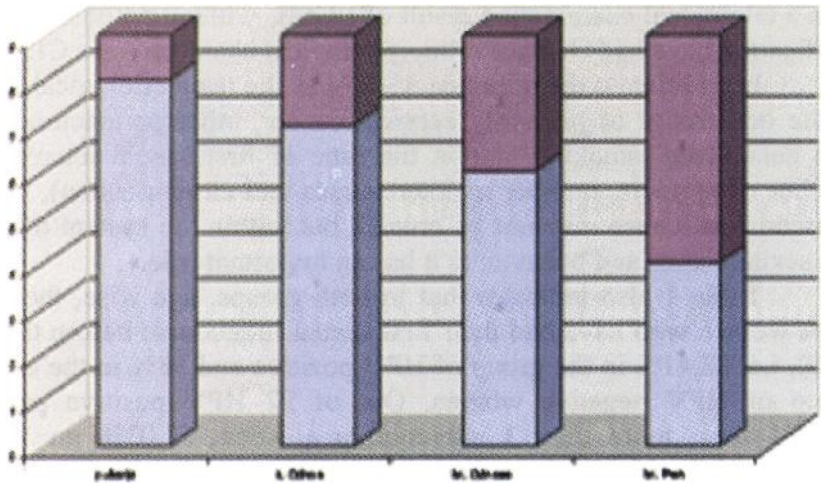
which towards CIN III and 2 towards CIS or heavier lesion. 2 patients with a citological examination result of LGSIL without patohistological verification have had changes of morphological characteristics CIN I.

In addition to the presence of HPV as the main etiological factor in the occurrence of primarily cervical cancer, other parameters were also considered (smoking, age at the time of first sexual intercourse, number of partners, number of intercourses and circum-cision), whose individual influence may not be crucial, but within the system of daily and sexual habits and behaviours it has an important role.

Table 1 also indicates that in both groups, age wise, there are more women who have had their first sexual intercourse before the age of 20, i.e. 82,41% in the group of HPV pozitive and 58% in the control group of HPV negative women. Out of 92 HPV positive patients 91,20% have more than 3 intercourses a week, 67,03% are active smokers while 46,15% have had or have more than 1 partner. In the control group the situation is almost completely the opposite; patients are non-smokers in u 66% cases, 74% have one relationship and sex partner, 71% of them have 1 to 2 intercourses per week. In both the examined and the control group male partners are circumcised (68,13% in group), and 67% (in the control group).

Table 1. *Other risk factors and their presence in the case of HPV positive women*

HPV positive		Factor	HPV negative	
N	%		N	%
61	(67,03)	yes ---smoking --- yes	34	(34,00)
31	(34,65)	No no	66	(66,00)
75	(83,41)	≤ 20 --- 1st intercourse --- ≤ 20	58	(58,00)
17	(18,68)	≥ 20 ≥ 20	42	(42,00)
50	(54,94)	1 --- No of partners ---- 1	74	(74,00)
42	(46,15)	≥ 2 ≥ 2	26	(26,00)
9	(9,89)	≤ 2 --- No of intercourses --- ≤ 2	71	(71,00)
83	(91,20)	> 3 > 3	29	(29,00)
62	(68,13)	yes --- Circumcision --- yes	67	(67,00)
29	(31,86)	No no	33	(33,00)
92	(100,00)	Total	100	(100,00)



Picture 3. *Influence of co-factors on the CIN among HPV negative patients*

Table 2. *Presence of other risk factors among HPV positive women*

Factor		Lesion		
		ASCUS*	LGSIL**	HGSIL***
Smoking	N	11	26	53
	Yes	8	14	33
	No	5	9	17
	Earlier	-	3	-
1st intercourse	N	13	48	95
	≤ 20 yrs.	11	26	53
	≥ 20 yrs.	2	22	42
No of partners	N	11	26	53
	> 1	6	14	30
	≥ 4	6	12	21
No of intercourses	N	13	26	53
	≤ 2	2	1.	6
	≥ 3	24	15	18
	> 5	5	9	26
	≥ 10	2	1	3
Circumcision	N	13	26	53
	Yes	7	17	38
	No	6	9	15

* Atypical squamous cell undetermined significant
** Low- grade squamous intraepithelial lesion
*** High- grade squamous intraepithelial lesion

Out of 9 HPV negative patients in the examined group with a present lesion of cervix 5 (83,33%) were smokers, with more than 5 intercourses a week or 66,66% and with < 20 years of age at the time of first intercourse or 77,7%. This can be noted in Picture 3.

The influence of individual risk factors in accordance with the present lesion has also been monitored among HPV positive patients, irrespective of the type of virus. Numerical presence and co-relations between different risk factors in relation to lesion can be seen in table 2.

Overall, patients are monogamous with 53,84% in the LGSIL and 56,60% in the HGSIL, but with a higher frequency of intercourses per week, or over 5 for ASCUS in 38,46% cases, and 49,05% for HGSIL, while patients with LGSIL have had a slightly lesser number of intercourses, between 3 and 5, with 57,69% and mainly with circumcised men. Irrespective of the present lesion, the largest number of patients are active smokers at 61,53% in ASCUS lesions, 53,84% in LGSIL and 62,26% in HGSIL. As in the previous analysis, these are mostly the cases where sexual life started early before the age of 20, which in the ASCUS and LGSIL groups stands at 84,61%, and in the HGSIL group at 79,24%.

Discussion

The fact that infection by humane virus of papilloma leads to different lesions of cervix, not to the same speed and degree of progression, but even to a spontaneous regression of certain changes under specific preconditions, points to the importance of other, additional factors; environment or endogenic factors, quite possibly able to modulate the evolution of HPV in the cervix tissue (Bosch at al., 1997.). A woman's risk from HPC infection is determined by her age, behaviour and both her own and her partner's sexual habits. The change in age limits is evident though, and so an increased CIN incidence was observed among younger women, especially that of high degree (Blohmer and sar,1999). In relation to the age this is the first consideration, age at the time of first sexual intercourse. According to our data, it is evident that irrespective of the presence of HPV, beginning of sexual intercourses at an early age considerably affects the creation and development of different lesions of cervix. At the same time, when comparing HPV positive and HPV negative patients with respect to the age factor, there is a more obvious discrepancy between HPV positive patients who started with sexual activity prior to the age of 20 and those who started it after the age of 20 (Iljazović-Latifagić, 2000). Biological basis for influence of age on the HPV prevalence is not known as yet. One explanation could be the reduced exposure to the virus, as a consequence of change in sexual behaviour at an older age (Ho and assts.., 1998;

Hildesheim and assts.,1993). Further, certain gained immunity mechanisms can develop after a period of exposure which can lead to a reduction in HPV prevalence at an older age (Schiffman, 1994). Persistent infection can increase the risk of development and lasting of squamous intraepithelial lesion. According to the study of multi-factorial influences on the lesions of cervix, Susane Kruger-Kjaer, among HPV positive and HPV negative women, it is exactly the early sexarha that is the crucial factor for creation of either ASCUS, LGSIL or HGSIL, and especially HGSIL at an early age. (Kruger-Kjaer,1998). The fact that female population of certain peoples or countries has a high incidence of cervical cancer, despite certain traditional norms of sexual behaviour, focuses the interest on the role of "the male factor" as an important element in the rate of risk for development of cervical lesion among women (Kruger-Kjaer and assts., 1991).

Primarily, and in relation to the mentioned age factor, the number of sexual partners is also important. According to our study of HPV negative patients, they are mainly monogamous (74%) with a lesser frequency of intercourses, which is probably an important preventive measure for STD. HPV positive patients are sexually more active (91,20% with more than 3 intercourses/week) and are almost in the same position in the examined groups whether with one or with more partners, which emphasises the role of a man. Certain characteristics of male sexual behaviour, such as genital hygiene and circumcision, STD and smoking, greatly affect the time of appearance and the development stage of a created cervical lesion.

Brinton and co-workers reported that men much more frequently have more sexual partners until they establish a permanent relationship, an earlier sexarha. And more frequently get STD, which, with joint sexual habits with a permanent sexual partner increases the risk of pre-malignant and malignant lesions (Brinton et al.,1989; Bosch et al.,1996). This position could, to a certain degree, be an explanation for high prevalence of HPV and lesions in our study. According to our results, an early sexual activity, more partners and higher frequency of intercourses are mutually unrelated factors in the creation of cervical cancer (de Sanjose and assts., 1997). The said factors and the high HPV prevalence is expected among the population of lower social-economic status (Ferrera and asst.., 1999; de Sanjose and assts, 1997) which may be a consequence of less developed preventive health care.

According to some studies, insufficient penilane hygiene, presence of STDs and bad education, i.e. "a highly risky man", increases the risk of his partner to get a disease of cervix. (Svare,1998 and Kruger-Kjaer, 1991). Although it has been proven that circumcision protects a men from HIV infection, from penilene cancer, infection of urinary system and ulcerous STDs (Moseset et al.,1998), our results as

well as the results in Panama, Costa Rica, Bogota,Columbia and Mexico City (Brinton,1991) with difficulty define the role and influence of circumcision on the creation and progressing of lesion, where there are more sexual partners and a highly risky sexual behaviour (not using condoms, more partners). In other words, it appeared that circumcision does not have a protective role in cases of highly risky sexual behaviour.

An almost identical percentage of circumcised partners (68% and 67%) in both groups of our study (HPV positive and negative patients) with specific difference in the presence of other risk factors, points to the importance of sexual habits/behaviour, as a factor in the creation of cervical intraepithel neoplasias and invasive cancer.

Certain earlier studies indicated that smoking was a protective factor for persistent HPV infection, without knowing if the protective mechanism was biological or some other unknown effect (Ho and assists., 1998). The percentage of 67,03% active smokers in the HPV group of positive patients in relation to 34% active smokers in the HPV group of negative patients in our study, points to an absolutely opposite effect and points to tobacco as a very potent carcinogene, able to lead to neoplastic progression and its significant joining with the cervical cancer (Ngelangel etal., 1998).

The presented results, as well as the results of numerous studies conducted world-wide point to the central role of the HPV in the pathogenesis of cervical lesions with an etiologically important co-action with other mutually independent factors, primarily factors related to sexual habits/behaviour. The factors from this group, in the frame of environment of highly risky sexual behaviour and without the presence of HPV, considerably affect the creation of cervical lesions.

Apstrakt

Cervikalni karcinom(CC) je, i po incidenci I mortalitetu drugi u svijetu, a prvi u zemljama u razvoju. Prema podacima iz literature, različiti faktori su manje ili više značajni u njegovom nastanku. Jedan od najznačajnijih faktora u nastanku ne samo malignih nego I premalignih lezija grlića jeste infekcija humanim virusom papiloma (HPV). Najjača epidemiološka korelacija nađena je za dob u vrijeme prvog odnosa, naručito prije 18, odnosno 21 godine života. Cilj ove studije je da determiniše uticaj različitih etioloških faktora (Pušenje, dob u vrijeme prvog odnosa, broj odnosa, broj partnera I status cirkumcizije) na nastanak premalignih I malignih lezija grlića kod pacijenata sa infekcijom HPV.

Ključne riječi: *cervikalne lezije, infekcija HPV, kofaktori nastanka*

References

Adam E, Berkova Z, Dexnerova Z, Icengole I, Revs WC, Kaufman RH. (2000). *Papillomavirus detection: Demographic and behavioural characteristics Influencing the identification of cervical disease.* Am J Obstet Gynacacol, 182 (2): 257-64

Anonymus. (1997). *Consensus steatment;* National Institutes of Health Consensus Development Conference Steatment on Cervical Cancer, Gynaecologic oncology 66,351 – 361.

Blohmer JU, Schmalisch G, Klette I, Grineisen Y, Kohls A, Guski H, Lichtenegger W. (1999). *Increased incidence of cervical intraepithelial neoplasia in young woman in the Mitte district, Berlin, Germany.* Acta Cytol, Mar-Apr; (2):195-200

Bosch FX, Castallsague X, Munoz N, de Sanjose S, Ghaffari AM, Gonzalez LC, Gilli M, Izarzugaza I, Viladiu P, Navarro C, Vergara A, Ascunce N, Guerrero E, Shah KV. (1996). *Male sexual behavior and human papillomavirus DNA: key risk factors for cervical cancer in Spain.* J Natl Cancer Inst, Aug 7, 88 (15): 1060-7.

Bosch FX, Munoz N, de Sanjose. (1997). *Human papillomavirus and other risk factors for cervical cancer.* Biomed&Pharmacother, 51:268-275.

Brinton LA, Reeves WC, Brenes MM, Herrero R, Gaitan E, et al. (1989).: *The male factor in the etiology of cervical cancer among sexually monogamous women.* Int J Cancer, 44(2): 199-203.

De Sanjose S, Bosch FX, Munoz N, Shah K. (1997): *Social differences in sexual behaviour and cervical Cancer.* IARC Sci Publ, 138:309-317.

Ferrera A, Velema J.P, Figueroa M, Bulnes R,Toro L.A, et al. (1999). *Human papillomavirus Infection, cervical dysplasia and invasive cervical cancer in Honduras: Case-control study.* Int J Cancer; 82, 799-803.

Griffiths M. (1991): *Nuns,Virgins, and Spinsters.* Rigoni-Stern and Cervical Cancer Revisited. Br J Obstet Gynecacol, 98:797-802.

Ho Gloria YF, Bierman R,M.D., Beradsley, N.P., Chee J, Burk RD,M.D. (1998) *Natural history of cervicovaginal papillomavirus infection in Young Women.* N Engl J Med;338:423-8.

Hildesheim A, Gravitt P, Schiffman M.N, et al. (1993) *Determinants of genital human papillomavirus infection in low-income women in Washington,* D.C. Sex Transm Dis 20:279-285.

Iljazović-Latifagić E. (2000): Doktorska disertacija; *Značaj identifikacije i tipizacije humanih virusa papiloma u patogenezi nemalignih lezija i karcinoma grlića materice.*

Kjaer SK, de Villiers EM, Dahl C et al. (1991): *Case control study of risk factors for cervical neoplasia in Denmark.* I: role of the male factor in women with one lifetime sexual partner. International Journal of cancer 48 (1):39-44.

Koutsky LA, Holmes KK, Critchlow MS et al (1992): *A cohort study of the risk of cervical intraepithelial Neoplasia grade 2 or 3 in relation to papillomavirus infection.* N Engl J Med, 327: 1272-1278.

Kruger-Kjaer S, van de Brule AJ,Svare EI,Engholm G, Sherman ME,Poll PA, Walboomers JM, Bock JE, Meijer CJ (1998): *Different risk factor patterns for High grade and low grade intraepithelial lesion on the cervix among HPV positive and HPV negative young woman.* Int J. Cancer; 76(5):613-619.

Moses S, Bailey RC, Ronald AR. (1998): *Male circumcision: assessment of health benefits and risk.* Sex Transm Infect; Oct; 74(5):368-73

Ngelangel C, Munoz N,Bosch XF, Genara ML, Festim MR, Deacon J,Jacobs MV, Santamaria M, Chris JLM Mejier, Walboomers Jan MM: Report. (1998): *Causes of cervical cancer in the Philippines:case-control study.* Journal of the Natiol Cancer Institute,Vol. 90, No1, pp 43-9.

Svare EI, Kjaer SK, Worm AM, Qsterlind A, Moi H, Christensen RB, Meijer Chris JLM, Walboomers Jan MM, Van de Brule A JC. (1998). *Risk factors for HPV infection in women from sexually transmited disease clinics: Comparasion between two areas with different cervical cancer incidence.* Int. J. Cancer; 75, 1-8.

Schiffman M.H, bauer H.M, Hoover R.N, Glass A.G, Cadell D.M, Rush B.B, Scott D.R, Sherman M.E, Kurman R.J, Wacholder S, Stanton D.R, Manos M.M. (1993) *Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia.* J. nat. Cancer.Inst.,85, 958-964.

Terris M, Wilson F, Nelson JH Jr. (1980): *Comparative Epydemiology of Invasive Carcinoma of cervix,* Carcinoma in Situ and Cervical Dysplasia. Am J Epidemiol 112:253-257.

William M.R. (1999) *Cancer of the Cervix.* Cancer net: Journal of cancer on line.

Zarcone R,Bellini P,Carfora E, Longo M, Tartaglia E, Monarca M.Vullo G. (1998) *Psychological cosequences in women with symptomatic HPV infection.* Minerva Ginecol, 50(6):235-7.

ANU
BiH
АНУ
БиХ

ETIOLOGY OF POSTTRAUMATIC STRESS DISORDER AND COMORBIDITY DURING 1992 – 1998

Ifeta Ličanin[1], Amira Redžić[2]

Abstract

Posttraumatic Stress Disorder (PTSD) represents a delayed response to a stress event, particularly of menacing and catastrophic nature, which most likely causes pervasive distress to almost everyone. This disorder is frequently associated with another psychiatric disorder.

As can be see from the name, PTSD does not have to be caused by the events of war, but by other traumatic events, as well.

The aim of this research is to show the main factors that cause PTSD and the ones that cause PTSD comorbidity.

This research was conducted in the period from April 1998 till October 1999. The participants were divided in two groups of 30: the first group consisted of 30 participants with symptoms of PTSD only and the second group included 30 participants with symptoms of PTSD and another psychiatric disorder. Both groups were quite similar with regard their gender and age. Research tools included: Standard Psychiatric Interview, Harvard Trauma Questionnaire (HTQ), Hamilton Anxiety Rating Scale (HAMA), Hamilton Depression Rating Scale (HAD), and Drug and Alcohol Abuse Checklist (1,2,3). The evaluation showed that PTSD was most common among the male, aged 25-45 in both of the groups.Participants from both groups were exposed to traumatic events at least once, but the most frequent was the case of exposure to etiologic factors. This is the case with the participants of the second group because they had greater exposure to multiple traumatic events. In that group, the most common disorders associated with PTSD were as following: personality disorders (46.6%); depression (29.9%); drug abuse (13.%); alcohol abuse (6.7%) and dissociative disorder (3.3%).

The key words: *PTSD, comorbidity, etiology, torture.*

[1] Psychiatric Clinic Clinical Centre University of Sarajevo Bosnia and Herzegovina
[2] Department of Biology with Human genetics of the Faculty of Medicine of the University of Sarajevo, Bosnia and Herzegovina

Introduction

Stress occurs in case of unbalance between the demands put forwards to a person and his/her capacity to deal with them. Excessive stress may effect both the mental and the physical health. Chronic stress effects even the immune system of a person my making it more vulnerable to many diseases.

The types of reactions to stress may be expressed in the following reactions: (4) Acute stress reaction, Adjustment disorders, Posttraumatic stress disorder (PTSD).

PTSD is delayed and (or postponed) response to a stress event or a situation (either of short or long duration) particularly of menacing and catastrophic nature, which most likely causes pervasive distress to almost everyone. The most common symptoms are: intrusive symptoms, flashback, nightmares, evading symptoms, loss of sensitivity and emotional emptiness, separation from people, indifference to environment, anhedonia and avoiding activities, as well as situations that remind him/her of the trauma.

If the symptoms of illness last less than three months we are speaking of an acute state but if they persist longer than that we are speaking of chronic disturbance. When six months pass between a traumatic event and breaking out of the symptoms of illness we have a case of PTSD with delayed initial stage. The duration varies with complete recovery during three months from the trauma experience in approximately half of the cases, but in persistence of symptoms longer than twelve months in many other cases. The intensity, duration and close exposure to trauma are the most significant factors in possible disorder development.

In general population the prevalence varies from 1% to 14%. The studies conducted on risk sample (for example: army veterans, population in areas engulfed by war, prisoners of concentrations camps, victims of natural disasters) showed prevalence from 3% to 58%. PTSD may happen at any age, even in childhood. The data achieved indicate that despite of the type of trauma, PTSD is greatly connected with other mental disorders including abuse of substances, depression, personality disorder.

PTSD was given different names probably as a result of different views through time. It was called "shell shock", "military heart", "war neurosis", "battle fatigue", "traumatic neurosis". Lately, due to rise of awareness of prevalence of this disorder and its damaging effect on the quality of life, it was named Posttraumatic Stress Disorder (PTSD). The American Psychiatric Association added PTSD into third edition in 1980 (Diagnostic Statistics Manual of Mental Disorders, DMS-III 1980) and classification schemes. Although considered a

controversial diagnosis at the time of initial presentation, PTSD filled in significant emptiness in psychiatric theory and practice (6).

This disorder, as can be seen from its name, does not have to be caused only by the war but many other traumatic events: natural disasters or those caused by men, serious injuries from fighting, witnessing horrible death of others, being a victim of violence, terrorism and rape or other criminal acts. So long as predisposition factors such as: personality traits (e.g. compulsive, astenic), with earlier case history of neurotic illness are present, the threshold for development of syndrome may be decreased. Many psychiatrists and psychologists examined soldiers and concluded that the symptoms were much stronger connected with the nature and place of war activities (7,8). Some authors have shown that low intelligence is a risk factor for development of this disorder and some again that the risk factor is low education level (9, 8, 10). As usual, the truth shows that the trend point to inclination to place it between the two opinions.

PTSD is one of the rare mental disorder defined through etiological factor: this disorder cannot exist without a significant stress event. However, the trauma itself is not sufficient to clarify this disorder, because not all individuals who are exposed to traumatic events are going to develop PTSD. Numerous works of authors bear witness to this. PTSD is unique among the traumatic diagnosis because of immense significance of etiological factor- traumatic stress. In fact, the PTSD diagnosis cannot be made until the patient experiences "the stress criteria" that is, until the person has been exposed to an event that is qualified to be traumatic.

The model by which this disorder is described would not be complete without taking into consideration the vulnerability of a person prior the trauma, the experiencing of trauma and posttraumatic factors. Most important determining factors rely on the nature and the intensity of traumatic events. The problem relies on operationalization of the intensity and the nature of stress events. Many studies have shown that the stress degree is closely related with the degree of PTSD. Cognitive understanding of factors also plays a significant role. Impressions of an individual to be in a safe place in time when he/she is experiencing a trauma or to fear greatly and feel helpless are strong risk factors. The research work conducted so far has pointed out some factors that contribute to emergence of this disorder: earlier elaboration on the same type of trauma, history of trauma in childhood, personality before the trauma, the age at the moment of exposure to trauma. Among the factors that follow the trauma social support is recorded and exposure to stress trauma, which may alleviate the trauma. Despite of that, some studies conducted with war veterans have shown the insignificance of these factors when the intensity of trauma is in growing: high level of

exposure during battle results with high percentage of PTSD, independent of premorbid factors.

At the beginning of development of diagnosis PTSD little has been thought about the events such as: war, torture, rape, holocaust, atomic bombing of Hiroshima and Nagasaki, natural disasters (earthquakes, hurricanes, volcano eruptions) and accidents caused by men (explosions, plane accidents, automobile accidents, different types of torture).

A catastrophe is an event in which an individual may react simultaneously or differently. Bio-psycho-social aspect of victims and accessing longitudinal development of their problems is particularly important (11).

The goal of the work is to show which etiologic factors are present in the development of PTSD and PTSD comorbidity.

The method

Research type:

The research conducted in the Psychiatric Clinic in Sarajevo was epidemiological, retrospective -prospective type, of analytic-descriptive feature and based on observation and analysis of relevant variables.

The model:

The subjects of this research were patients of the Psychiatric Clinic-Department for Psychiatric disorders caused by stress with expressed psychopathology PTSD and comorbidity. Distribution among the gender and age was almost equal (mainly between 20 and 40 years of age). We divided them in two groups: the first group of 30 participants with typical symptoms of PTSD only and the second group of 30 participants with symptoms of PTSD and another psychiatric disorder. The research was conducted in the period from April 1998 till October 1999.

Research tools:

Research tools included: Structure Clinical Interview, Harvard Trauma Questionnaire (HTQ), Hamilton Depression Rating Scale, Hamilton Anxiety Rating Scale and Checklist. HTQ is an instrument adjusted to and intended to clinic personnel who are working with persons who experienced traumas caused by war and exile (1,2,3). The evaluation showed that PTSD was most common among the male, aged 25-45 in both of the groups.

Working diagnosis has been given, irrespective of the subjectivity of the psychiatrists, based on structured clinical interview

founded on DSM-IV criterion and ICD-X and the HTQ enquiry form, and the final one once both criteria were fulfilled (6, 4).

The results

The below given Table 1 shows relevant data concerning the age and gender and Table 2 the etiological factors relevant for PTSD and comorbidity, including comments. Then we conducted correlation of the meaning and quality of etiological factors of both groups. The first group is marked by I and the Second group by II.

Review of participants with regard to gender and age

Table 1. Age and gender of participants

	Age							
Gender	Up to 24 year Group I Group II		25-44 years Group I Group II		25- 64 years Group I Group II		Total Group I Group II	
Male	2 (100,0)	5 (100,0)	13 (72,2)	14 (93,3)	6 (60,0)	17 (85,0)	21 (70,0)	26 (86,6)
Female	0 (0)	0 (0)	5 (27,8)	1 (6,7)	4 (40,0)	3 15,0)	9 (30,0)	4 (13,4)
Total	2 (6,6)	5 (16,6)	18 (60,0)	15 (50,0)	10 (33,3)	20 (66,6)	30 (100)	30 (100)

$\chi^2 = 15{,}107$ p= 0,0091
χ^2 m = 1,845 p= 0,397
χ^2 f = 1,034 p= 0,5963

The Dominating group of patients is the one of 25-44 years of age with 18 patients (60%) in Group I and 15 (50%) in Group II. The group of patients of 45-64 years of age with 10 (33,3%) in Group I and 20 participants (66,6%) in Group II, was on the second place. There were two participants under 24, 2 (6,6%) in Group I and 5 (16,6%) in Group II.

Types and frequencies of traumatic events

The Tables 2.1. and 2.2. show all traumatic events with special retrospection of those patients with head trauma with and without loss of consciousness, analogue to requirements of HTQ.

Table 2.1. *Traumatic Events*

PartI: Trauma Events

	Have you experienced	Group I PTSD	Group II PTSD comor.
1.	*Lack of shelter*	25	30
2.	*Lack of food or water*	25	30
3.	*Ill health without access to medical care*	17	25
4.	*Confiscation or destruction of personal property*	20	22
5.	*Combat situation (e.g., shellin and grenade attacks)*	25	28
6.	*Used as a human shield*	9	5
7.	Exposure to frequent and unrelenting sniper fire	25	30
8.	*Forced evacuation under dangerous conditions*	23	20
9.	*Beating to the body*	11	8
10.	Rape	3	8
11.	*Other types of sexual* abuse *or sexual humiliation*	4	5
12.	*Knifing or axing*	6	2
13.	*Torture (i.e., while in captivity you received deliberate and systematic infliction of physical or mental suffering)*	11	10
14.	*Serious physical injury from combat (e.g., shrapnel, burn, bullet wound, stabbing, etc.) or landmine*	12	9
15.	*Imprisonment*	12	8
16.	*Forced labor (like* animal or slave*)*	11	5
17.	*Extortion or robbery*	12	20
18.	*Brain washing*	13	8
19.	*Forced to hide*	24	25
20.	*Kidnapped*	11	10
21.	*Other forced separation from family members*	15	20
22.	*Forced to find* and bury *bodies*	13	0
23.	*Enforced isolation from others*	10	20
24.	*Present while someone searched for people or things in your home (or the place where you were living)*	15	20
25.	*Forced to sing songs you did not want to sing*	10	3
26.	*Someone was forced to betray you* and *place you at risk of death or injury*	8	5
27.	*Confined to home because of danger outside*	24	30
28.	*Prevented from burying someone*	18	25
29.	*Forced to desecrate or destroy the bodies or graves of deceased persons*	3	0

	Have you experienced	Group I PTSD	Group II PTSD comor.
30.	*Forced to physically harm family member or friend*	7	5
31.	*Forced to physically harm someone who is not family or friend*	5	27
32.	*Forced to destroy someone* else's property *or* possessions	1	8
33.	*Forced to betray family member, or friend placing them at risk of death or in'u*	4	10
34.	*Forced to betray someone who is not family or friend placing them at risk of death or injury*	4	10
35.	*Murder or death* duc to *violence of spouse*	0	5
36.	*Murder or death due to violence of son or daughter*	1	3
37.	*Murder or death due to violence of other family member or friend*	12	26
38.	*Disappearance or kidnapping of spouse*	1	0
39.	*Disappearance or kidnapping of son or daughter*	3	1
40.	*Disappearance or kidnapping of other family member or friend*	21	20
41.	*Serious physical injury of family member or friend due to combat situation or landmine*	27	26
42.	*Witness beatings to head or body*	16	10
43.	*Witness torture*	13	15
44.	*Witness killing or murder*	18	23
45.	*Witness rape or sexual abuse*	3	8
46.	Describe: *Any other situation that was very frightening or in which you felt your life* was in *danger. Specify:*		

Table 2.1. on traumatic events shows that that the Group II patients' exposure to traumatic events is 5 times and even more greater in questions number: 1, 2, 3, 7, 10, 17, 23, 24, 27, 28, 31, 32, 33, 34, 35, 37, 43, 44 and 45.

The First group's exposure to stresses is five times greater in following questions: 16, 18, 22, 25 and 42. Consequently, the Second group was exposed to 19 stress events five times time more and the First group to five stress events. Equal or almost equal exposure to traumatic events with patients of both groups was in the 13 items, under the following questions: 4, 5, 6, 8, 11, 13, 19, 20, 36, 38, 40, 41 and 43.

Table 2.2. *Head Trauma*

		Experienced?		*Loss of consciousness?*		*If yes, for how long?*	
		Group I PTSD	*Group II PTSD comor.*	Group I PTSD	*Group II PTSD comor.*	Group I PTSD	*Group II PTSD comor.*
1.	*Beatings to the head*	14	16	5	5	2 x 10' 2 x 15'	2 x 15' 1 x 10'
2.	*Suffocation or strangulation*	7	4			2 x 3'	1 x 5'
3.	*Near Drowning*	2				1 x 5'	
4.	*Injury to the head from nearby explosion*	6	4				
5.	*Other types of injury to the head (e.g., shrapnel, bullet wound, stabbing, burns, etc.)*	5	3				

The patients of both groups have had head injuries: in the first group fourteen and the Second group sixteen. Of that number, two patients in each group had loss of consciousness. Seven patients in First group and four in second group were exposed to suffocation and strangulation.

Table 3. *Types of psychological disorders associated with PTSD*

	Diagnosis							
Pol	F10	+F11	F32	F33	F44	F60	F62	Ukupno
Male	4 (100)	2 (100)	5 (62,5)	1 (100)	0 (0)	10 (100)	4 (100)	26 (86,6)
Female	0 (0)	0 (0)	3 (37,5)	0 (0)	1 (100)	0 (0)	0 (0)	4 (13,4)
Totalo	4 (13,3)	2 (6,7)	8 (26,6)	1 (3,3)	1 (3,3)	10 (33,3)	4 (13,3)	30 (100)

$\chi^2 = 4{,}021$ p=0,134

Table 3 shows that psychological disorders associated with PTSD are: personality disorder bears by far the greatest percentage 46,6%. Depression is on the second place being: depressive episodes without psychotic symptoms 26,6% and depression with psychotic symptoms 3,3%. Drug abuse is on the third place with 13,3% and alcohol abuse on fourth place with 6,7%. Dissociative disorders with 3,3% are on the last place.

Discussion

The results show that the majority of patients with PTSD are men of age between 25 and 40.The majority of studies show significantly greater vulnerability of women to PTSD development then men (12). This difference can be explained by participation of men in war in environments in which the research had been conducted. All patients were exposed to stress events at least once and in most case to multiple stress events, such as: lack of shelter, exposure to sniper fire, lack of food and water, ill without access to medical care, suffering material losses, witnessing grotesque killings, exposed to different types of torture, and similar.

The Table on trauma events shows that all of them were exposed to all forty-six traumatic events. The patients of the second group were exposed to traumatic events five times more than the patients of the first group, for the following questions: 1 (lack of shelter), 2 (lack of food and water), 3 (Ill health without an access to medical care), 7 (exposure to frequent and unrelenting sniper fire), 10 (rape), 17 (Extortion or robbery), 21 (forced separation from family members), 23 (Enforced isolation from others), 24 (experienced searches for people or things in their own homes), 27 (confined to home because of danger outside), 28 (prevented from burying someone), 31 (forced to physically harm someone), 32 (forced to destroy someone else's property or possessions), 33 (forced to betray a family member in order to put his life in jeopardy), 34 (forced to betray someone who is not family in order to put his life in jeopardy), 35 (finding out about murder or death of spouse due to violence), 37 (finding out about murder or death of a family member due to violence), 44 (witness torture of others), 45 (witness rape and sexual abuse).

The patients of the first group were exposed to stress events five times more than the patients of the second group, for the following questions: 12 (injured by a knife or an ax), 16 (forced labor- exhausting or like a slave), 18 (brain washing), 22 (forced to collect and bury bodies), 25 (forced to sing songs one does not want to sing), 42 (witness beating to head or body). Consequently, the patients of the second group had been exposed to twelve stress events five time more then the patients of the first group and the first group the same in five stress events. When comparing etiology factors that lead to PTSD of these patients with similar research work conducted in the world for the same problem so far, we can se that they are practically the same in 90% of cases, in the type and the intensity. Among the etiologic factors the most frequently quoted are: sexual abuse, wars and war imprisonment in over 70%.

The available world literature in this area points that frequent cause of PTSD is material loss with prevalence of 76% of patients (13,14). There are numerous studies on sexual abuse as etiological factor (15, 16, 17, 18, 19). Many authors have written about brain injury as stresses of PTSD (20). To some authors witnessing humiliation or killing of a person, witnessing assaulting, torture and sexual abuse are the most frequent stress events for PTSD (21). Many authors have conducted research studies on veterans who were engaged in the Gulf war, as PTSD cause (22). The Vietnam veterans were also studied by many authors who concluded that PTSD was associated with different aspects of war stresses (23). War traumas and prevalence PTSD with civilians was researched by many authors (24, 25, 26, 27).

A large number of research work has shown how important is the exposure to battle activities with a focus on researching of specific aspects of war experiences: killing of civilians, witnessing and participating in torture and violence (28, 29).

From the comorbidity group we recorded the following: personality disorder 46,6%; depression 29,9%; (depression without psychotic symptoms 23,3% and depression with psychotic symptoms 6,6%); drug abuse 13,3%; alcohol abuse 6,7% and dissociative disorder 3,3%. PTSD and personality disorders have been subjects of many studies (30, 31). The world literature has shown similar results: personality disorder of Borderline type at 69,5% (31, 32). In our research work Borderline is most prevailing by 23,3% compared to the total number of participants with personality disorder. Multiple literature data shows comorbidity with huge depression and PTSD at 50% (33, 34).

The result of 50% of depression with PTSD as well association of other disorders with PTSD was proved by a group of authors in their research work (35). As stated in the result of our research work the drug and alcohol abuse has been identified to be associated with PTSD, on the third and fourth place of frequency scale (13,3% drug abuse and 6,7% alcohol abuse) and only with male participants. The similar also was found by group authors (36). Some authors found even 60% and 80% of drug and alcohol abuse with PTSD in treated Vietnam veterans (37, 38).

Drug abuse which appeared after self-medication along with heroin medication, methadone and cocaine has been researched by many authors (39).

Conclusion

The dominating participants with PTSD were men, 25-40 years old.

Multi etiological factors have crucial role in the occurrence of treated disorders.

Namely, the participants were exposed to stress events at least once and in most cases to multiple stress events, such as: lack of shelter, exposure to sniper fire, lack of food and water, ill without access to medical care, to suffer material losses, to witness grotesque killings, exposed to different types of torture, and similar.

The study of the patients with PTSD comorbidity proved that multiple exposure to traumatic events had an essential role in emergence of the given disorder.

Of the comorbidity symptoms together with the known PTSD symptoms, according to frequency we recorded the following: personality disorder 46,6%; depression 29,9%; (depression without psychotic symptoms 23,3% and depression with psychotic symptoms 6,6%); drug abuse 13,3%; alcohol abuse 6,7% and dissociative disorder 3,3%

Apstrakt

Posttraumatski stresni poremećaj (PTSP) je odloženi odgovor na stresogeni događaj, osobito prijeteće ili katastrofične prirode, koji najverovatnije prouzrokuje prevazivni distres kod svakog pojedinca. Ovaj poremećaj se često javlja udružen sa drugim psihičkim oboljenjima.

Kao što se iz imena vidi, PTSP ne mora biti izvazvan samo ratom, nego i mnogim drugim traumatskim događajima.

Cilj rada je pokazati koji su etiološki faktori zastupljeni u nastanku PTSP, a koji u razvoju PTSP komorbiditeta.

Ispitivanje je urađeno u periodu od aprila 1998. do oktobra 1999. godine. Ispitanici su podijeljeni u dvije grupe po 30: prva grupa je formirana od pacijenata sa simptomima PTSP, a druga od ispitanika sa PTSP i nekim drugim psihičkim poremećajem. Podjednako su zastupljena oba spola unutar obje grupe bez bitnije prevalencije dekadnog životnog doba. Od instrumenata istraživanja korišten je standardni psihijatrijski intervju, HTQ, Hamiltonove skale: za depresiju i za anksioznost, Check lista na ovisnosti (1, 2, 3). Nakon evaluacije dobijenih rezultata ustanovljeno je da se obje forme PTSP češće javljaju kod osoba muškog spola, dobi od 25-45.

Kod ispitanika obje grupe ustanovljena je izloženost najmanje jednom od traumatskih događaja, ali je najčešća zastupljenost više etioloških faktora. Upadljivo je da ispitanici sa PTSP i komorbiditetom imaju veću izloženost multiplim traumama. U toj grupi ispitanika najčešći komorbiditeti PTSP nađeni

su: poremećaj ličnosti sa 46,6%; depresije sa 29,9%; zloupotreba droga sa 13,3%; zloupotreba alkohola sa 6,7% i disocijativni poremećaj, sa 3,3%.

Ključne riječi: *PTSP, komorbiditet, etiologija, tortura*

References

Mollica, R.F., Caspi-Yavin Y, Bollini, P., Troung, T., Tor, S. & Lavelle, J.: *Harvard Trauma Questionnaire: Validating a Cross-Cultural Instrument for Measuring Torture and PTSD in Indochina Refugees.* Journal of Nervous and Mental Disease 1992; 180:111-116.

Hamilton, M. (1959): *The assessment of Anxiety States by Rating.* British Journal of Medical Psychology, 32, 50-55

Hamilton, M. (1960): *A Rating Scale for Depression. Journal of Neuorology,* Neurosurgery and Psychiatry, 23, 56-62

International Clasification of Diseases, X edition. Geneva: World Health Organisation, 1994.

American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, third edition revised (DSM-III-R).* Washington, D. C.: A. P. A., 1987.

American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, third edition (DSM-III).* Washington, D. C.: A. P. A., 1980.

Weine, S. M., Vojvoda, D., Becker, D. F., McGlashan, T. H., Hodžić, E., Laub, D., Hyman, I., Sawyer, M., Lazrove, S. (1998): *PTSD Symptoms in Bosnian Refugees 1 Year After Resettlement in the United States.* Am. J. Psychiatry 155 (4): 562-4

Guerin, E. J. (1999): *Air Disaster and Posttraumatic Stress Disorder.* Am. J. Psychiatry. Aug; 156 (8): 1290-1

Macklin, M. L., Metzger, L. J., Litz, B. T. et al., (1998): *Lower Precombat Intelligence is a Risk Factor for Postraumatic Stress Disorder.* J. Consult. Clin. Psycholo. Apr; 66 (2): 323-6

Breslau, N., Davis, G. C., Andreski, P. (1998): *Risk Factors for PTSD-Related Traumatic Events: a Prospective Analysis.* Am. J. Psychiatry Apr; 152 (4): 152 (4):529-35

Yehuda, R. (1999): *Bilogical Factors Associated With Susceptibility to Posttraumatic Stress Disorder.* Can. J. Psychiatry, Feb; 44 (1): 34-9

American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV).* Washington, D.C.: A. P. A., 1994.

Maes, M., Delmeire, L., Schotte, C. (1998): *Epidemiologic and Phenomoenological Aspects of Posttraumatic Stress Disorder: DSM-III-R Diagnosis and Diagnostic Criteria not Validated*

Thulesius, H., Hakansson, A. (1999): *Screening for Posttraumatic Stress Disorder Symptoms Among Bosnian Refugees.* J. Trauma Stress. Jan; 12 (1): 167-74

Robin, R. W. et al., (1997): *Prevalence and Characteristics of Trauma and Posttraumatic Stress Disorder in a Southwestern American Indian Community.* Am. J. Psychiatry. Nov.; 154 (11):1582-8

Regehr, C. et al., (1999): *Response to Sexual Assault: a Relational Perspective.* J. Nerv. Ment. Dis. Oct.; 187 (10): 618-23
Friedman, M. J. (1997): *Posttraumatic Stress Disorder.* J. Clin. Psychiatry, 58 Suppl. 9:33-6. Review
Wilson, A. E. et al., (1999): *Risk Recognition and Trauma-related Symptoms Among Sexually Revictimized Women.* J. Consult. Clin. Psychol. Oct; 67 (5): 705-10
Matsunga, H. et al., (1999): *Psychopathological Characteristics of Recovered Bulimics Who Have a History of Physical or Sexual Abuse.* J. Nerv. Ment. Dis. Aug; 187 (8): 472-7
McMillan, T. et al., (1999): *Traumatic Brain Injury and Posttraumatic Stress Disorder.* Br. J. Psychiatry. Mar; 174: 274-5
Gibson, L. E., Holt, J. C., Fondacaro, K. M., Tang, T. S., Powell, T. A., Turbitt, E. L. (1999): *An Examination of Antecedent Traumas and Psychiatric Comorbidity Among Male Inmates with PTSD.* J. Trauma Stress. Jul; 12 (3): 473-84
Wolfe, J. et al., (1999): *Course and Predictors of Posttraumatic Stress Disorder Among Gulf War Veterans: a Prospective Analysis.* J. Consult. Clin. Psychol. Aug; 67 (4):520-8
McFall, M. et al., (1999): *Analysis of Violent Behavior in Vietnam Combat Veteran Psychiatric Inpatients with Posttraumatic Stress Disorder.* J. Trauma Stress. Jul; 12 (3): 501-17
Michultka, D. et al., (1998): *Responses to Civilian War Experiences: Predictors of Psychological Functioning and Coping.* J. Trauma Stress. Jul; 11 (3): 571-7
Weine, S. M. et al., (1998): *PTSD Symptoms in Bosnian Refugees 1 Year After Resettlement in the United States.* Am. J. Psychiatry. Apr; 155 (4): 562-4
Becker, D. F. et al., (1999): *Case Series: PTSD Symptoms in Adolescents Survivors of "Ethnic Cleansing".* Results from a 1-Year Folow-Up Study. J. Am. Acad. Child. Adolesc. Psychiatry. Jun; 38 (6): 775-81
Weine, S. M. et al., (1998): *Psychiatric Consequences of "Ethnic Cleansing": Clinical Assessment and Trauma Testimonies of newly Resettled Bosnian Refugees.* Am. J. Psychiatry. Apr; 152 (4): 536-42
Urlic, I. (1999): *Aftermath of War Experience: Impact of Anxiety and Aggressive Feelings on the Group and teh Therapist.* Croat. Med. J. Dec. 15; 40 (4): 486-492
Allen, J. G. et al., (1999): *A Self-Report Measure to Screen for Trauma History and its Application to Women in Inpatienet Treatment for Trauma-Related Disorders.* Bull. Menninger Clin. summer; 63 (3): 429-42
Zimmerman, M., Mattia, J. I. (1999): *Axis I Diagnostic Comorbidity and Borderline Personality Disorder.* Compr. Psychiatry. Jul-Aug; 40 (4): 245-52
Bleich, A., Koslowsky, M., Dolev, A., Lerer, B. (1997): *Post-traumatic Stress Disorder and Depression.* An Analysis of Comorbidity. Br. J. Psychiatry. May; 170: 479-82
Mollica, R. F., Mcinnes, K., Poole, C., Tor, S., (1998): *Dose-Effect Relationships of Trauma to Symptoms of Depression and Posttraumatic Stress Disorder Among Cambodian Survivors of Mass Violence.* Br. J. Psychiatry. Dec; 173: 482-8

Shalev, A. Y., et al., (1998): *Prospective Study of Posttraumatic Stress Disorder and Depression Folowing Trauma.* Am. J. Psychiatry. May; 155 (5): 630-7

Brown, P. J., Stout, R. L., Gannon-Rowley, J. (1998): *Substance Use Disorder-PTSD Comorbidity.* Patients' Perceptions of Symptom Interplay and Treatment Issues. J. Subst. Abuse Treat. Sept-Oct; 15 (5): 445-8

Dow, B., Kline, N., et al., (1997): *Antidepressants Treatment of Posttraumatic Stress Disorder and Major Depression in Veterans.* Ann. Clin. Psychiatry. Mar; 9 (1): 1-5

Quimette, P. C. et al., (1998): **During Treatment Changes in Substance Abuse Patients with Posttraumatic Stress Disorder.** The Influence of

Dansky, B. S. et al., (1998): *Untreated Symptoms of PTSD Among Cocaine-Dependent Individuals.* Chages Over Time. J. Subst. Abuse Treat. Nov-Dec; 15 (6): 499-504.

Zimmerman; M., Mattia, J. I. (1999): *Axis I Diagnostic Comorbidity and Borderline Personality Disorder.* Compr. Psychiatry. Jul-Aug; 40.

Coffey, S. F., et al., (1998): *Screening for PTSD in a Substance Abuse Sample: Psychometric Properties of a Modified Version of the PTSD Symptom Scale Self-Report.* Posttraumatic Stress Disorder. J. Trauma Stress. Apr; 11.

Reports

BALKAN ENDEMIC NEPHROPATHY IN THE BOSNIA AND HERZEGOVINA

Academy of sciences and arts of Bosnia and Herzegovina, Sarajevo,
October 08, 2002

ANU
BiH
АНУ
БиХ

Review

CONTEMPORARY APPROACH TO BALKAN ENDEMIC NEPHROPATHY

Goran Imamović[1], *Senaid Trnacević*[1], *Ahmet Halilbašić*[1]

Abstract

Balkan endemic nephropathy (BEN) is a progressive disease with insidious course inevitably leading to terminal uremia. Histology reveals focal tubular atrophy, focal non-destructive, hypocellular interstitial sclerosis, focal segmental and global glomerulosclerosis of collapsed type and intimal hyalinosis of arterioli and interlobular arteries. Association of upper urothelial tumors (UUT) with BEN is striking. It seems that the most likely underlying pathological mechanism is the slow intoxication in genetically predisposed subjects. Hypothetical agents act upon tubular epithelial and endothelial cells. It exerts pathological effects via interfering with metabolism and through direct genotoxicity thereby disturbing cell cycle and initiating apoptosis that appears to be the underlying mechanism of atrophic changes. Target cells express the genes that are normally inactive and subsequently produce cytokines and complement thereby transforming into proinflammatory cells. Affected cells seem to undergo transdifferentiation, i.e. expression of mesenchymal markers with subsequent production of collagen as extracellular matrix that triggers sclerosis. The mechanism of malignant alteration seems to be the same and agent to induce it also seems to be the BEN-inducing agent. Regarding etiology, epidemiological studies have revealed elevated concentrations of many putative nephrotoxins in BEN area, but their particular concentrations still do not reach the toxic ones. Nevertheless, it seems reasonable to keep insisting on pathogenesis of known models of toxic nephropathies, especially in the light of recent advances in molecular biology. Ochratoxin A (OTA), aristolochic acid (AA) and polycyclic aromatic hydrocarbons (PAH) represent such models. Further research will help us understand them better, understand their toxic metabolites that are supposed to contribute to development of the disease as well as to better understand other concomitant

[1] Department of Nephrology and Dialysis, University Medical Center, University of Tuzla, Tuzla, Bosnia and Herzegovina

and synergistic factors from endemic areas that might participate in that development.
Key words: *Balkan endemic nephropathy, epidemiology, pathogenesis, etiology, ochratoxin A, aristolochic acid, polycyclic aromatic hydrocarbons, molecular biology*

Introduction

Balkan endemic nephropathy (BEN) is an unknown disease that ultimately leads to terminal uremia. It affects rural population of the following countries in the Balkan Peninsula: Croatia, Bosnia and Herzegovina, Yugoslavia, Romania and Bulgaria. Distribution pattern of the villages is mosaic, with affected and non-affected ones adjacent to each other. The same applies to households within affected villages. Main epidemiological criteria are: living in the endemic region, familial occurrence, farming and occurrence of upper urothelial tumor (UUT) in a family. Functionally, it is characterized with tubular proteinuria type and morphologically with focal proximal tubular atrophy, focal non-destructive, hypocellular interstitial sclerosis, focal segmental and global glomerulosclerosis of collapsed type and intimal hyalinosis of arterioli and interlobular arteries.

Main topics at International Workshop held recently in Belgrade (April, 2002.) pertained to epidemiological and pathogenetic aspects of this disease. On the basis of the most recent epidemiological studies it seems that the incidence is decreasing and the onset of the disease has been moved towards older ages. It was accounted for by considerably improved living standard and subsequent possible reduced exposure to hypothetical causative agent. Concerning pathogenesis, the prevailing opinion nowadays is in favor of slow intoxication in genetically susceptible subjects. Regarding patho-physiology, immunological aspect does not seem to play important role. The same applies to inorganic and viral causes from etiological standpoint. Much attention is nowadays being paid to organic compounds such as ochratoxin A (OTA), aristolochic acid (AA) and polycyclic aromatic hydrocarbons (PAH)

Pathogenesis

Genetic hypothesis

On the basis of their cyto-genetic studies, Bulgarian authors have been providing the evidence for almost last two decades that the long arm of one chromosome of the 3rd pair at 3q25 is shortened. Therefore, they consider BEN inherited disease and believe the type of inheritance is autosome-dominant (1). In accordance with the development of recombinant DNA technology they conducted PCR

study that showed significant positive association of alleles C4 and A6 with BEN at loci ACPP and D3S1282, respectively at 3q22.1-3q26.2 and significant negative association of the allele a2 with BEN at locus D3S1509. The idea was to search for genetic marker for BEN. They concluded that their former cyto-genetic results on the significance of 3q24-3q26 region for BEN have been confirmed by this study, but proposed further research (2).

There is nowadays the prevailing consensus that genetic predisposition to develop BEN plays an important role in BEN development, but only in conjunction with hypothetical factor from the environment. Thus, it has been suggested that the various metabolic activities of xeno-biotic enzymes such as cytochrom P450-dependant mono-oxygenase (CYP) and glutathion-S-transferase (GST) might render selected subjects candidates to develop BEN. Various activity rates of these enzymes indicating genetic predisposition to develop BEN was demonstrated on a drug debrisoquine as a substrate (3). That activity would determine particular persons to be either fast or slow oxidizers of the putative environmental factor. The factor, itself does not necessarily even need to be toxic in its native form, unlike it's metabolite that might accumulate at a high rate. It has been demonstrated that BEN patients are fast oxidizers of the drug debrisoquine, thereby suggesting increased activity of their xeno-biotic enzymes (4). A significant polymorphism of the genes encoding for the synthesis of these enzymes has been demonstrated. Thus, CYP2D6 alleles with complete deficit or ultra-fast enzyme activity are associated with oncogenesis (5).

Intoxication

Slow intoxication seems to be the most likely pathogenetic mechanism in BEN development.

Illustrative example to demonstrate this is an ochratoxin A (OTA) nephropathy induced in rats. OTA produces following effects in rats:

1. inhibits aerobic respiration in mitohodria thereby lowering ATP
2. inhibits an enzyme tRNA synthetase thereby decreasing protein synthesis
3. increases lipid peroxidation
4. is genotoxic; evidence on genotoxicity is the finding of OTA-DNA adducts in mice treated with OTA (7). OTA-DNA adducts represent co-valent complex of OTA and guanine base in the DNA molecule and they initiate cancerogenesis (8).

Toxic effect of hypothetical agent produces genomic disorder whereby cell cycle becomes subject to change (proliferation vs.

apoptosis). Apoptosis has been demonstrated in the pathogenesis of toxic nephropathies other than BEN, i.e. analgesic (10), ochratoxin A (11) and cyclosporine (12), but it has been shown in 1998. to play a significant role in BEN, too (13). The finding was confirmed three years later and demonstrated to take place in tubular epithelial cells (14). In general nephropathology it has been shown that endothelial cells also undergo apoptosis, which then induces ischemic changes. Subsequent hypoxia accelerates tubular epithelial cells apoptosis that results in tubular atrophy and interstitial sclerosis (15). Moreover, damaged tubular cells become activated to produce complement, cytokines and extra-cellular matrix as a result of the expression of genes that are otherwise inactive in normal conditions (16, 17).

Expression of various intermediary filaments of the cytoskeleton is characteristic for the particular types of cells. Thus, *keratin* is the marker normally found in tubular cells whereas *vimentin* can be found in mesenchymal cells. It has been widely accepted that damaged tubular cells can express various markers during their regeneration. Thus, co-expression of keratin and vimentin can occur irrespective of a causative agent (18).

It has been demonstrated that tubular cells express in the early phase BEN vimentin along with keratin (19). Given mesenchymal cells produce *collagen* as an extra-cellular matrix and given tubular cells produce *laminin*, it has been suggested that transdifferentiation of tubular cells into myofibroblasts might be responsible for interstitial fibrosis in BEN (20).

Significant contribution of vascular lesion with this respect has been suggested by Ferluga and Vizjak who believe the same process might simultaneously affect endothelial cells and result in interstitial sclerosis (21, 22). They found in BEN patients collagen IV and laminin over-expression in thickened tubular basement membrane of atrophic tubules as well as in interstitial capillaries. They believe that an unknown agent could simultaneously act upon tubular epithelial cells and upon vascular endothelial cells thereby inducing their hyperactivity with overproduction of extra-cellular matrix that results in sclerosis, characteristic for BEN. Moreover, vimentin was expressed in non-sclerosed glomeruli, interstitium, blood vessels as well as in co-expression with cytokeratin in epithelial cells of the damaged tubuli whereas negative in intact tubuli. Also, they found complement C3 deposits that correlated with the most intense tubulo-interstitial histological changes as well as with high degree of proteinuria. They concluded that injured and activated tubular epithelial cells could produce complement (23).

Pathogenesis of BEN and BEN-associated tumors

Striking association between BEN and UUT has become particularly interesting in the light of advances in molecular biology. Gen p53 and its protein are important mediators of cell cycle. They oppose cell proliferation and initiate apoptosis. The rate of apoptosis is a biological marker of tumor progression because of horizontal spreading of oncogenes from an apoptotic body of one tumor cell which is taken up by the other tumor cell (24). Savin and Petronic found an increased expression of p53 in epithelial tumors from BEN area and suggested p53 mutation. This finding is certainly not BEN-specific since half of human tumors have p53 affected, but they also found less frequent apoptosis in BEN-associated UUT from BEN region vs. UUT outside that region and concluded that this accounted for former less invasive tumors (25).

As for the striking association between BEN and UUT, Bulgarian researches keep reaffirming the genetic background of both diseases. Toncheva et al. found loss of heterozygosity in one out of 3 analyzed patients affected with BEN-associated tumors at locus D3S1299 at 3q24 thereby supporting their previous data on that region being associated with BEN. Loss of heterozygosity test is the method to prove an increased incidence of tumor occurrence in certain families and method that discovered the existence of suppressor genes. They suggested the existence of a new tumor-suppressor gene at 3q24 (26).

Etiology

A lot has been done so far studying various inorganic substances (heavy metal, ionizing substances, microelements, selenium deficiency etc.) as well as viruses, but no causative relationship with BEN has been established. In the light of previous, but also recent researches of organic compounds such as: ochratoxin A (OTA), aristolochic acid (AA) and polycyclic aromatic hydrocarbons (PAH), much attention has been paid to them, accordingly. All three are nephrotoxic and oncogenic which fits BEN clinical presentation, too.

Ochratoxin A (OTA)

OTA is secondary metabolite of fungi Penicillium and Aspergilus. It is natural contaminant of food and feed. According to studies conducted so far, population has been exposed to OTA to a greater extent in BEN than in non-BEN region, but still in insufficient amounts to produce toxicity in humans (27).

There is an established animal model of OTA-related nephropathy in pigs which is called Porcine nephropathy, discovered in Denmark by Krogh and characterized with proximal tubular atrophy and

interstitial sclerosis. It can be encountered in West and Mid Europe. Krogh happened to spend some time in the Balkans and then started to work on the issue of BEN bearing in mind already existing porcine nephropathy in Denmark. His research on BEN brought him to the solution of porcine nephropathy enigma in his country (Ozegovic* - personal communication). On the other hand, it seems that pigs from former Yugoslavia do not suffer from OTA-related nephropathy (28).

OTA-related nephropathy patho-histology in mice differs from the ones in pigs as well as from BEN. It is characterized with "post-proximal nephron" injury, with impaired concentrating capacity (29), unlike the latter ones that are characterized with proximal tubular injury and related Fanconi syndrome.

Prof. Ladislav Ozegovic, Member of the Academy of Sciences and Arts of Bosnia and Herzegovina

OTA is carcinogenic in mice. It induces renal parenchymal cancer by creating OTA-DNA adducts (7). OTA-DNA adducts were also found in human UUT (30), but there is still not enough evidence of OTA–related pathology in humans. There were only two cases in Tunisia suspect on OTA-related nephropathy, then a case of acute renal failure in Italy after inhalation exposure and the cases of a brother and a sister in France with high blood ochratoxin A levels and karyomegaly (31).

Aristolochic acid (AA)

Ivic found AA in wheat flour in BEN area in 1970. He presumed that AA originated from the plant *Aristolochia clematitis* that can be encountered in BEN region, but is ubiquitous, too. He was feeding thereafter rabbits with Aristolochia clematitis seeds. The result was tubulo-interstitial nephropathy very similar to BEN (32).

It was demonstrated then that AA was cancerogenic in rats (33) and that AA activates gene *ras* in experimental animals tumors (34).

Thereafter, AA has been virtually forgotten as a serious candidate for BEN elucidation until an incident that took place at one Belgian weight-loss clinic in 1992. As many as 80 women undergoing slimming regimen with Chinese herbs containing AA developed rapid progressive terminal renal failure (35), accompanied by UUT in 40% of them (36).

Morphologically, it was tubulo-interstitial nephropathy, tubular proteinuria was found and no hypertension was observed thereby resembling BEN very much. AA-DNA adducts were found in a controlled study in the same patients (37). Then, rabbits and rats were fed with AA and the results were interstitial fibrosis and UUT. The conclusion was that AA caused the disease.

So, the new disease was discovered and was designated as Chinese Herbs Nephropathy (CHN), even though minimal nephrotoxic doses in rats were still several times higher than those found in slimming pills. Moreover, doses administered in slimming pills did not differ from the ones used to be prescribed in traditional Chinese medicine, with no adverse health effects (39, 40). Therefore, it has been suggested that it was not only AA to result in CHN nor in UUT development. The supplemental slimming regimen consisting of sympathomimetics (appetite suppressors), purgatives and diuretics (acetazolamide) could have contributed to the pathology (41, 42).

Due to similarities between CHN and BEN, the idea of relating AA to BEN was proposed again (38).

Polycyclic aromatic hydrocarbons (PAH)

Chemical analysis of water samples from BEN and non-BEN villages in Romania showed the presence of nephrotoxic and cancerogenic organic compounds (naftilamin, anilin, aminofenol and PAH) in much higher concentration in endemic vs. non-endemic villages (43).

Low-rank Pliocene lignite correlates with BEN areas. Weathered lignite deposits contain the above mentioned compounds that are hydro-soluble and thus transported by the local ground water flow system thereby contaminating shallow water wells of the BEN-affected households.

Feder et al. tested methanol lignite extracts from BEN and non-BEN areas. In BEN area benzene and naphthalene were found and they were rich in functional groups: methoxy, acetyl, keto and hydroxy. Those compounds are hydro-soluble and some of them are nephrotoxic and cancerogenic. In the same samples terpane/steranic groups were found. They are the markers of the poor quality of the coal that resembles very much a fossil wood. Extracts from non-BEN areas neither contained toxic functional groups nor were hydro-soluble (44). Therefore, the authors proposed explanations for the geographical restriction of BEN. Two of them appear most attractive. First, BEN area lignite has to have specific features and has to differ from non-BEN area one. Pliocene lignite location in the Balkans corresponds to the southeastern and southern margins of the Tertiary Panonian Basin and overlaps with BEN areas. A specific coalification process, which has been incomplete, could have taken place under local climatic and geological conditions in the Balkan Peninsula, thereby leaving partially decayed compounds such as *terpenoids* – biomarkers of incomplete degradation of fossil fuels. Some of those compounds, such as *terpineol*, are mutagenic and others are nephrotoxicants. They were found in coal samples of BEN unlike in non-BEN area.

The another explanation for BEN geographical restriction pertains to ground waters that leach the toxic organic compounds from the rocks and transport them to shallow wells/springs thereby determining endemic villages as well as BEN-affected households within an endemic village. Those waters determine affected households with their flows and various concentrations of the toxic compounds, which depends on local soil characteristics (permeability, rainfall etc.) This explanation nicely accounts for both the existing mosaic distribution of endemic villages in the Balkan Peninsula as well as for mosaic distribution of particular affected households within an endemic village.

Conclusion

Genetic predisposition to develop BEN in susceptible subjects who are exposed to hypothetical factor from the environment is nowadays prevailing approach to this disease. It has been suggested that the susceptibility might be the result of the various metabolic activities of xeno-biotic enzymes such as cytochrom P450-dependant mono-oxygenase (CYP) and glutathion-S-transferase (GST). The rate of activity of these enzymes would qualify particular persons to either fast or slow oxidizers. It has been suggested that BEN-susceptible candidates are fast oxidizers of the agent that is not necessarily nephrotoxic and/or oncogenic in its native form, but its metabolite that is being accumulated at a high rate might be so (3).

Regarding pathogenesis, it seems that slow intoxication of metabolism and/or direct genotoxicity of the hypothetical agent/s in genetically susceptible subjects are the underlying mechanisms of BEN.

The outcome of toxic activity of a hypothetical toxic metabolite is a gene control disorder of the cell cycle (proliferation vs. apoptosis) which results in the expression of a gene responsible to trigger apoptosis via an enzyme that initiates atrophy of the target tissue. Target tissue is postulated to be renal epithelial and endothelial cells (13-17). Cells initially damaged express during their regeneration various markers that are otherwise not characteristic for that tissue (transdifferentiation) and produce mediators such as cytokines, complement and extra-cellular matrix which gives rise to interstitial sclerosis (16, 17).

The association with UUT is striking and its elucidation might contribute to better understanding of BEN through molecular genetic studies. Thus, the finding of AA-DNA adducts in patients that were taking AA and developed consequently CHN and UUT was quite sufficient (along with successful animal experimentation) to relate CHN and UUT to AA (36, 37). OTA-DNA adducts were related to cancerogenesis in mice (8) and were found in human UUT (30).

So, in some patients a hypothetical metabolite can be nephrotoxic inducing atrophy and fibrosis due to activation of normally inactive genes. Those genes encode for biosynthesis of proteins that trigger apoptosis and extra-cellular matrix production, respectively. In other patients carcinogenesis might take place via either activation of proto-oncogenes into oncogenes, inactivation of tumor-suppressor genes or via damaging the genes responsible for reparation of DNA replication errors in S stage of cell cycle. The mechanism might be point mutation, chromosomal rearrangement or gene amplification/deletion. Accordingly, p53 over-expression was found in BEN-associated UUT and its mutation was suggested (25). Also, loss of heterozygosity (LOH) was found in BEN-associated UUT and presence of a new tumor-suppressor gene at 3q24 was suggested. It was found in the same chromosomal region which Bulgarian authors considered chromosomal marker for BEN (26). It has been also suggested that the same agent induce both BEN and UUT. As in a number of UUT associated with toxic nephropathies, such as analgesic, CHN and OTA (the latter one confirmed in animals, only) apoptosis plays an important role in tumor progression in BEN, too (25).

No results have been achieved after a half of a century of thorough investigations searching for a single BEN-causing agent. Much effort has been made working on OTA, AA and PAH.

OTA intake is higher in BEN vs. non-BEN area, but it still fits into safety limits and the lowest toxic OTA doses in animals are still 4-5 times higher than those found even in hyper-endemic areas. The latter applies to AA, too, but such findings should not be discouraging for further research. First, further work on OTA, AA and PAH-related nephropathies, respectively will bring us closer to better understanding of pathogenesis of toxic interstitial nephropathies, which will be helpful to possibly get closer to BEN enigma solution, itself. Second, it is not necessarily that the parent compound from the environment, irrespective of its concentration, could be responsible for the pathology, but its metabolite that is accumulated at a high rate in susceptible subjects. Good example to illustrate this provides model of OTA intoxication in rats. Preliminary results suggest that oxidative pathways in OTA metabolism generate genotoxic metabolite via ko-oxidation of the metabolic pathway of prostaglandin synthetase (6). Administration of prostaglandin-synthetase inhibitors such as Aspirin or Indomethacin significantly reduced the amount of those adducts (9).

Third, patho-histological presentation of OTA-related nephropathy in animals differs from BEN, but on the other hand, OTA-related rat nephropathy differs from porcine nephropathy, too.

Fourth, OTA-related porcine nephropathy in Denmark differs at ultrastructural, mitochondrial level from the one in Bulgaria. Thus,

electron-dense formations in the nuclei, surrounded by a small electron-dense mass as well as myelin figures in the cytoplasm and mitochondria were not demonstrated in Denmark (unlike in Bulgaria). The authors suggested that described differences were probably due to some interference between ochratoxin A and other mycotoxins so that some synergistic effects between ochratoxin A and various other nephrotoxic mycotoxins produced by the same ochratogenic fungi might have taken place (45). Such other nephrotoxic mycotoxins could be: citrinin, penicilic acid, rubratoxin A, B, viomellein and xanthomegnin (31). Accordingly, Abouzied et al. suggested that OTA might not cause BEN alone, but in synergistic action with other toxicants in genetically predisposed subjects (27). That is why mycotoxin hypothesis for BEN elucidation should not still be necessarily ruled out.

The same applies to aristolochic acid that has been proved to cause Chinese Herbs Nephropathy in humans, even though particular ingested doses have not been considered toxic to humans, but they still caused the disease in conjunction with dehydration (purgatives and diuretics) as well as peripheral vasoconstrictors (appetite suppressors) administered at a Belgian weight-loss clinic. BEN resembles CHN, too and AA has been identified as early as in 1970. as its possible causative agent. Since farming is one of the epidemiological criteria for BEN diagnosis and in the light of insufficient sole AA dose to cause CHN, it is worthwhile to note that farmers are sweating a lot in the field! The main difference between those two diseases is that CHN presents clinically as rapidly progressive tubulo-interstitial nephropathy after only 8 months of AA ingestion, as opposed to BEN which takes up to two decades. This could reflect a higher level of toxic exposure in CHN than in BEN patients (47). Therefore, the role of AA in BEN development can not still be ruled out if it was a matter of slow intoxication with minimal doses over the course of two decades and without additional rigorous slimming treatment applied at Belgian weight-loss clinic. The final demonstration that AA plays a role in BEN requires the evidence that patients with an unequivocal diagnosis of BEN have ingested foods containing AA, present the typical biological and morphological characteristics of CHN and harbour AA-DNA adducts in their renal tissue (46).

PAH-related nephropathy as the consequence of ground waters that leach the toxic organic compounds from the rocks and transport them to shallow wells/springs thereby determining endemic villages as well as BEN-affected households within an endemic village provides an attractive example of geographically restricted disease. It is more difficult to deal with OTA and AA in this regard because they are ubiquitous, but genetic predisposition, along with concomitant and synergistic effect of known nephrotoxins might account for BEN as

INSTRUCTIONS TO CONTRIBUTORS

Posebna izdanja ANUBiH is ownwd and controlled by the Medical Department of Academy of Sciences and Arts of Bosnia and Heryegovina and is devoted to the publications of work relating primarily all kinds of medicine. It will be accepted all kind of medical presentations (case reports, scientific article, review articles, historic article and so on) depending on quality . Articles of unusual cases and technical notes on special instruments or equipment that might be useful to others in the field of any kinds of medine and surgery are also acceptable. Case reports should be limited in length and should not include extensive review of literature.

Manuscripts are accepted in two copies, one in Bosnian language and other in correct English. Manuscripts must be typewritten in duplicate, double spaced on one side of the paper with 2,5 cm wide margin on top, button and both sides, accompanied by the identical file on the diskette, and submitted to a academic Džmeal Rezaković, Medicinsko odjeljenje ANUBiH, Akademija nauka i umjetnosti Bosne i Hercegovine, Bistrik 7, 71000 Sarajevo, Bosna i Hercegovina. Manuscripts must be arranged as follows:

1. Title page: This should include the full title on the paper, short clear and specific, and author's full names without academic degrres and institutial affiliation.
2. Abstract: This should be submitted on a separate page, and should describe in less than 250 words exactly what was done, the author's idea, aims results obtained and conclusions drawn. Following the abstract, and on the same page a list of key words (maximum six) for coding and indexing in Alphabetical order. If the text is in Bosnian language, abstract must be in English, and vice versa.
3. Text of the paper: Tables should be presented on the separate page and have a brief descriptive title.
4. References cited: Footnotes are not accepted.
5. Photographs: Legends should be on the separate paper and numbered consecutively.
6. Acknowledgements if there is any, also should be on the separate paper.
7. Separate page giving the name, address, including zip code and telephone number of the main author for correspondence.

All the pages should be numbered consecutively, starting with the title pages as page one.

Any medications materials and devices must be identified by full nonproprietary or generic name.

Reference numbers in the text and among the literature could be cited in two manners, by alphabetical order or according in order to citation, better by alphabetical order. All references must be cited by the same way and it means for example for paper in the journal: 17. Kassel NF, Turner HI, Size of intracranial aneurysm. Neurosurgery, 9:466, 1981.

Contributors are responsible for accuracy of the English text.

ANU
BiH
АНУ
БиХ

opposed to non-endemic areas where sufficient amount of various nephrotoxins and/or their even more toxic metabolites in genetically predisposed subjects is possibly not reached. Schmeiser, the prominent molecular biologist from Heidelberg (Germany), has recently found DNA adducts of both AA and OTA in renal tissue in 2 out of 3 patients from BEN area that were suffering from UUT and ureteral stenosis. In the third case OTA-DNA adduct was not found and presumed AA-DNA adduct was rather faint for complete identification to be confirmed. This finding was still not conclusive in terms of causative relation to BEN because the study was neither controlled nor was it the matter of confirmed BEN cases (47), but we know that UUT is highly associated with BEN in BEN regions and this study could give us the guidelines for further research.

Therefore, in order to keep on trying to elucidate BEN enigma, the research on the described toxins should not be given up.

Apstrakt

Balkanska endemska nefropatija (BEN) je progresivno oboljenje podmuklog toka koje neumitno vodi u terminalno bubrežno zatajenje. Histološki se radi o fokalnoj tubularnoj atrofiji, fokalnoj nedestruktivnoj hipocelularnoj intersticijskoj sklerozi, fokalnoj segmentnoj i globalnoj glomerulosklerozi kolapsnog tipa i intimalnoj hijalinozi arteriola i interlobularnih arterija. Izrazita je udruženost sa tumorima gornjeg urinarnog trakta. Čini se da je najvjerovatniji patološki mehanizam u podlozi ove bolesti spora intoksikacija u genetski predisponiranih osoba. Hipotetički agens djeluje na tubularni epitel i endotel. On ispoljava patološki efekat interferirajući sa metabolizmom kao i direktnom genotoksičnošću zbog čega se remeti ćelijski ciklus i injicira apoptoza za koju se smatra da je u osnovi atrofičnih promjena. Ciljne ćelije istovremeno aktiviraju gene koji su normalno inaktivni te proizvode citokine i komplement transformišući se u pro-inflamatone ćelije. One trpe transdiferencijaciju, tj. Ekspresiju mezenhimskih markera zbog čega proizvode kolagen kao ekstra-ćelijski matriks, što je uvod u sklerozu. Čini se da je mehanizam maligne aliteracije isti, kao i da je agens koji ga pokreće isti. Što se tiče etiologije, epidemiološke studije su pokazale povišene koncentracije mnogih nefrotoksina na BEN terenu, ali njihove pojedinačne koncentracije još uvijek ne dosežu toksične doze. I pored toga se čini opravdanim i dalje insistiranje na patogenezi poznatih modela toksičnih nefropatija, naročito u svjetlu skorih dostignuća u području molekularne biologije. Ohratoxin A, aristolohijska kiselina i policiklički aromatski ugljeni hidrati predstavljaju takve poznate modele. Buduća istraživanja će doprinijeti njihovom boljem razumijevanju, zatim razumijevanju njihovih sekundarnih toksičkih metabolita za koje se pretpostavlja da bi mogli doprinijeti razvoju bolesti, kao i boljem razumijevanju drugih konkomitantnih i sinergističkih faktora sa endemskog terena koji tom razvoju doprinose.

References

Dimitrov T: *Balkan Endemic Nephropathy in Bulgaria.* Facta Universitatis Vol. 9 No 1: 7-14, 2002. (www.freemedicaljournals.com)

Toncheva D, et al: *Association study of 3q microsatellite loci in Bulgarian patients with Balkan endemic nephropathy.* Facta Universitatis Vol. 9 No 1:124, 2002. (www.freemedicaljournals.com)

Nikolov I, et al: *Genetic predisposition to Balkan endemic nephropathy: Ability to hydroxylate debrisoquine as host risk factor,* in: Mycotoxins, *Endemic nephropathy and Urinary Tract Tumours (115),* edited by IARC Scientific Publications, 1991, pp 289-296.

Ritchie JC, et al: *Evidence for an inherited metabolic susceptibility to endemic (Balkan) nephropathy.* Proceedings of the 5th symposium on endemic (Balkan) nephropathy. Nis, 1983.

Atanasova S, et al: *Genotyping of CYP2D6 mutant alleles in BEN patients.* Facta Universitatis Vol. 9 No 1: 125-126, 2002. (www.freemedicaljournals.com)

Pfohl-Leskowicz A: *Ochratoxin A, ubiquitous mycotoxin contaminating human food.* S. R. Seances Soc Biol Fil 188 (4): 335, 1994.

Pfohl-Leszkowicz, et al: *DNA adduct formation in mice treated with ochratoxin A,* in: Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours (115), edited by IARC Scientific Publications, 1991: 245-253.

Maaroufi K, et al: *Ochratoxin A genotoxicity, relation to renal tumors.* Arch. Inst. Pasteur. Tunis 71 (1-2): 21, 1994.

Obrecht Pflumio S: *Protection by indomethacin and aspirin against genotoxicity of ochratoxin A, particularly in the urinary bladder and kidney.* Arch. Toxicol. 70 (3-4): 244, 1996.

Rocha GM, et al: *Direct toxicity of NSAID for renal medullary cells.* Proc. Natl. Acad. Sci. USA: 200: 98(9): 5317-5322

Schwerdt G, et al: *The nephrotoxin ochratoxin A induces apoptosis in cultured human proximal tubule cells.* Cell Biol Toxicol 15(6): 405-15, 1999.

Thomas S, et al: *Accelerated apoptosis characterizes cyclosporine-associated interstitial fibrosis.* Kidney Int 53(4): 897-908, 1998.

Mantle PG, et al: *Does apoptosis cause renal atrophy in Balkan endemic nephropathy?* Lancet 3, 352 (9134): 1118-1119, 1998.

Savin, et al: *The significance of apoptosis for early diagnosis of Balkan nephropathy.* Nephrol Dial Transplant 16 (Suppl 6): S30-S32, 2001.

Khan S, et al: *Hypoxia induces renal tubular epithelial cell apoptosis in chronic renal disease.* Lab Invest 79(9): 1089-1099, 1999.

Van Kroten C, et al: *Role of tubular cells in progressive renal disease.* Kidney Blood Press Res 22: 53-61, 1999.

Daha MR, van Kroten C: *Is the proximal tubular epithelial cell a proinflamatory cell?* Nephrol Dial Transpl 15 (Suppl 6): S41-S43, 2000.

Grone, et al: *Coexpression of keratin and vimentin in damaged and regenerating tubular epithelia of the kidney.* Am J Pathol 129: 1-8, 1987.

Stefanović V, et al: *Coexpression of vimentin and cytokeratin in damaged tubular epithelia of kidney in Balkan nephropathy.* Nephron 72: 119-120, 1996.

Marković-Lipkovski J: *Tubuloepithelial-myofibroblast transdifferentiation - possible pathogenic mechanism of interstitial fibrosis in Balkan endemic nephropathy.* Facta Universitatis, Vol. 9 No 1: 79-81, 2002. (www.freemedicaljournals.com)

Ferluga D, Hvala A, Vizjak A, et al: *Renal function, protein excretion and pathology of Balkan endemic nephropathy. III Light and electron microscopic studies.* Kidney Int 40 (Suppl 34): S57-S67, 1991.

Ferluga D, Vizjak A, Hvala A, et al: *A kidney biopsy study of early endemic nephropathy,* in Čvorišćec D et all. Endemic nephropathy in Croatia, edited by Academia Croatica Scientiarum Medicarum, Zagreb, 1996, pp 43-72

Vizjak A, Trnačević S, Halilbašić A, Ferluga D: *Immunohistologic kidney biopsy study of Balkan endemic nephropathy.* Facta universitatis, Vol.9 No1: 88-91, 2002. (www.freemedicaljournals.com)

Bergsmedh, et al. *Horizontal transfer of oncogenes by uptake of apoptotic bodies.* Proc Natl Acad Sci USA 98: 6407-6411, 2001.

Savin M, Petronić V: *The significance of molecular-biological characteristics of upper urothelial carcinomas associated with the Balkan endemic nephropathy.* Facta universitatis, Vol 9. No1: 95-97, 2002. (www.freemedicaljournals.com)

Toncheva D, et al: *Molecular genetic studies of BEN tumors.* Facta universitatis, Vol 9. No1: 125, 2002. (www.freemedicaljournals.com)

Abouzied M, et al: *Ochratoxin A concentrations in food and feed from a region with Balkan endemic nephropathy.* Facta Universitatis Vol 9. No1: 129, 2002. (www.freemedicaljournals.com)

Mantle PG, et al: *Penicillium aurantiogriseum-induced persistent renal histopathological changes in rats: An experimental model for Balkan endemic nephropathy competitive with ochratoxin A,* in: Castegnaro M, et al: Mycotoxins, Endemic nephropathy and Urinary Tract Tumours (115), edited by IARC Scientific Publications, 1991, pp 119-127

Gekle M, Silbernagl S: *The role of the proximal tubule in ochratoxin A nephrotoxicity in vivo: toxodynamic and toxokinetic aspects.* Renal Physiol Biochem 17: 4049, 1994.

Pfohl-Leszkowicz A, et al: *Ochratoxin A-related DNA adducts in urinary tract tumors of Bulgarian subjects.* IARC Sci. Publ. 124: 141, 1993.

Atkins J: *Balkan Nephropathy,* 1999. http://www.wramc.amedd.army.mil/departments/Medicine/Nephrology/education/Lectures/balkan/sld001.htm

Ivić M: *The problem of aetiology of endemic nephropathy.* Acta Fac Med Naissensis 1: 29-38, 1970.

Mengs U, Lang W, Poch JA: *The carcinogenic action of aristolochia acid in rats.* Arch Toxicol 61: 107-119, 1982.

Schmeiser H: *Aristolochic acid activates ras genes in rat tumors at deoxyadenosine residues.* Cancer Res 50: 5464-5469, 1990.

Vanherweghem J-L et al: *Rapidly progressive interstitial renal fibrosis in young women: association with slimming regimen including Chinese herbs.* Lancet 341: 387-391, 1993.

Cosyns JP, et al: *Urothelial malignancy in nephropathy due to Chinese herbs.* Lancet 344: 188, 1994.

Schmeiser HH, et al: *Detection of DNA adducts formed by aristolochic acid in renal tissue from patients with Chinese herbs nephropathy.* Cancer Res 56: 2025-2028, 1996.
Cosyns JP, et al: *Chinese herbs nephropathy: A clue to Balkan endemic nephropathy?* Kidney Int 45: 1680-1688, 1994.
Kee Chang Hung: *The Pharmacology of Chinese Herbs.* Boca Raton, FL: CRC Press, Inc 1993.
Vanhaelen M, et al: *Identification of aristolochic acid in Chinese herbs.* Lancet 343: 174, 1994.
Dharmananda S. *Are Aristolochia plants dangerous?,* 2001. http://www.itmonline.org/arts/aristolochia.htm,
Norier JL, et al: *Urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi).* New England Journal of Medicine 23 (342): 1682-1692, 2000.
Feder GL, et al: *Relationship between weathered coal deposits and the etiology of Balkan endemic nephropathy.* Kidney Int 40 (Suppl. 34): S9-S11, 1991.
Feder G, Tatu C, Orem W: *Weathered Coal Deposits and Balkan Endemic Nephropathy.* Facta Universitatis, Vol.9. No1: 34-38, 2002. (www.freemedicaljournals.com)
Stoev S, et al: *Ultrastructural and toxicological investigations in spontaneous cases of porcine nephropathy in Bulgaria.*Veterinarski arhiv 68 (2): 39-49, 1998. (http://www.vef.hr/vetarhiv/68-2/stoev.htm)
Cosyns JP: *Human and experimental features of aristolochic acid nephropathy: Are they relevant to Balkan Endemic Nephropathy?* Facta Universitatis, Vol. 9 No 1: 49-52, 2002. (www.freemedicaljournals.com)
Schmeiser H. et al. *Aristolochic acid as a risk factor for Balkan endemic nephropathy.* Facta Universitatis, Vol. 9 No 1: 53-56, 2002. (www.freemedicaljournals.com)

MOLECULAR BIOLOGICAL INVESTIGATIONS OF ENDEMIC NEPHROPATHY

Đemo Subašić[1], *Kemal Šerić, Amela Ljubović-Dedelić*

Abstract

Today, the investigation of different human diseases cannot be successful without involving of this disease molecular aspect. It means modern medicine and molecular biology is fortunately irreversible connected disciplines that together provide disease investigation on the level of informatics macromolecules (DNA and RNA). It is now possible to detect causative agent of some disease4 very fast with sensitivity and specificity almost 100%. Molecular biology provide important inform ations about many human disease such as Diabetes mellitus-1, Familial adenomatous polyposis, Cystic fibrosis and in the same time is crucial in prenatal diagnostics and transplantation medicine.

Molecular aspect of endemic nephropathy investigation, of course, provides also confirmation of wide accepted hypothesis about genetic predispositions of humans to this disease. BEN (Balkan endemic nephropathy) is disease of not yet known ethiology. By using of several molecular methods it is determined that 3q21.3-3q27.3 region of human 3 chromosome is connected with BEN. The investigation of polymorphic microsatelite DNA sequences of this region will be useful in solving of BEN genetic background. But priority surely will be finding of main aethiological factors included in making of BEN specific genetic disorders. Only multidisciplinary approach in solving of this disease will be successful, in which molecular biology will play very important role.

Introduction

Many epidemiological aspects of Balkan endemic nephropathy (BEN) are very similar to those determined in countries in which it occurs (Romania, Bulgaria, Yugoslavia, Croatia and Bosnia). Despite of

[1] Clinical Centre University of Sarajevo, Institute for Microbiology, Immunology and Parasitology-Clinical Centre University of Sarajevo, Bosnia and Herzegovina

numerous investigations, the aetiology of this disease remains not yet known. The main clinical feature is tubulo-interstitial nephropathy. All things considered many of scientist included in research of BEN agreed that secondary metabolite of Aspergillums and Penicillium species, possible viral infection, environmental conditions as well as genetic predispositions are important crucial factors in BEN development.

Recently, modern molecular-biological investigations of BEN provide new important informations about phenotype-genotype correlations associated with this disease. But, unfortunately, molecular mechanisms which cause specific genetic changes of BEN responsible genes and main aehiological factors which cause that, remain a big misters and will be the object of molecular investigations in the near future.

Molecular methods of BEN investigation

Fluorescent in City Hybridisation (FISH) – is method that is used of DNA target sequences in chromosome cell or tissue. By FISH is possible direct identification of candidate genes, part gene, specific chromosome regions or whole chromosome. It is base on hybridisation of two complementary sequences in metaphase or interphase nuclei: FISH analysis provides also information's about caryotype changes that are invisible by classical cytogenetics method. For BEN investigations all FISH probes were applied in combination with alternatively labelled chromosome 11 centromeric probes, because of FGF3 and FGF4 genes (Fibroblast growth factor protein genes) location at human 11 chromosomes. (*Toncheva etal., 2002.)*

Polymerase Chain Reaction (PCR) – By PCR is investigating polymorphism of microsatelite DNA sequences (associated with BEN) at human 3 chromosome (3q 21.3.3q 27.3 region).

Isolation of human DNA is performed usually by QIAGEN extraction kit.

Amplification in thermal cycle according specific primers and detection of PCR products by gel electrophoresis with specific DNA markers.

Phlyacrylamid – gel electrophoresis (PAGE) is important in separation of nucleic acid, by using of silver staining procedure and specific markers.

By DNA sequencing it is possible to detect mutations of genes and their analysis as well.

But it is necessary before DNA sequencing perform PCR amplification of target nucleotide sequences.

Discussion of obtained results of BEN molecular investigations

Genetic predispositions and different environmental conditions are surely factors very important for BEN appearance including possible viral aetiology. By using of molecular methods (FISH, PCR, DNA-sequencing, PAGE-polyacrylamid gel electrophoresis) there is provided new information's crucial for solving and better understanding of BEN genetic background. Now, it is widely accepted hypothesis about multifactorial aetiology of this disease.

Molecular investigation of BEN patients provide the following information's:

- the polymorphisms of Nat 2 and MDR genes (3 chromosome) can mean increased possibility for BEN development.
- CYP2DG allele distribution, other human 3 chromosome anomalies in the region 3q24 – 3q27.3 may be markers for BEN susceptibility.
- Frequent changes of chromosome X could be associated with the female BEN predominance. *(Atanasova et al., 2002)*
- Investigation of FGF3 and FGF4 genes (Fibroblast growth factor genes located at 11Q13 region may be useful for better understanding of BEN tumors.

Modern investigation of BEN cannot be imagined without great possibilities at molecular biology. In fact recent medicine and molecular biology go together into 21 century. Molecular biological methods provide presimtomatic, early fast and high specific diagnostics and realisation of preventive therapy.

Investigation of many diseases such as Diabetes mellitus-tipl, familiar adenomatous polyposis, Cystic fibrosis, Hemophilia is successful by using of molecular methods that are very important also in prenatal diagnostics and transplantation medicine as well.

The realisation of BEN investigation project must include molecular aspect and multidisciplinary approach in solving this great medical problem and institutions that already have infrastructure for specific aspect of researches. *(Zahaaireva et al., 2002; Atanasova et al., 2002;)*

Apstrakt

Savremena istraživanja različitih humanih oboljeja nebi bila tako uspješna bez uključivanja molekularnog aspekta. To podrazumjeva da su moderna medicina i molekularna biologija neodvojive discipline koje zajednički omogućavaju istraživanje bolesti na nivou informacionih makromolekula (DNK i RNK). Moguće je detekovati uzročnika bolesti veoma brzo sa senzitivnošću i specifičnošću od skoro 100%.

Molekularna biologija obezbjeduje važne informacije o mnogim humanim oboljenjima kao što su *Diabetes melitus* tip I, Familijarna adenomatozna polipoza, Cistična fibroza, a istovremeno je od krucijalnog značaja u prenatalnoj dijagnostici i transplantacionoj medicini.

Rezultati molekularnog aspekta istraživanja endemske nefropatije omogućavaju potvrdu opšteprihvaćene hipoteze o genetičkim predispozicijama ljudi na ovu bolest. BEN (Balkanska endemska nefropatija) je oboljenje još uvijek nepoznate etiologije.

Upotrebom nekoliko molekularnih metoda determiniran je 3q21.3-3q27.3 region na 3 humanom hromosomu koji je povezan sa BEN. Istraživanje polimorfizma mikrosatelitskih DNA sekvenci koje se nalaze u ovom regionu, biće korisna u rješavanju genetičke osnove BEN. Međutim, svakako da će prioritet biti pronalaženje genetičke promjene specifične za Balkansku endemsku nefropatiju. Jedino će multidisciplinaran pristup istraživanja ove bolesti biti uspješan u kome će molekularna biologija imati značajnu ulogu.

Literature

Toncheva, D.I., Zahaireva, B.M., Todorov, T., Georgiev, Ch., Sauter, G., 2002: *Fish study of llQ13 amplification on tissue microarray (TMA) of BEN tumors.* Facta Universitatis-University of NiŠ, Vol.9, No.1, (Abstracts of the meeting: Balkan endemic nephropathy an uptate).

Atanasova, S., von Ahsen, N., Dimitrov, T., Ocllerich, M., Toncheva, D., 2000: *Genetic polymorphisms of xenoobiotic enzymes and transporter proteins in bulgarian patients with Balkan endemic nephropathy.* Facta universitatis – University of Niš, Vl.9,No. 1 (Abstracts of the meeting: Balkan endemic nephropathy an uptate).

Zahaireva, B., Simon, R., Gasser, T.,Sauter, G., Toncheva, D., 2002: *High trough put tissue misroassay technology for molecular profiling of a large number of bladder cancer.* Facta Universitatis-University of Niš, Vol. 9,No.1 (Abstracts of the meeting: Balkan endemic nephropathy an uptate).

Atanasova, S., von Ahsen, N., Dimitrov, Tl, Ocllerich, M., Toncheva, D., 2002: *Genotyping of CYP2DG mutant alleles in ben patients.* Facta universitatis – University of Niš (Yugoslavia, Vol.9, No.1 (Abstracts of the meeting: Balkan endemic nephropathy an uptate).

Original article

PEDOLOGIC SOIL CONTENT IN THE LOCALITIES OF ENDEMIC NEPHROPATHY IN THE MIDLAND BOSNIAN POSAVINA

Ibrahim Osmić[1], Danijel Šimunović[2], Želimir Stipančić[2], Bahrija Šaćiragić[3], Emina Leto[1], Emir Avdić[1]

Abstract

Introduction. Area of Midland Bosnian Posavina belongs streamwater of rivers Sava and Bosna. The soil composed of aluvial and diluvial segments, specially carbonated composition. The soil is good drenaged and belongs walley soil group.

Epidemiologically, that soil belongs to areas with endemic locality.

Aim. The aim was to investigate dependence of soil and incidence of endemic nephropathy.

Methods. For pedologic work of soil were used pedological charts section Vinkovci 3 and 4, Slavonska Požega 1 and 2, Bosanski Brod 3 and 4 and Tuzla 1 and 2, working maps and aerophotopictures and pedological parameters: profile and type of soil, drenage of soil and water solution pH value. A systematic examination of population was executed (aged 6-66) as «screening» tests: a) uroprotcins b) urinary sediment. Perlustrated population is divided into 3 groups: 1) healthy 2) exactly ill 3) endemic nephropathy suspected. At last two groups were done additional laboratory investigations: CBC, urineculture and antibiogram, BUN and creatinine, electrophoresis of concentrated urine proteins and ß-2 microglobulin. Student t-test is used for statistical analysis of given results.

Results. The land of both side of river-bed Bosna make iAK, LP^b and PDg soil. Turning from river-bed pedological characteristics of soil were changed. Average water solution pH value of 7,39 in iAk soil dropped on 5,49 in PDg soil; natural drenage of easily drenaged iAK soil to badly drenaged PDg soil dropped. The gretest incidence of endemic nephropathy is on LP^b soil (5,28%), 2,3% on PDg soil and 1,54% on iAK soil.

Conclusion. The soil content of both side of river-bed Bosna is the same. The coast is iAK soil, than LP^b soil and most far is PDg soil. The greatest incidence of endemic nephropathy is

[1] Hemodialysis Center-Clinical Center University of Sarajevo
[2] Hemodalysis Center-Department of Health Odžak
[3] Institute for pedology-Faculty of Agriculture Sarajevo

on LP^b soil, less on PDg and iAK soil. It refers on direct dependence of soil and this disease. Our aim is to continues to investigate in the same way.
Key words: *pedological investigation, soil content, endemic nephropathy.*

Introduction

Problem of endemic nephropathy is actual and present more than 50 years. This disease is not enough known etiologically that's why is still in center of occupation our and worldwide experts in area of nephrology.

Our study represent analysis results given from unfinished investigation during 1990 till 1993. Results given than are great value till today.

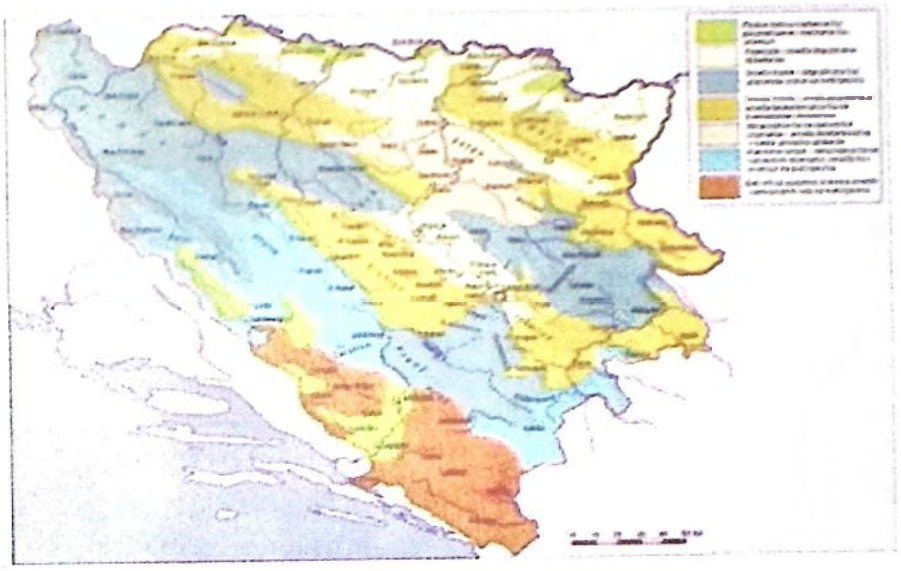
All previous studies lead to on existence of direct dependence of endemic nephropathy and enviroment, respectively presence of causes of this hard renal disease in the soil and in drinking water (4,7,8). On acceptability this way of thinking refere localities of area Midland Bosnian Posavina, where river Sava flow through northern part and the midlle part river Bosna. Geological substrate of this area is made of aluvial and diluvial sediments, especially carbonated composition. The soil is good natural drenage and belongs walley soil group.This soil has large amount of accumulated subterranean water, which is its bigger part infiltrated from river-bed, and its less part from supply superficial waters from badly drenaged soil area (5,6). Epidemiologically, that soil belongs to areas with endemic localities (1).

Aim of study

Aim of stuy was pedologic work of soil in endemic localities and perlustration of population accord to valid criteria and on given results basis to estimate dependence of soil and incidence of this etiologically still unkonown kidney disease.

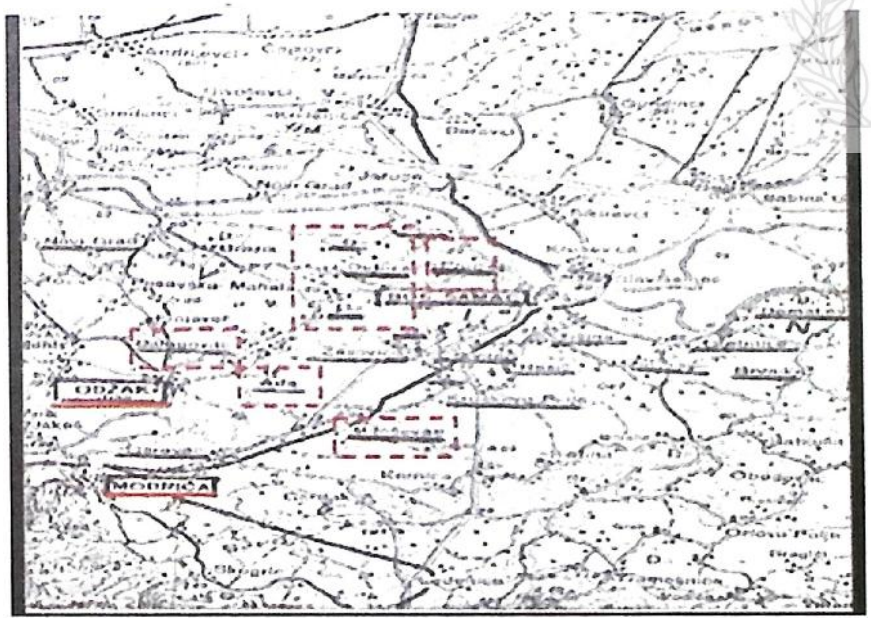
Material and methods

Looking for the soil content, we have done pedologic work of soil in the next willages: Bosanski Miloševac, commune of Modriča on the right and Ada, Novo Selo, Gornja Dubica, Donja Dubica, Prud, commune Odžak on the left side river Bosna (Pictures 1 and 2).



Picture 1: *Pedologic charts of Bosnia and Herzegovina*

Picture 2: *Endemic nephropathy on area Modriča and Odžak (Midland Bosnian Posavina)*



Pedological chards section Vinkovci 3 and 4, Slavonska Požega 1 and 2, Bosanski Brod 3 and 4 and Tuzla 1 and 2 were used through investigation. Terrain work map (1:50000) with all topographic details is used for pedological investigation. In case of walley soil group we

used aerophotopictures (1:35000) what made possible an undisturb working on terrain and properly systematic unit separation. Each systematic or mapped unit had signed by chart which was symbol for type of soil (3,5). As pedologic parameters were used number of profile and pedologic mark of soil, drenage rate and water solution pH value.

Systematic examination of population and laboratory investigations were done by WHO Conference expert recommendation for balkan endemic nephropathy. Conference have been in Dubrovnik, 1964 (2).

According that recommendation we have perlustrated of population (aged 6-66)). For perlustration we used «screening» tests: a) uroproteins b) urinary sediment. Perlustrated population divided into 3 groups: 1) healthy 2) exactly ill 3) endemic nephropathy suspected. An additional laboratory investigation were done in the group 2 and 3: CBC, BUN, creatinine, urineculture and antibiogram, electrophoresis of concentrated urine proteins and β-2 microglobulin.

For statistical analysis of given results we have used: 1) x-median 2) s- standard deviation, 3) sx- standard error and 4) t-test.

Results

Table 1. *shows pedologic characteristics of soil in the endemic localities of Midland Bosnian Posavina area.*

Table 1.: *Pedologic characteristics if soil*

Profile number	Pedologic mark of soil	Sea attitude (m)	Natural drenage	pH reaction in the water
1	2	3	4	5
151	iAK	91	Pd_3	7,50
150	iAK	91	Pd_1	7,40
142	iAK	95	Pd_2	7,28
143	LP^b	95	Pd_3	6,80
144	LP^b	95	Pd_4	6,40
149	LP^b	95	Pd_3	6,35
146	PDg	95	Pd_6	5,35
147	PDg	95	Pd_5	5,49
148	PDg	95	Pd_5	5,45

P<0,001

Profiles No 151, 150 and 142 belong to aluvial soil group with good drenage, which growing up by deepnes and expressive alkaline

reaction, specially in upper levels. That are aluvial carbonated clay soils, marked iAK.

Profiles No 143, 144 and 149 represent graybrown (meadows) non-carbonated soil marked Lp^b. They continuous on the aluvial carbonated soil and stay away from river-bed Bosna. They have an average drenage, in lower levels are softly acid or even neutral pH reaction.

Profiles No 146, 147 and 148 are podzolast pseudoglean soils. Thay spread on walley aluvium and continuous on graybrown non-carbonated soils.They are the most far of river-bed Bosna.They have never been overflowed.They are acid soil marked PDg.

Using table 1. data analysis we can see the same content of soil on both sides river-bed Bosna. Near coast, 1-1,5 km latitude is iAK soil, 2-5 km is LP^b soil and 5-7 km is PDg soil. As we can staying away from river-bed, pedologic characteristics of soil have changed in the investigation area. Average pH value of 7,39 in the iAK soil dropped on 5,43 in PDg soil, and also natural drenage dropped from easily drenaged iAK soil to badly drenaged PDg soil.

Table 2 shows number of perlustrated persons, soil types and incidence of endemic nephropathy.

Table 2.: *Soil types and incidence of endemic nephropathy*

Number	Pedologic mark of soil	Number of perlustrated persons	Number of illnes	Number of suspected	Sum of illnes and susp.	(6/3)x100
1	2	3	4	5	6	7
1.	iAK	388	2	4	6	1,54%
2.	LP^b	1136	44	16	60	5,28%
3.	PDg	824	4	15	19	2,30%
UKUPNO		2348	50	35	85	3,62%

P<0,001

Using table2 data analysis we can see that 2348 persons with incidence of endemic nephropathy of 3,62% were perlustrated, accordingly with other autors reports (1,7,8). The greatest incidence of endemic nephropathy is 5,28% on LP^b soil (2-5 km distance of river-bed Bosna), incidence of 2,30% on PDg soil (5-7, rarely 10 km distance of river-bed Bosna) and incidence of 1,54% on iAK soil (near coast 1-1,5 km).

Discussion

From acid unpassed diluvial origin stratum soil (PDg) superficial waters flow and stay in lower levels and progressive filtrate to old aluvial soil marked LP^b. Infiltrated waters from river-bed Bosna and superficial waters from unpassed soil is place for collision (5,6). We can expect that in this area named "contact zone" happened sedimentation of define substance suitable oxidative and/or reductive processes where appeard determinated nephrotoxic substances (5,6). Entering that substances into human body during long time (10-15 age) using water and food become renal tubular damage and endemic nephropathy progress.

Conclusion

The soil content of both side of river-bed Bosna is the same. The coast is iAK soil than LP^b soil and the most far is PDg soil. The area of «contact zone» respond to LP^b soil. That's the greatest incidence of 5,28% for difference of 2,30% for PDg soil and 1,54% for iAK soil. It seems that there is direct connection between soil and incidence of etiologically unknown renal disease till now.

It is the same or similar soil content in another endemic localities it's still unknown but our aim is to continues to investigate in the same way.

Apstrakt

Uvod. Područije srednje bosanske Posavine pripada vodotocima rijeke Save i rijeke Bosne. Tlo se sastoji od aluvijalnih i diluvijalnih segmenata, prvenstveno karbonatnog sastava.

Zemljište je dobro drenirano i pripada grupi dolinskog tla. Epidemiološki, pripada područijima sa endemskim lokalitetima.

Cilj rada. Cilj rada je bio ispitati zavisnost tla i učestalost endemske nefropatije.

Metodi. Za pedološku obradu zemljišta korišćene su pedološe karte sekcija Vinkovci 3 i 4, Slavonska Požega 1 i 2, Bosanski Brod 3 i 4 i Tuzla 1 i 2, te radne karte i aerofotosnimci, a od pedoloških parametara profil i tip tla, dreniranost i pH vrijednost vodenog rastvora. Sproveden je sistematski pregled stanovništva u dobi od 6 do 66 godina, te "screening" testovi: a) bjelančevina u urinu i b) sediment urina. Perlustrirano stanovništvo je razvrstano u 3 skupine: 1) zdravi, 2) sigurno bolesni i 3) suspektni na endemsku nefropatiju. U posljednje dvije skupine učinjene su i dodatne laboratorijske pretrage: KKS, Kultura urina i ABG, Urea i Kreatinin u krvi, Elektroforeza bjelančevina koncentrovanog urina i β-2 mikroglubulin. Statistička obrada dobivenih rezultata je učinjena t-testom za razliku aritimetičkih sredina.

Rezultati. Zemljišta sa obje strane korita rijeke Bosne čine tla iAK, LP^b i PDg. Sa udaljavanjem od riječnog korita mjenjaju se i pedološke karakteristike. Opada prosječna pH vrijednost vodenog rastvora od 7,39 u zemljištu iAK na 5,49 u zemljištu PDg, opada i prirodna dreniranost od lako dreniranog iAK do veoma slabo propusnog PDg. Najveća učestalost endemske nefropatije je 5,28% na zemljištu LP^b, nešto manja 2,30% na zemljištu PDg, a najmanja 1,54% na zemljištu iAK.
Zaključak. Isti je sastav tla sa desne i lijeve strane korita rijeke Bosne. Priobalno je iAK, slijedi a najudaljenije zemljište je PDg. Najveća učestalost endemske nefropatije je na zemljištu, a nešto manja na PDg i iAK, što upućuje na direktnu zavisnost tla i ovog oboljenja. Naš je zadatak nastaviti sa daljnim istraživanjima, upravo, u ovom pravcu.
Ključne riječi. *Pedološko istraživanje, sastav tla, endemska nefropatija.*

References

Aranicki, M. Gaon, J. Šerstney, E. *Dosadašnja epidemiološka ispitivanja endemske nefropatije u SR BiH.* Med. arh., 1961; 3: 98-131.
Hall, P.W. et al.: *The endemic nephropathy of South-eastern Europe.* Report on ae planning conference. Dubrovnik 1964. Nephron, 1978; 22 : 281-284.
Mehmedbašić, A. *Pedologic maps of Yugoslavia, sections Vinkovci 3 and 4, Slavonska Požega 1 and 2, Bosanski Brod 3 and 4 and Tuzla 1 and 2.* The Institute for agriculture. Sarajevo, 1974.
Osmić, I. Nijemčević, S. Krekić, S._Božičković, M. *The soil and water was examined as potency bearer causal of endemic nephropathy.* 6 th, conference of the endemic nephropathy, 13 – 15 . Mey 1987, Niš – Niška Banja.
Osmić, I. Nijemčević, S. Ambrozić, Lj. *Dodir dva tipa zemljišta i endemska nefropatija.* Zbornik radova, Skopje, 1987: 451-453.
Osmić, I. Nijemčević, S. *"Contact zone" in the soil of Midland Posavina and the incidence of endemic nephropathy.* Period. Biol., 1988; (vol. 90), 4:503-504.
Pihler, O. Bobinac, E. Miljuš, B. Sindik, A. *O učestaloj pojavi bubrežnih oboljenja u okolini Slavonskog Broda.* Lij. Vjes., 1959; 81:296-306.
Radošević, Z. Radonjić, M. Horvat, Z. *Klinička zapažanja o endemskoj nefropatiji u Hrvatskoj.* Lij. Vjs., 1959; 81:445-456.

OCHRATOXIN A (OTA) AND BALKAN ENDEMIC NEPHROPATHY (BEN)

Ladislav Ožegović[1]

Abstract

OTA as the aetiological agent of BEN could not be treated as the exclusive agent responsible for BEN, because OTA is already estabilished all around world including neighbourhood areas surrounding BEN endemic regions. His presence in tissues-organism of animals who took OTA and which were used for cooking or other procedure by men, diminished after 2-3 hours of cooking and as a result it could not be determined in substrate-beans. Seeing the existing close correlation of high aflatoxin concentration and B and C hepatitis in incidence of primary liver carcinoma, maybe it should be taken as an example of synergism of two factors which could be explored in strictly localised regions of BEN with adequate valid methods and possible exploration of all existing etiologic agents (soil related or other).

Actual aethiology hypothesis of BEN includes genetic, toxic and virous ones and amongst them for the last 20 years the toxic hypothesis represents the most significant one. Seeing that OTA is present all over the world, as in the substrates contaminated with the molds and in most common producers of OTA, Asp. ochraceus, Pen.viridicatum as well as in animal's products (pigs, poultry) in these regions, they determine pathologic changes in kidneys (liver as well) and as such are connected aethiologically with BEN. Such a hypothesis is quite understable, when other hypothetic role of genetic and virous agents-causes could not be connected with BEN (1, 2, 3).

From this point of view, there are not unexpected investigations from BEN regions in countries of former Yugoslavia, where OTA presence was found in the great number of grains and the beans, as well as in human blood and blood and tissue of animals who ingested OTA by food (4-24).

It is known, that OTA in animals is resposible also for other derrangements, leading to the noneconomic animal production. In the

[1] Academy od Sciences and Arts of Bosnia.and Herzegovina

same time, the presence of OTA in animal products represents danger for the people consuming these food.

Investigations on OTA presence were done on animals and blood and other samples. The main goal of all these investigations was to determine correlation and the causal connection between the presence of OTA in food, the quantities and the changes in organs of people and animals and to determine if such a connection exists in the more and significant measure on BEN regions. Numerous investigations have been done and are cited in relevant literature (Ožegović L., Pepeljnjak, 26).

These investigations were in relation to mass loss of turkey poults in Britain in 1960 (called «X disease of turkeys») later determined as poisoning with toxins from peanut, respectively mycotoxin aflatoxin, with later liver lesions and intoxication with Asp. flavus toxin-aflatoxin pure toxin with incidence of livre carcinoma. These continued investigations of aflatoxin hepatotoxicity concering aethiology of primary liver cancinoma in humans especially concentrate on certain regions where the peanuts and products alike are the only protein source of nutrition. Legislation and investigations on toxicity for individual species and cathegories of animals and for people led to the new knowledges of the way sof production and the toxinogenic species and varieties of Asp. flavus, from methods and ways of production and measurements of decreasing concentrations in these products to new approaches to detoxification of indigested aflatoxin (25). Aflatoxin is not only one mycotoxin regarded for regional increased frequency of carcinoma in people. Same approach goes for fumonisin, who is presumably responsable for incidence of carcinoma of oesophagus and stomach in certain regions of the world.

Subsequent investigations proved that the initial euphorya on the exclusively role of aflatoxin in the aethiology of liver carcinoma in people was premature seeing that in certain regions with high incidence of liver carcinoma and high content of aflatoxin present and corn, there was high incidence of hepatitis B and C too, thus proving the fact that the primary liver carcinoma is the result of mutual activities of aflatoxin and hepatitis viruses. Not surprising seeing that in these regions factors included besides mycotoxin aflatoxin and B and C hepatitis concerned powerty and malnutrition. Theory-hypothesis of OTA aethiology of BEN is really not whitout good reason, because this nephrotoxin is really present in grains and food-products of animals fed with such a contaminated food, but this hypothesis is confronted by the fact, that in the great part of the world, especially in Danmark and Poland, as well as in many other regions in Balcans, in spite of really high concentrations of OTA in grains, there is BEN nonexisting. That includes OTA presence in almost every region of former Yugoslavia.

OTA presence was proved in humans outside BEN regions who never showed any BEN clinical signs but subclinical course more than 20 years course . Critical appreciation on the OTA presence in food and the quantity of the food people are taking on a dayly basis and eventual connetion with BEN is of great interest. By counting on these parameters (OTA food concentration / necessary OTA quantities to cause kidney damage), the day average dose of contaminated food was estabilished: 95 μg/kg of corn or 85 μg/kg of bean meaning 6kg of corn or 6,6kg of beans as a toxic OTA dosage (14).

It is well known, that the food preparation procedure on daily basis has influence on present OTA activity. People from BEN regions do not consume OTA contaminated food uncooked and cooking procedure diminishes the quantity and activity of present OTA: by length of food preparation and by temperature used in this cooking process. 2 - hours post cooking period shows small OTA presence in cooked beans (experimentally with the toxic strain of Asp. ochraceus) and no activity present (32).

According to these results, with existing possibility of taking small quantities of OTA and its slower or faster desintegration in organism and later newfilling with further dosages, still there is too much «space» to the dosage what should be absolute toxic. Although it is relative in respect to every individual organism and his possibilities to degrade mycotoxins, depending on its actual metabolism. Although, OTA presence in great concentrations in grains or other food in wide regions of the world is in relations to certain factors; agricultural procedures, climate factors, abilities to metabolise mycotoxin (s), it represents a problem unsolved.

The reason for these expressed manifestations should be present and «the cause» should be common to all BEN localitions. If we bear in mind lever carcinoma and its etiology (hepatitis B and C as well as aflatoxin) we should ask ourselves is OTA responsibile for BEN? In the case of liver carcinoma and aflatoxin and the connection with the virus of hepatitis B and C viruses, it is obvius that one of these two factors is «trigger» and the other is «killing» factor or «executor». If the hypothesis of OTA signifanse is looked at the same way, we should ask ourselves which is the «trigger» for BEN in OTA presence and which is the other trigger agent, strictly bound to restricted BEN regions. Otherwise speaking, we should continue our investigation of all relevant and possible causes-agents which could work in synergism, if the hypothesis of OTA is still one of possible factors of BEN.

Apstrakt

OTA kao uzročnik BEN ne može biti smatran isključivim agensom odgovornim za to oboljenje, jer OTA je utvrđen u cijelom svijetu, pa i u neposrednom susjedstvu područja u kojem je BEN endemičan. Osim njegove prisutnosti u tkivima – organima životinja, koje su uzimale OTA, a takvi produkti se uzimaju od ljudi isključivo pripremanjem hrane (djelovanje topline i eventualno dodanih začina), žitarice i grah se također kuhaju – pripremaju, a dokazano je da toplina kuhanja graha uništava OTA već nakon 2 sata kuhanja. Prema tome način ishrane stanovništva produktima u kojima se nalazi OTA isključuje njegovo toksično djelovanje i ne može se OTA smatrati uzročnim agensom BEN. Možda bi se iz sličnosti primarnog karcinoma jetre u područjima u kojima je utvrđena visoka koncentracija aflatoksina i virusa hepatitisa B i C mogla uzeti kao primjer sinergizma djelovanja dva moguća agensa, što bi u područjima BEN trebalo utvrditi s obzirom da je BEN vezan uz strogo i usko ograničena područja u kojima nisu odgovarajućim validnim metodama istraženi svi mogući etiološki agensi, vezani možda uz tlo ili drugi uzrok – uzročnik.

Literature

Srpska Akademija nauka i umjetnosti: *Simpozijum o endemskoj nefropatiji,* Beograd 1973.

Srpska Akademija nauka i umjetnosti: *II Simpozijum o endemskoj nefropatiji,* Beograd 1979.

Academie Srbe des Sciences et des Arts, *Bulletin Tome LXIX,* Classe des sciences medicales, No.12, Beograd 1980.

Ožegović L.: *Mikroflora kukuruza, pšenice i graha u području endemske nefropatije u Bosni i Hercegovini.* ANUBiH, Posebna izdanja Knjiga LX, Knjiga 10, Simpozijum o mikotoksinima, 55-62, 1982.

Pepeljnjak S.; Mablzer I.: *Pregled mikoloških i mikotoksikoloških istraživanja sa nefropatičnog i anefropatičnog područja Hrvatske,* Ibid. 75-80.

Hlubna D.: *Istraživanja aflatoksina, zearalenona i ohratoksina na žitaricama u grahu u području endemske nefropatije u Semberiji,* Ibid. 82-86, 1982.

Pepeljnjak S., Cvetnić Zdenka: *Mikološka i mikotoksikološka kontaminacija žitarica na širem anefropatičnom području Hrvatske.* ANUBiH, Posebna izdanja knjiga LXXX, OMN Knjiga 12, II simpozijum o mikotoksinima, 29-42, 1986.

Hlubna D., Ožegović L.: *Rezultati istraživanja mikotoksina (aflatoksina B1, ohratoksina A i zearalenona u Bosni i Hercegovini.* Ibid. 65-72. 1986.

Pepeljnjak S., Čuljak K.: *Nalaz rezidua ohratoksina A u organima svinja na širem anefropatičnom području Sr Hrvatske,* Ibid. 71-76, 1986.

Mašić Z., Rajić I., Knežević N.: *Rezidue ohratoksina A u mesu i unutrašnjim organima svinja,* Ibid. 77-82, 1986.

Rajić I., Mašić Z., Knežević Z., Obradović V., Jezdić R., Matić G.: *Intrauterina kontaminacija prasadi ohratoksinom A i njegovo izlučivanje kolostrumom i mlekom krmača.* Ibid. 83-88, 1986.

Fluks R., Habazin- Novak Vlasta, Radić Božica, Peraica Maja, Pleština R.: *Distribucija ohratoksina A u životinja,* Ibid. 89-92, 1986.
Peraica Maja, Habazin-Novk Vlasta, Fuks R., Radić Božica, Pleština R.: *Djelovanje ohratoksina A u bubregu sisavaca,* Ibid. 93-96, 1986.
Hlubna D.: *Istraživanje prisustva ohratoksina A u žitaricama i grahu na području endemske nefropatije u SR Bosni i Hercegovini.* Disertacija, Sarajevo, 1986.
Šoštarić B., Radić Božica, Pavličak A., Peraica Maja, Fuks R.: *Mikotoksinska nefropatija svinja u intenzivnom uzgoju, III Simpozijum o mikotoksinima,* ANUBiH, 161-170, 1986.
Škrinjar M., Vujičić I., Stublefield R., Jurić V., Stojanović E., Mašić Z.: *Kontaminacija hranjiva ohratoksina A u Vojvodini,* Ibid. 171-180, 1986.
Hlubna D., Ožegović L.: *Dinamika kontaminacije graha ohratoksinom A na području endemske nefropatije u Bosni i Hercegovini.* Ibid. 181-184, 1986.
Rajić i., Ninkov D., Mašić Z.: *Uticaj ohratoksina A na prirast i konverziju hrane u janjadi u tovu,* Ibid. 185-192, 1986.
Milutinović Desanka, Rajić I., Panin Milica, Mašić Z.: *Uticaj ohratoksina A na prirast i konverziju hrane pilića u tovu,* Ibid. 193-202, 1986.
Rajić I., Panin Milica, Radić Božica, Milutinović Desanka: *Ispitivanje rezidua ohratoksina A u mesu i unutrašnjim organima pilića hranjenih kontaminiranom hranom,* Ibid. 193-202, 1986.
Mašić Z:, Rajić I., Mrđen M., Škrinjar Marija: *Uticaj ohratoksina A na neke parametre krvnog seruma odlučene prasadi,* Ibid. 207-214, 1986.
Mašić Z., Rajić I., Škrinjar marija, Pavkov S., Gagrčin M.: *Rezultati pregleda mesa svinja na rezidue ohratoksina A u jednoj industrijskoj klaonici un SAP Vojvodini,* Ibid. 215-218, 1986.
Ožegović L., Hlubna D.: *Efekti aplikacije kuhanog graha kontaminiranog ohratoksinom A u miševa,* Ibid. 219-224, 1986.
Cvetnić Zdenka, Pepeljnjak S.: *Toksinogenost sojeva Aspergillus ochraseus nefropatičnog i anefropatičnog područja SR Hrvatske,* Ibid. 225-234., 1986.
Ožegović L., Pepeljnjak S.: *Mikotoksikoze,* Školska Knjiga Zagreb, 1995.

CASE REPORTS

ANU
BiH
АНУ
БиХ

GENETIICAL MUTATION OF HROMOSOME 1 - MORBUS GAUCHER TYPE I

- Case report -

Amira Redžić[1], *Fatima Begić*[22],
Jasminka Dželilović-Vranić[33], *Amila Alikadić-Husović*[44]

Introduction

Modern diagnostics, with aid of advanced instrumental technics, almost ever day discovers new forms of pathological conditions in human population caused by various genetical factors.

Up to date has been registered more than 3 600 various forms of diseases determined by various forms of gentical disorder in genotype (McKusick, 1982). Their distribution and frequency is various, and it depends on both endogenous and egzogenous factors, and even on belonging to the certain ethnical groups (Kingama et al. 1998).

Investigations of the frequency of congenital malformations is today one of the high priority tasks in Bosnia and Herzegovina. This enables development of successful prevention, as well as finding of adequate measures of modern therapy on the basis of achievements of gene therapy.

One od such diseases is a very rare genopaty Morbu Gaucher type 1 which will be presented in this paper in more details.

What is Morbus Gaucher type 1?

Morbus Gaucher is lisosome disease of lypide deposition. It was described by French dermatologist Ph. Gaucher in his dissertation from 1882. Its family character and more detailed description was given by German scientist F. Schlagenhaufer in 1907. Defect of enzyme betaglycozydase (glycocerebosidase), which play a role in decomposition of

[1] Katedra za biologiju sa humanom genetikom Medicinskog fakulteta Univerziteta u Sarajevu, BiH
[2] Kantonalna bolnica «Dr.Irfan Ljubijankić», Bihać, BiH
[3] Neurološka klinika Kliničkog centra Univerziteta u Sarajevu, BiH
[4] Oftalmološka klinika Kliničkog centra Univerziteta u Sarajevu, BiH

cerebroside lypides and it is located in autosome 1, is caused by this disease.

This leads to the accumulation of parts of not completely decomposed cerebrosides, which are particularly numerous in reticule-endotel cells of spleen and bone marrow - Gaucher cells. The disease is relatively benign, and ita has gradual progression and it last for decades. Particular attention is paid to the therapy, that is to the supstitution of adequate enzyme, which is very expensive. (Grabowski, 1998).

It should be noted that never in the same family was described more than one type of Gaucher disease, although this disease is inherited via autosomal-recesive way.

There are three forms of this disease, and all of thema are the result of mutation on hromosome 1. This leads to decrease of activity of glucocerebrosidase. This enzyme cuts glucose from glucocerebroside (glycozylceremide) composed of equimolar amounts of amino alcohols of long chains of sphingosine, fatty acid in long chain, and glucose. Described lypide is accumulated mainly in cells of reticular endotels, which become alike Gaucher cells size frm 20 to 100 microns. They could be easily found in red pulp of spleen, in bone marrow and in sinusoides of liver (Grabowski, 1998).

Type 1 is a chronic or adult relatively benign form without consequences on CNS, which could give syptoms from school children age or later on. It is manifested through increase of spleen size as the result of increase of level of glucosil-ceremides in cells of RES. The next stage is hepatomegaly, and pains in bones and joints begin due to the infiltration of bone marrow with pathological fractures and aseptic osteonecrosis. The disease lasts for decades, but possible complications in osteoarticular system make prognostics more difficult.

Acute malign form, type II, begins between one and six months of age with apathy, strabism, opistotonuse, and attacks of laringospasm. Sometime appears convulsions. A large number of children dies at age of nine months, and main cause od death is anorexy and lungs infection.

Juvenile, subacute, neuronopathical form of the disease (type III) represents transition between ttype I and type II of the disase. Only a few cases of this form of the disease was described. A lrage number of children has convulsions with abnormal EEG. It begins after age of three years and neurological symptomes have slow progress in comparison to the acute form, and for these reasons patients could reach adult age.

Defintive diagnosis could be obtained by proving of decrease or lack of activity of enzyme in leucocites in blood or fibroblast of skin. Enzyme test is the only one specific method for diagnostics. In addition, DNA analysis could be carried out. Prenatale diagnostics of the disease could be determined by cultivation of cells of amnion liquor and by the

determination of activity of beta-glycosidase in them There is also apparent increase of acid phosphatase activity. Investigation of activity of that enzyme in leucocites and fibroblasts could help in determination of heterozygote vectors of this disase (Pastores, 1998).

Materials and methods

Gaucher morbus type 1 was determined in girl (age 14) in Cantonal hospital «Dr. Irfan Ljubijankić" in Bihać (Fig. 1). The clinical investigations were carried out at the same hospital, and samples necessary for the further detailed biochemical analysis were taken together with all other relevant data for this disease.

Fig. 1. *Patient with Gaucher morbus type 1*

A contact with the Institute for metabolic disorders in Austria was i made in order to obtain a definitive diagnostics. It was necessary to determine enzymes in skin fibroblast. Biopsy was made and material was sent for analysis to professor Paschke, Lab. for metab. Disorders UNI Graz.

All available genealogical methods were used in order to determine «genetical identity» and source and form of transfer of Gaucher gene.

Results and discussion

Case description

A girl (age 14) came to the Department of pediatrics clinic of the Cantonal hospital "Dr. Irfan Ljubijankić" due to the abundant and prolonged menstrual bleeding, increased liver, spleen and anemia (Fig.1). The girl was born as seventh child in healthz mother. Perinatal and postnatal period were regular. Up to this period the girl was not ill. The first menstruation appeared at age of 13.5. The second menstraul bleeding came after five months and it lasted for six days. After the break that lasted for two days bleeding started again with short breaks until coming to the hospital.

In status: afebrile, pale, lower height at brests, finding is regular. There is a noise at heart, on the left parasternal, of intensity II/VI, with functional characteristics. Abdomen is slightly distended, liver is palped for 2 cm, and spleen for 7 cm. Lymphe glands on predilection sites are not palped. The skin is clean, without signs of bleeding.

Laboratory results: E: 2.9 L: 8.9 hct 0.21 MCV 72 Tromb 265. DBP without any changes. Urea, creatinin, bilirubin, urin, proteinogram, transaminaze, mineralogram, koagulogram, finding regular. Gynecological finding regular. Ultrasound of abdomen showed increases liver with pronounced intrahepatical portal blood vessels. Portal vein has limited width. Spleen is significantly enlarged. Kidney ultrasound is regular. Ultrasound of thyroid gland has shown a bit decreased size of the gland. Since in the punctate of bone marrow and spleen exist large foamy cells there are some indices on Niem-Pick disease. After transfusion of depalsmatic eritrocites the signs of hemoragical dietesis have stoped, and the patient was sent home. She receives iron peroraly and vitamine C. Blood picture and trombocites are controlled once per month. She ocassionaly suffers pains in spleen region. Menstraul cycles are regular.

Table 1. *Values of analysed enzymes*

Enzyme	Patient	Control	Normbereich	Nachweis von
β-Glucosidase	0	1.41	1.5-4.7	M.Gaucher
Sphingomyelinasc	7.56	9.99	9.4-15.6	M.Niemann-PickA/B
β-Galaktosidasc	8.04	4.32	3.2-24.0	GMI-Gangliosidose MPS IVB
β-Hexos. Thermostabilitata (%)	42	53		M.Tay-Sachs
β-Hcxos., 4-MU-GlcNAc-6-S	5.79	6.38		M.Tay-Sachs

Values of enzymes in cultivated skin fibrobalsts are presented in Table 1. Activity of beta-glycosidase is not measurable. This is the evidence of Gucher disease. Value of acid phosphatase was high.

There is no significant difference between M.Gaucher and M. Nieman-pick.

Genetical aspects

Gaucher gene is type of proteine and this gene is defective and it is not capable to fulfill its normal function.

The patient was born as the seventh child in the family. Parents, brothers and sisters are healthy, except for one child which died at age of three months due to suffocation. One child in the family of uncle has epilepsy and it is psychically retarded.

"Gaucher gen" is located on autosome number 1. Patient with Gaucher disease must inherit two deffective copies of the gene (one from both parents). If both parents have normal genes, and one of them is carrier of the gene for Gaucher disease while and other is not, there are 50% chances that child will inherit gene and become disease carrier ("Geucher gena"). Children of parents carriers will not have Gaucher disase since the children will have normal gene collection from other parent.

If both parents are carriers of Gaucher gene there are 25% with each pregnancy that children will inherit that gene and in that way they will become disease carriers.

In this case, taking into acount rules of distribution of certain genes responsible for transfer of characteristics and processes, and following genealogical series, it could be concluded that parents of the patient are heterozygotes (healthy) and carriers of Gaucher gene, and that ¼ of their children are dominant homoyzgotes (healthy), 2/4 are heterozygotes (healthy) and carriers of Gaucher gene, and ¼ of children, together with the patient, are recessive homozygotes, and carriers of Gaucher gene with expression of the type 1 of this disease. If both parents are carriers of Gaucher disease there is 25% of probbility in each pregnancy that child that inherited that gene would become disease carrier.

Disscusion

Clinical situation

The most pronounced is cerebral syptomatology: disturbance, high sensitivity, brain spasms, opistotonus, stabism. The resistance to ifections is decreased. All these changes decrease working capabilities

and make problems. Prenatal diagnostics is possible (Kičić&Krajinčanić, 1989).

Constant attention of hematologist is necessary due to the trombocytopenia. It would be necessary to pay attention to the development of anemia. Splenectomy could yield good results when there are signs of hyperspalnism. However, this operation should be postponed as long as it is possible, since after splenectomy begins increased production of Gaucher cells in liver and bone marrow which accelerate insufficience of these organs. The only adequate therapy is supstantion of enzymes which is in wide use today. This therapy is very expensive and it is implemented in the form of injections of purified glucocerebrosidase obtained from human placente (ceredasa, and modified form of glucocerebrosidase - Ceresyme, which use is decreased price of therapy and there are no evidence of increase of antibodies in relation to aglucerase (Table 2).

Table 2. *Therapeutical results of use of certain drugs on Gaucher disease*

	Ceredase	**Cerezyme**
U.S. Generic: Name	*Aglucerase injection*	*Imiglucerase for injection*
Molecule	*Human placental protein (endogenous); trace human chorionic gonadotropin*	*Recombinant; one amino acid change (R495H)*
Vial size (units)	*400;50*	*200*
Formulation	*Solubilized*	*Lyopphilized*
Additives	*Citrate Human serum albumin (1%)*	*Citrate Mannitol Polysorbate 80*

The therapy with this enzyme was for the first time carried out in 1989, and since 1991 it is in wide use. Therapeutical dose is 60U/kg i.v. every other week. There were certain attempts to implement twice smaller dose, but there were no expected results. More than 2000 patients from all over the worls was treated with this enzyme. The study carried out by Gregory A. Grabowski in Human Childrens Hospital, Cincinnati, Ohio, USA involved 17 female and 13 male patients which have been parallely treated with aglucerase and imiglucesae. The study included measurements of values of acid phosphatase, bilirubin, hemoglobyne, trombocytes, leucocytes, iron, volumen of liver and spleen estimated with CT and MRI. Antibodies maesurements were also carried out. Evaluation included period of nine months. The dose was 60 U/kg every second week (Grabowski, 1998).

Increase of values for hemoglobine and number of trombocites after sic months and after nine months of therapy is presented in Table3. There are no significant differences in values between therapy with ag-

lucerose and imiglucerose. The volume of liver and spleen decreased for 34.7% after six months of therapy. Acid phosphatase decreased for more than 30%. Antibodies at beta-glycosidase was present in six patients treated with aglucerase and in three patients treated with imiglucerase.

Table 3 - *A comparative presentation of the results of the therapy with aglucerase and imiglucerase (Grabowski, 1998)*

Enzyme	Hemoglobin			Platelets		
	Initial (Δg/dL)	6 mo (Δg/dL)	9 mo (Δg/dL)	Initial (x10³/mm³)	6 mo (% Δ)	9 mo (% Δ)
Aglucerase	10.77 (8.7 to 12.8)	↑1.60 (-0.35 to 3.1) 13/15>1.0 9/15>1.5	↑2.28 (0.5 to 4.25) 13/15>1.5	70.9 (28 to 138)	19.600 (↑33.5%) (1.6 to 123%) 7/15 > 20%↑	30.100 (↑53.2%) (-23 to 210%) 7/15 > 40%↑
Imiglucerase	10.71 (6.0 to 13.6)	↑1.82 (0 to 4.3) 12/15 > 1.0 8/15>1.5	↑2.54 (0.4 to 5.8) 10/15>1.5	72.1 (28.5 to 133.5)	16.100 (↑21.5%) (-21 to 87.5%) 7/15>20%↑	28.773 (↑43.5%) (-6.3 to 95%) 7/15 > 40%

Possible therapy in our patient

Cells obtained from punctate of bone marrow or spleen are similar, but there is an difference regarding therapy. Gaucher disease is the first lysosome disease where enzymatical therpay was implemented. There are enzymes in the market, but treatment is very expensive. Annual costs of the treatment for our patient would be some 250000 euros. There are no registered other cases of this disease in other pediatrics clinics in Bosnia and Heryegovina, and in neighbouring Croatia. Three cases are registered in Austria, where all costs of treatments are coverd by the state. Of course, we do not have such a possibility, and therefore the girl could rely only on symptomatical therapy. Since Gacuher disase type 1 has slowly progress, the prognosis is not so bad. It is not possible to determine when the first signs of hypersplenism, bone marrow changes and other complication will take place.

Genetical aspects

Having one child with Gaucher disase does not mean that next three children will inherit this disease. If one of the parents has this disease or he/she is the carrier of the disease, all children will get the gene for Gaucher disease, and they could become ill, or they could become carriers of gaucher gene inherited from the parents. Therefore, gaucher disease is a form of "Genetical disorder" and there is a Gaucher gene in the family.

Families with anamnesis of Gaucher disease should participate in discusion on this disease with the doctor. It would be necessary to take a blood sample for the analysis of glucocerebrosidase in order to determine carrier. Blood tests are not always suitable due to the variations at enzyme level, level of acid phosphatasis, and glucocereb-rosidase.

Amnioentesis and causes of chorion samples could be used for determination of Gacuher disease in earlz stage of pregnancy.

Genetic consulting is possible for pairs that are carriers or that have in family cases with Gaucher disease (Rice et Barranger, 1996).

Conclusion

Maucher gaucher is a rare disease. It occures once in 40000 to 65000 persons in the whole world. This is the most frequent lysosome disease in the clinics. This is the first lysosome disease used in perinatal diagnostics and the first one where enzyme therapy was implemented.

Case of gaucher disease morbus type 1 was found in girl of age 14 in Cantonal hospital «Dr. Irfan Ljubijankić" in Bihać. The clinical investigations were carried out at the same hospital, and samples necessary for the further detailed biochemical analysis were taken together with all other relevant data for this disease.

In this case, taking into acount rules of distribution of certain genes responsible for transfer of characteristics and processes, and following genealogical series, it could be concluded that parents of our patient are heterozygotes (healthy) and carriers of Gaucher gene, and that ¼ of their children are dominant homoyzgotes (healthy), 2/4 are heterozygotes (healthy) and carriers of Gaucher gene, and ¼ of children, together with the patient, are recessive homozygotes, and carriers of Gaucher gene with expression of the type 1 of this disease.

For therapy of Gaucher disease there are in the market two enzyme. Aglucerosis obtained from human placente and imiglucerasis obtained from cells of ovaria of hamster. The second preparation is cheaper and it is in wide use. There are no differences in the activity, as well as in creation of antibodies - the enzyme entered in use for the first time in 1989, and since 1991 is in wide use. In Bosnia and Herzegovina, therapy is not possible due to the financial restrictions.

Apstrakt

Maucher gaucher je rijetka bolest. Javlja se jedan slučaj na 40000 do 65000 osoba cijelog svijeta. To je njačešća lizozomalna bolest u klinikama cijelog svijeta. To je prva lizozomalna bolest koja se koristi u perinatalnoj dijagnostici i prva kod koje je primijenjena enzimatska terapija.

Slučaj Gaucher morbus tip 1 je utvrđen kod djevojčice stare 14 godina u Kantonalnoj bolnici «Dr. Irfan Ljubijankić" u Bihaću. U istoj bolnici djevojčica je klinički ispitana, uzeti su neophodni uzorci za detaljnije biohemijske analize kao i drugi relevantni podaci za ovu bolest.

U ispitivanom slučaju, uz uvažavanje zakonitosti distribucije pojedinih gena odgovornih za transfer osobina i procesa, te genealoškim slijedom, može se konstatirati da su roditelji naše pacijentice heterozigoti (zdravi) i nosioci Gaucherovog gena, da je ¼ njihove djece dominantni homozigoti zdravi, 2/4 heterozigoti zdravi i nosioci Gaucherovog gena, a ¼ djece u koju spada i ovo dijete, su recesivni homozigoti, nosilac Gaucherovog gena kod kojeg je došlo do ekspresije ove bolesti tipa 1.

Za terapiju Gaucherove bolesti, na tržištu postoje dva preparata enzima. Agluceraza koja se dobiva iz humane placente i imigluceraza koja se dobiva iz ćelija ovarija hrčka. Ovaj drugi preparat je jeftiniji, te je naišao na široku primjenu. Nema razlike u načinu djelovanja, kao ni u stvaranju antitjela- enzim je prvi put naišao na primjenu 1989 godine, a od 1991 godine je u širokoj upotrebi. Kod nas iz finanasijskih razloga ta terapija je još neprihvatljiva.

Literature

Grabowski, A.G. (1998): *A comparasion of imiglucerase and aglucerase therapy for Type 1 Guacher disease.* Gaucher Clinical Perspectives – Molecular medicine & Therapeutics, 6(1): 1-4.

Kičić, M., Krajinčanić, Branka (1989): *Medicinska genetika.* Zavod za udžbenike i nastavna sredstva – Beograd, 1-160.

Kingama, W., Rosenberg, M, Richards, S.M. (1998*): Antibody formation in patients receiving imiglucerase and impact on safeti and clinical response.* Gaucher Clinical Perspectives – Molecular medicine&Therapeutics, 6(1): 8-11.

McKusick, V.A. (1982): *Mendelian inheritance in man.* Catalogs of autosomal, dominant, autosomal recessive, and X-linked phenotypes. Sixth edition. The Johns Hopkins University Press, Baltimore and London.

Pastores, G.M. (1998): *Recombinant enzyme therapy for Gaucher disease: long-term clinical experience and therapeutic outcomes with cross-over from alglucerose.* Gaucher Clinical Perspectives–Molecular medicine&Therapeutics, 6(1): 5-8.

Rice, E., Barranger, A. (1996): *Laboratory diagnosis of end genetic counseling for Gaucher disease.* Gaucher Clinical Perspectives – Molecular medicine & Therapeutics, 4(2): 1-4.

ELBOW INTRAARTICULAR LOCATION OF OSTEOID-OSTEOMA

- Case report -

Ismet Gavrankapetanović[1], *Faris Gavrankapetanović*[1], *Mufid Lazović*[1], *Besima Hadžihasanović*[1]

Abstract

Numerous locations of Osteoid-osteoma are described in our pervious literature. This benign tumor is followed by strong night pains, which are making more difficult life for the patients. It still presents a big challenge for surgeon.
This case report has intention to show ectopic intraarticular location in elbow with consecutive synovial reaction.

Young man 21 years old, was treated two years in other hospital. In September 2002 year he is admited in our Clinic. He had constant pains in the right elbow, especially by night.

We done RTG, which shows changed structure of bone tissue, than we done CT, MRI and Scintigraphy. Radiologists suspected on scarce form of Osteoid osteom in elbow with synovial reaction.

Young man had operation on 8th November 2002 year with hind access on elbow. We accessed in pit olecrani, and than under control of RTG (in more direction), we opened an aperture on hind rind of distal humerus (1 – 60).

Intraoperatively we found nest, smaller venose bleeding, and than we extirpated tumour. We did not remove perisclerotic part.

The pains stopped promptly, just after operation in the same night.

Patohistological analyse was showed osteoid osteoma too.

Discussion

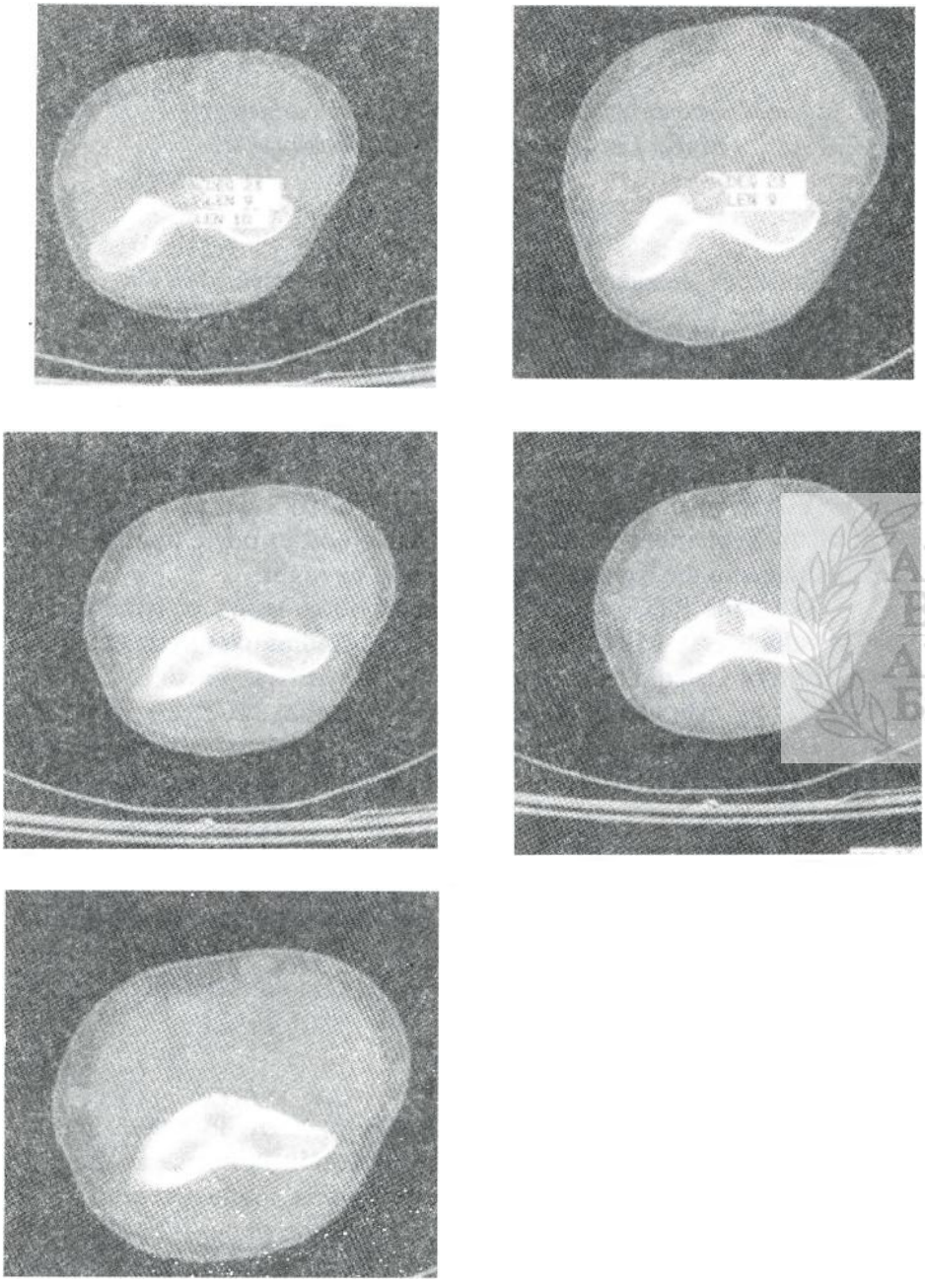
Osteoid-osteom, bone lesion characterized by little nest. For the first time was described by Jaff 1935y. A lesion is not scarce, and it includes this bones: femur and tibia, less humerus, fibula and talus.

From our pervious literature, this location is described in 1981 year, but there is not any confirmation by CT or MRI.

[1] Clinical Center of the University of Sarajevo, Clinic for Orthopaedy and Traumatology

Our opinion is that this case report can accentuate the importance of preoperative cooperation between radiologist and surgeon.

That co-operation is making surgery easier, it is shortening time of operation and makes surgery more comfortable.



CT scans of elbow joint which present osteoid osteoma tumor.

Apstrakt

Mnogobrojne lokacije Osteoid-osteoma su opisane u nama dostupnoj literaturi.Ovaj benigni tumor je praćen jakim noćnim bolovima, koji otežavaju život pacijenata.Ovo još uvijek pretstavlja veliki izazov za hirurga.

Ovaj prikaz slučaja ima za cilj da pokaže ektopičnu intraartikularnu lokaciju u laktu sa posljedičnom sinovijalnom reakcijom.

References

Barnes, R. *Osteoid osteoma,* J. R. Coll. Surg.: 2114, 1956

Bleifirld, C. *Osteoid osteoma,* Articular communication and minimal pain.:N.Y. State.J.Med. 81.1371, 1981.

Braun. S., Chevrot.A, Tomeno., B. Delbarre F., Pallardy G., Mountounnet J. and Kulas-Durand R. *A propos des osteomes osteoides phalangiens (13 cases personnels).* Rev rhum., 46: 225, 1979.

Byers P.D. *Solitary benign osteoblastic lesions of bone Osteoid osteoma and benign osteoblastoma.* Cancer, 22:43, 1968.

Carroll, R.E.: *Osteoid osteoma in the hand.* J Bone Joint Surg.; 35-A-888, 1953.

Clark, C.R., Ozonoff, M.B, and Drennan, J.C. *Case report 157: Osteoid osteoma of the femoral neck with localized synovitis.* Skeletal Radiol., 6:286, 1981.

Colton, C.L. and Hardy, J.G. *Evaluation of a sterilizable radiation probe as an aid to the surgical treatment of Osteoid osteoma.* Technical note. J.Bone Joint Surg. 65-A:, 1019, 1983.

Cronemeyer, R.L., Kirchmer, N.A.De Smet, A. and Neff, J.R. *Intra-articular Osteoid osteoma of the humerus simultaing synovitis of the elbow.* A case report. J.Bone Joint Surg. 63-A:1172,1981.

Daly, J.G. *Case report; Osteoid osteoma of the skull.* Br. J. Radiol., 46:392, 1973.

De Wet, I.S.: *Osteoid osteomata.Review of the literature with a report of five cases.* South Afr.J.Surg., 5:13, 1967.

Dreznik, I., Grunebaum, M. and Horodniceanu, C.: *Radiographic aspects of Osteoid osteoma in childhood and adolescence.* Harefuah, 99:349., 1980.

Estersohn, H.S., and Day, J.c., Jr.: *Osteoid osteoma: Benign osteoblastic lesion of Bone.* J. AM. Pediatry Assoc., 71:568., 1981.

Ferrer, J.M.: *Osteoid osteoma of the vertebral column.* Rev. Clin. Esp., 77:10, 1960.

Foss, E.I., Dockerty., M.B. and Good, C.A.: *Osteoid osteoma of mandble; Reprt of case.* Cancer, 8:592, 1955.

Freidberger, R.H, Loitman, B.S., Helpern, M., and Thompson, T.C.: *Osteoid osteoma. A Report on 80 cases.* A.J.R., 82:194, 1952.

Geshickter, C.F., and Copeland M.M. *Tumors of bone, 3rd edition.* Philadelphia. Lippincott, 1949, p.366.

Gliday, D.L. *Diagnosis of obscure childhood osteoid osteomatis with the bone scan(abscract).* J. Nucl. Med. 15:494, 1974.

Goldberg, V.m., and Jacobs, B. *Osteoid osteoma of the hip in the children.* Clin. Orthop. 106:41, 1975.
Gould, N. *Articukar Osteiod osteomaof the talus. A case report.* Foot ankle. 1:284, 1981.
Halperin, N., Gadoth, N., Reif, R. and Aver, A.: *Osteoid osteomna of the proximal femur stimulating spinal root compression.* Clin. Ortoph., 162:191, 1982.
Helms, C.A., Hattner, R.S. and Volger, J.B. *Osteoid osteoma. Radionuclide diagnosis.* Radiology. 151:779, 1984.
Horwitz.,T.: *Osteoid osteoma of the astragalus.* Radilogy. 39:226, 1942.
Jackson, I.J.: *Osteoid osteoma of the lamina and its treatment.* AM. Surg., 19:17,1953.
Jaffe, H.L.: *Primary bone tumors.* Chicago Med. Soc. Bull., 50:10, 1947.
Johnson, G.F.: Osteoid osteoma of femoral neck. Report of three cases. A.J.R. 74:65, 1955.
Kehl, D. K., Alonso, J. E., and Lovelly,W. W.: *Scolis secondary to an osteoid-osteoma of the rib.A case report.* J. Bone Joint Surg.,-a:159, 1975.
Kenzora, J.E. and Abrams, R.C.: *Problems encountered in the diagnosis and treatment of Osteoid osteoma of the talus.* Foot ankle, 2:172, 1981.
Kleinberg.S.: *Osteoid osteoma of femur. Report of case.* AM.J. Surg., 53:168, 1941.
Knotsson, F.: *Roentgenographic appereance of Osteoid ostema in children.* Acta radiol., 45:125, 1956.
Lapidus, P.W. and Salem, E.P.: *Osteoid osteoma. Report of case with probable double lesion.* Arch.Surg., 58:318, 1949.
Levine, E. and Neff, J.R. *Dynamic computed tomography scanning of benign bone lesion.* Preliminary results. Skeletal radiol., 9:238, 1983.
Levy, Y., Rosenheck, S., Torok, G.: *Osteoid osteoma of the distal phalanx of the thumb.* Acta ORTHOP. Scand., 50:667, 1979.
Lichtenstein,L.: *Bone tumors,* 5th Ed:St. Louis. Mosby, 1977, pp. 78-91.
Lisbona,R., and Rosenthall, L.: Role of radionuclide imaging in Osteoid osteoma.A.J.R., 132:77, 1979.
Loundeen,M.A. and Herring.J.A.: *Osteoid osteoma of spine: Scolinosis in two levels. A case report.* J.Bone.Joinrt.Surg., 62-A:476, 1980.
Marcove, R.C. and Freiberger, R.H.: *Osteoid osteoma of the elbow-a diagnostic problem.* J. Bone. Joint. Surg., 48-A:1185, 1966.
Moberg, E.: *The natural course of Osteoid osteoma.* J. Bone. Joint. Surg., 33-A:166, 1951.
Morrison, G.M., Hawes, L.E. and Sacco, J.J.: *Incomplete removal of Osteoid osteoma.* A.M.J. Surg., 80:476, 1950.
Munk. J., Peyser, E. and Gallei, B.: *Osteoid osteoma of the frontal bone.* Br. J. Radiol., 33:328, 1960.
Nikitin, M.N. and Ermolaev. I.U.F.: *Osteoid osteoma of the pedicle of third cervical vertebral.* Arch. Orthop. Traumatol. Protez., 7:60, 1983.
O Hara, J.P., Tegtmeyer.C., Sweet, D.E. and McCUE: *Angiography in the diagnosis of Osteoid-osteoma of the hand.* J. Bone. Joint. Surg., 57-A-165, 1975.
Ottolenghi, C.: *Osteoma osteoide del calcaneo.* Bol. Trab. Acad. Argentina Cr., 24:533, 1940.

Rand, J.A., SIM, F.H. and Unni, K.K.: *Two osteoid-osteoma in one patient. A case report.* J.Bone.Joint.Surg., 64-A:1243, 1978.
Reed, R.J.: *Fibrous dysplasia of bone.* Arch.Pathol. 75:480, 1963.
Rivero,S. and Catolla Cavalcanti, G.F.: *Varieta articolare del granuloma osteoide di Jaffe.* Mincrva. Ortop., 17:32, 1966.
Samoillova, L.I., Beliaeva, A.A. and Posternikova, T.T.: *Painless osteoid osteoma.* Ortop. Traumatol. Protez., 5:471, 1981.
Scaglietti, O. and Calandriello, B. *Sulla varieta articolare del «granuloma osteoide».* Arch. Putti, 12:9, 1959.
Sevitt, S. and Horn, J.S.: *A painless and calcified Osteoid osteoma of the little finger.* J. Pathol. Bacteriol., 67:571, 1954.
Sherman.M.S.: *Osteoid osteoma associated with changes in adjacent joint. Report of two cases.* J. Bone. Boint. Surg., 29:483, 1947.
SIM, F.H., Dahlin, D.C. and Beabout, J.W.: *Osteoid osteoma: diagnostic problems.* J. Bone. Joint. Surg., 57-A-154, 1975.
Smith,F.W. and Gliday.D.L.: *Scintigraphic appearances of Osteoid osteoma.* Radiology, 137:191. 1980.
Snarr, J.W., Abell, M.R. and Martel. W.: *Lymphofollicular synovitis with Osteoid osteoma.* Radiology. 106:557, 1973.
Spjut, H.J., Dorfan. H.D., Fechner, R.E. and Ackerman, L.V.: *Atlas of tumours of bone and cartilage.* Washington. D.C., Armed Forces Institute of Pathology, 1970, pp 120.140.
Stojanović, J., Papa., J. and Cicin-Sain.T.B.: *Computer tomography and angiographic pictures of en Osteoid-osteoma of the spine.* R.O.F.O., 137:226, 1982.
Vauzelle, J.R. and Caille, J.P.: *Paraosseus Osteoid osteoma. Anatomo-pathological study of a case.* Arch. Anat. Cytol. Pathol. 30:307, 1982.
Walker, J.W. *Experiances with benign bone tumours in pediatric practise.* Radiology, 58:662, 1952.
William,H., Simon. M.D. and Bellier, M.L.: *Intracapsular epiphiyseal Osteoid osteoma of the ankle joint. A case report.* Clin. Orthop., 108:200, 1975.
Winter, P.F., Johnson. P.M.Hilal, S.K. and Fekllдman: *Scintigraphic detection of Osteoid osteoma.* Radiology. 122:177, 1977.
Kozlowski, K.: *Osteoid osteoma (some diagnostic problems).* Radiol. Diagnost. 23:317, 1982.
Sherman.M.S. and McFarland, G.Jr.: *Mechanism of pain in osteoid osteoma,* South.Med.J., 58:163, 1965.

ANU
BiH
АНУ
БиХ

This Paper is based on a presentation by Midhat Haračić at the NATO-Civil Military Blood Conference Washington D.C. November 2000.

ANU
BiH
АНУ
БиХ

DEALING WITH THE UNEXPECTED IN EUROPE: THE CHALLENGE TO ENSURE SUFFICIENT QUANTITY OF SAFE BLOOD DURING THE WAR AND SIEGE OF SARAJEVO 1992-1995

Midhat Haračić[1], *Stephanie Simmonds*[2]

Abstract

A key principle that guides the work of every blood facility is that blood must wait for patients, not patients wait for blood. This principle was severely put to the test during the war and siege of Sarajevo, Bosnia and Herzegovina. At the onset of war the Blood Transfusion Institute in Sarajevo formulated a second principle. That its staff must take the risk of being injured not blood donors. As a result of our experience some recommendations are given for war/seige blood policy.

Post conflict 3 medium term strategies were developed. They focus on the motivation of blood donors, safe blood and the management of blood services. The strategies will contribute to achieving a long term perspective or vision that models blood services in Bosnia and Herzegovina on European laws, regulations, standards, best practices, and education and training.

Key words: *Blood supplies. War. Siege. Policy*

Introduction

Box 1. In the last decade of the 20th century

More than three years of war, siege and disruption of electricity and other utilities in Europe is a unique experiment in-vivo in modern day medicine.

Preplanning for the unexpected was always recommended in ex-Yugoslavia. But it was never imagined that in the last decade of the 20th century such preplanning would be put to the test because of war.

[1] Federation Blood Transfusion Institute, Sarajevo, Bosnia and Herzegovina.
[2] London School of Hygiene and Tropical Medicine, University of London and World Health Organization Special Representative for Bosnia and Herzegovina 1994-95 on secondment from the UK Department for International Development.

A war that lasted not just for a few days or weeks but for nearly 4 years. In Sarajevo, with no water, gas or electricity the epidemiologists expected communicable disease epidemics. But the reality was different – there were no such epidemics. The overriding problems were death and disability due to war injuries.

Four years of bombardment, massacres and snipers resulted in about 11,000 civilians being killed of whom more than 1,500 were children less than 12 years of age. And about 60,000 civilians were injured.

During those 4 years more than 80,000 blood units were taken and given to the wounded during 60,000 operations undertaken at the University Clinical Centre Hospital. Of those needing an operation 10 per cent were in reversible oligemic shock. And 10 per cent needed more than 10 units of blood.

Guiding principles

Box 2. Two key principles in war

1. Blood must wait for patients, not patients wait for blood
2. Blood Transfusion staff must take risks not donors

In box 2 are the key principles that guided the work in ensuring sufficient quantities of safe blood for anyone that needed it.

At the first International War Surgery Conference in Sarajevo in 1996 researchers confirmed excellent war injury survival results. This was attributed to two factors:

- First, that from the time of injury to being seen by a specialist in the University Clinical Centre was no more than thirty minutes- the range was ten to thirty minutes.
- Second, that blood was always available for those that needed it.

Rather than have members of the public risk their lives by coming to the Blood Transfusion Institute, the staff of the insitute went out in mobile teams to get blood from people near where they were living or working. This was because the targeting of people with shells and by snipers as they went to collect food rations, waited in queues for water, or were rushed to hospital with a war injury was a huge problem. As was the shelling of hospitals. The maternity hospital was destroyed and the two other hospitals constantly shelled.

Key challenges

Box 3. Three questions

1. How to ensure sufficient quantities of blood transfusion related supplies?
2. How to mobilise blood donors safely?
3. How to ensure safe blood supplies?

In ex-Yugoslavia based on cooperation between civil and army blood facilities, each civil blood transfusion centre was encouraged to keep a reserve of blood taking and testing supplies. The recommended amount of supplies depended on factors such as the average number of blood donors per month..

During the first year of the war the siege of Sarajevo was such that it was impossible to deliver any new supplies to the Institute. But because of the efficient implementation of the preplanning policy we had 4 to 5 times greater the quantity of the recommended reserve. Second year onwards the International Committee of the Red Cross and the World Health Organization in particular brought supplies into the besieged city.

Advertising to mobilise donors was not possible as the information might have attracted snipers and shelling. And so young people went with the mobile teams to get members of the public in apartments, shops, underground garages etc. to give blood. We built upon the enthusiasm and willingness of everyone, especially women who were not on the front line, to donate blood. The young people who went out with the professional teams were all volunteers. They were committed to helping despite the risk of injury or death.

The mobile teams were organised in such a way that they went out in small, highly mobile groups. This enabled a greater number of different locations to be covered and reduced the risk of attracting the attention of snipers and shells. The places were changed daily and the blood transfusion vehicle was parked in a different place from where the mobile team was working to minimise the risk of targeting and injuring/killing of donors. During this work all the Institute's blood transfusion vehicles, clearly marked with a Red Cross, were hit, and some staff injured. And when, in 1995, the World Health Organization brought some much needed blood from England into Sarajevo one of their UN vehicles was shot at.

Box 4. How did we ensure safe blood supplies?

For most of the 4 years there were no electricity, gas or water supplies

- Just one and a half years before the outbreak of war the new Blood Transfusion Institute building in Sarajevo was finished, the design fortunately included 3 inter-connecting underground chambers
- With a small fuel supply (20-30 litres for 36 days) a generator put on for up to one hour per day could maintain essential temperatures
- When the generator was turned on all blood samples were ready for ELISA and all other essential tests

There is a myth or presumption that in war there will be a higher than normal incidence of blood transmissable diseases and mistakes in blood grouping. At the first International War Surgery Conferencementioned earlier researchers highlighted that because of attention to detail and quality there was no increase in the incidence of either transmissable diseases or mistakes in blood grouping. The blood taking was always of high quality, in double blood bags, tested for transmissable diseases and divided into components. Because supplies were very low at times we had to use diagnostic materials months past their expiry date. Sensitivity and specificity tests on these products showed that they were OK.

The Blood Transfusion Institute in Sarajevo has 3 inter-connecting underground chambers. Each of the 3 chambers is designed to be maintained at either +2-5 degrees Celsius, -5 degrees Celsius, or -23 degrees Celsius. In summer, when there was no electricity, and the temperature in the first chamber, where blood was stored, was creeping above 5 degrees Celsius, we opened the middle chamber to cool the first. Sometimes, if the outside temperature was very high for example, more than 30 degrees Celsius, it was necessary to open the third chamber to cool the second and first one.

Recommendations for war/siege blood policy

The following are 6 key recommendations. We are hopeful that the experiment in-vivo that we experienced will not be repeated in Europe. But our lessons learnt are relevant to other parts of the world and must also be remembered as a contribution to science and medicine.

No guidelines existed for modern day Europe or from elsewhere when Sarajevo was in a state of siege and war.

1. Centralisation of some aspects of a blood service, such as an information system, is efficient and effective but each smaller facility must be able to carry out a basic service
2. Keep a good reserve of blood transfusion related supplies
3. Blood donor recruitment must rely on taking any and every opportunity
4. Underground inter-connecting chambers for storing blood can be very useful
5. Large amounts of albumin are not required. Whole blood, fresh frozen plasma, fibrinogen, and cryoprecipitating plasma are the key requirements
6. Leadership, risk taking, innovation, and flexibility are key management requirements

Post war strategies

Post Dayton three medium term strategies have been developed:

- Motivation of blood donors
- Safe blood
- Management of blood services

These were chosen as needing priority attention because of the very specific, complex environment that existed post conflict. A long term perspective or vision that explores and questions priorities for the future has also been taken.

Motivation of blood donors

Post conflict there is far less motivation to give blood. The people of Sarajevo no longer see massacres or have family or friends killed or injured due to shelling or snipers. They are also tired after nearly four years of much stress and danger. There was many a sleepless night due to heavy bombing and gunfire, the energy expended just to get a few litres of water was enormous, and people lived on food rations, mostly tinned or dehydrated long life food. Post war stress levels remain high due to uncertainty and poverty. The sum of all these factors is fewer blood donors.

But there is still a demand for blood. The hospitals have undergone a transition period from when almost 100 per cent of the work was trauma surgery to a more 'normal' mix of patients with malignancies, needing haemodialysis etc. Mobilisation of blood donors is therefore now a very specific post conflict challenge, different from either pre-war or during the war.

Safe blood

Post conflict a different type of blood is needed. In war the overwhelming need was for whole blood because of oligemic shock. Now the need is for blood products. This has resource implications. During the second half of the war there were sufficient quantities of safe, basic supplies such as bags and some testing materials thanks to the Red Cross and the World Health Organization. But post war this supply has dried up and the cash strapped post war economy means there is almost no money available to buy materials on the international market. Being in the capital city the Institute can lobby politicians and others but blood facilities in smaller, poorer towns have a major problem.

The management of blood services

Among the many pressing management issues facing the blood service are four outstanding ones: staff morale, laws and regulations, planning blood supplies, and communication.

Good staff morale in 'normal' times is a crucial factor in running a successful blood facility. This issue faces similar problems to those mentioned earlier about the motivation of blood donors. Staff are overwhelmed with thinking not about surviving but about the 'what' and 'how' of the present. How to earn a reasonable salary, what to do about elderly parents now trying to live on a very low pension, how to ensure children get a good education, etc., etc. And is still too much uncertainty about the future because of post conflict political, economic and social factors.

Laws and regulations are fundamental to ensuring a safe blood service. Bosnia and Herzegovina was a new country at the time of the outbreak of war. There was no time, and there was too much uncertainty, to spend time on adapting laws and regulations. Post war we have been exploring relevant European laws and regulations.

Planning blood supplies remains a difficult task. An epidemiological profile of the population that allows for some estimates of the type and number of blood and blood related supplies needed is not possible. There are still movements of people such as refugees and returnees. And an added complication is that there may now be a higher than normal incidence and prevalence of diseases such as cancer and other malignancies due to war related factors such as diet, stress and the environment.

Communication in Bosnia and Herzegovina is not easy especially between the two entities. And more specifically between blood facilities in the two entities. But contact is slowly increasing.

What of the future?

The following box highlights policy and management issues that now need to be addressed in order to put blood services in Bosnia and Herzegovina within a long term strategic framework.

Box 5. A long term strategic framework

- Laws and regulations needed, adapted from European ones
- Change method of financing blood services
- More emphasis on monitoring and evaluation, and control
- Standardisation of procedures and of the quality of products
- Computerised, country-wide, user-friendly information system
- Adapt undergraduate and postgraduate medical training and education curricula to European standards
- One main centre at State level needed but no centralisation of blood services

Apstrakt

U svim transfuzijskim institucijama važi bazični princip: krv mora čekati pacijenta – pacijent ne smije čekati krv. Ovaj princip bio je izuzetno značajan za vrijeme rata i opsade Sarajeva. Time je namatnut i drugi princip: osoble transfuzijske institucije mora preuzeti rizik povrjedivanja i ranjavanja – darivacci krvi moraju biti sigurni. Na bazi realizacije ovih principa u toku rata u BiH i rata u okruženom gradu, izgradili smo vlastite principe i vlastitu doktrinu.

Ona uokviruje motivaciju darivalaca krvi, aplikaciju sigurne krvi i upravljanje transfuzijskom institucijom. Ova strategija dporinjet će u perspektivi etabliranju modela transfuziološke prakse i organizacije u BiH na bazi evropskih regulativa, standarda, dobre prakse, edukacija I traninga.

ANU
BiH
АНУ
БиХ

Book Rewiew

Husref F. Tahirović and associates "Clinical Toxicology in Pediatrics", Medical Faculty of the University of Tuzla, 2002. Circulation: 1000 copies.

The book contains 434 pages, 32 figures, 40 tables and 604 references. Its size is 24cm x 15cm. Its divided into 18 chapters as follows: Epidemiology, Toxicokinetics, Diagnosis, Treatment, Prevention, Drug poisoning, Poisoning with psychoactive substances, Poisoning with household chemical, Poisoning with alcohol and glycols, Pesticides poisoning, Poisoning with carbon monoxide, Poisoning with mushrooms, Plant poisoning, Food borne diseases, Animal toxicology, Lead toxicity, Mercury toxicity, Neonatal poisoning. Index covers 15 pages.

The book «Clinical Toxicology in Pediatrics» is a valuable and extensive and rare work in the pediatric literature. The editor is Mr. Husref F. TAHIROVIĆ, MD, ph.D., pediatrician, Member of Academy of Sciences and Arts of B&H, Professor, Dean of the Medical Faculty of the University of Tuzla. The following, collaborators have contributed in preparing of this book: Pediatricians: Nedima ATIĆ, MD, Assistand (Professor), Medical Faculty, University of Tuzla; Hidajet BEGIĆ, MD, MS, Assistand (Professor), Medical Faculty, University of Tuzla; Izeta SOFTIĆ, MD Head of Department of Neonatology; alma TOROMANOVIĆ, MD, MS, Assisstant (Professor), Medical Faculty, University of Tuzla; Infectologists: Sead AHMETAGIĆ, MD, MS, Assistant (Professor), Medical Faculty, University of Tuzla; Ekrem JUSUFOVIĆ, MD, Pph.D., Associate rofessor, Medical Faculty, University of Tuzla; Lejla MACKOVIĆ, MD, Assisstant (Professor), Medical Faculty, University of Tuzla; Pharmacologists: Emil SREBOČAN, Ph.D., Professor of Pharmacology and Toxicology, Institute of Pharmacology and Toxicology, Veterinary Faculty, University of Zagreb; Vjekoslav SREBOČAN, Ph.D., Retired Full Professor of Pharmacology and Toxicology, Institute of Pharmacology and Toxicology, Veterinary Faculty, University of Zagreb; Otorhinolaryngologist: Besim HAJDAROVIĆ, MD, Ph.D., Retired Professor, Medical Faculty, University of Tuzla; Internist: Enis MEŠIĆ, md, ms, Ph.D., Associate Professor, Medical Faculty, University of Tuzla; Neuropsychiatrist: Osman SINANOVIĆ, md, ms, Ph.D., Full Professor, Medical Faculty, University of Tuzla; Specialist of Occupational Medicine: Nurka PRANJIĆ, md, ms, Ph.D., Associate Professor, Medical Faculty, University of Tuzla.

The book contains thorough contemporary knowledge of clinical toxicology in pediatrics. Texts are presented clearly, distinctly, and in detail, both in conception and linguistically. Physic and chemical properties

of poisons are sufficiently presented, with a special emphasize on pathophysiology, clinical picture, diagnosis and intensive treatment.

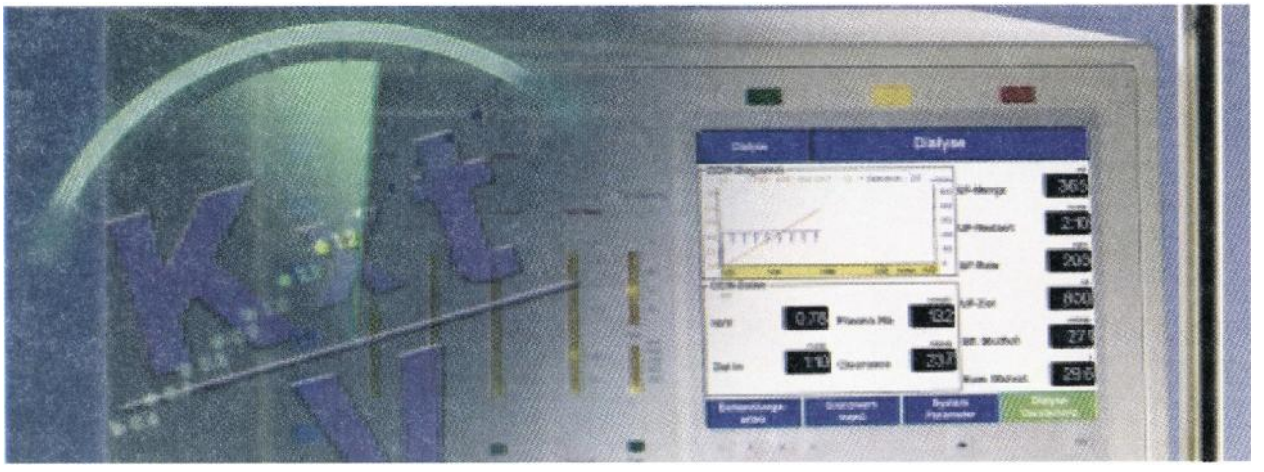
This book is special for its clinical descriptions of individual patients poisoned by certain agents, either from personal clinical experience or from literature, presented in corresponding parts. This strongly enhances the didactic value of the book, making the text more vivid and "digestible" for students. It informs the students of medicine, especially those learning pediatrics, about the problems of clinical toxicology in pediatric practice. It is also informative for clinicians who treat poisoned patients: internists, infectologists, pneumologists, otorhinolaryngologist, ophthalmologists, and other clinicians. This book will be a great stimulus for better prevention, diagnosis, and treatment of poisoned children. Presented problems significantly meet requirements of everyday practice. A special importance is given to a very interesting and unsual chapter on poisoning of newborns.

Although the book did not pretend to be "perfectly rounded off", it enlightens nearly all the problems of poisoning in pediatrics. The book of Professor Husref Tahirović and associates, being the pioneer in medical literature in this area and one of the rare in the world, will undoubtedly make valuable contribution to the education of medical students in graduate and postgraduate studies.

The book has been approved as an official textbook in clinical toxicology in pediatrics for medical students and postgraduate doctors.

Editorial Bord

Give your patients and yourself the confidence of good dialysis



Online Clearance Monitoring

To enable continuous monitoring of the dialysis dose during treatment Fresenius Medical Care has developed the Online Clearance Monitoring – a new option for the 4008 H/S haemodialysis machines – offering you many advantages in daily dialysis practice:

- Non-invasive, automatic online monitoring of effective in-vivo urea clearance (K), accumulated cleared plasma (Kt) or current dialysis dose administered (Kt/V)
- Direct adjustment during the course of treatment to meet prescribed therapy goal
- Online measurement of the plasma sodium concentration
- No additional staff involvement
- No additional disposables
- No additional treatment costs

oCm Kt/V

Impulses to improve quality of life

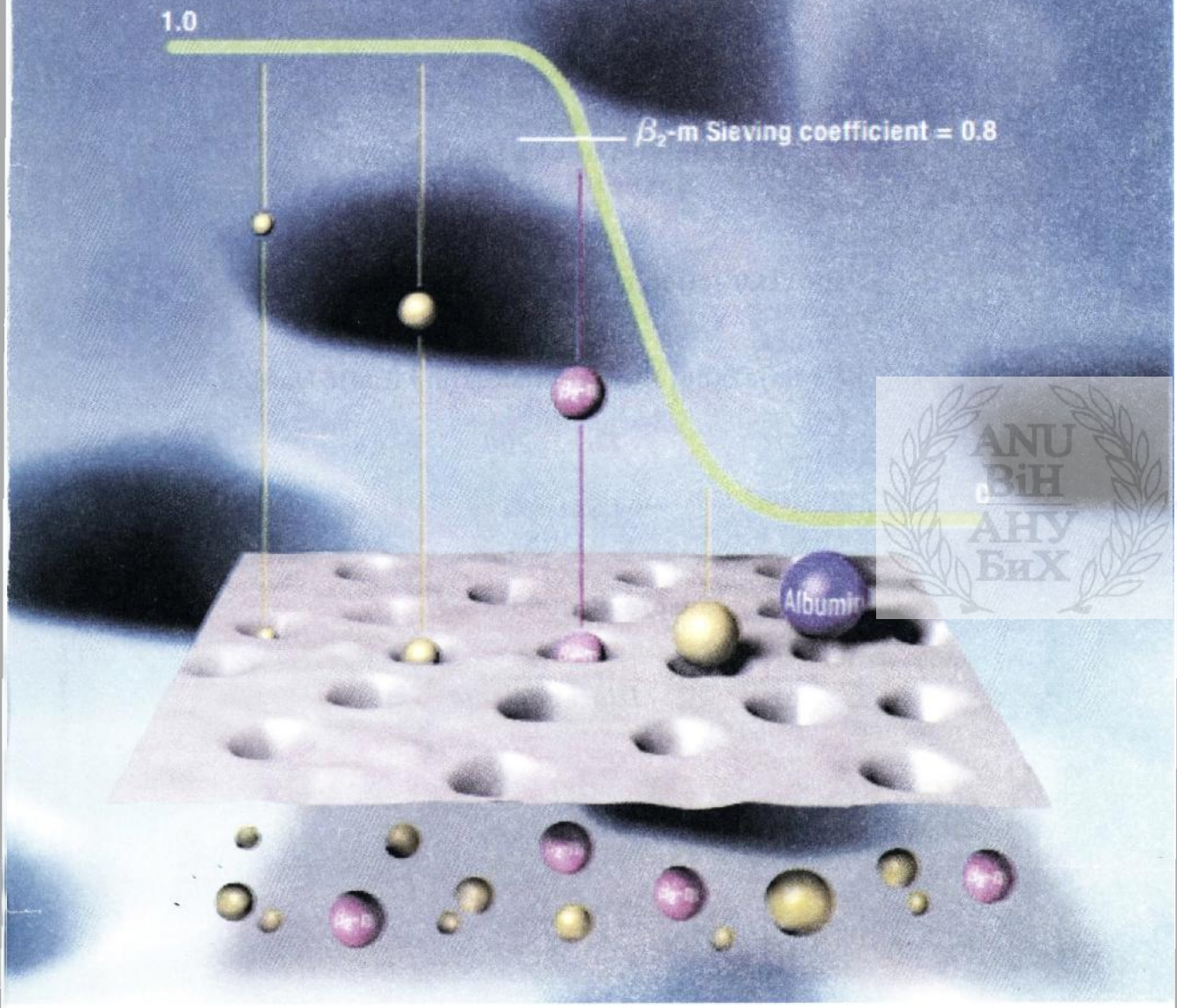
Fresenius Medical Care

Fresenius Medical Care, Else-Kröner-Strasse 1, D-61352 Bad Homburg, Germany
Phone: +49 (0) 61 72-6 09-0, Fax: +49 (0) 61 72-6 09-21 91, E-mail: dialysis-marketing@fmc-ag.com, Internet: http://www.fmc-ag.com

ANU
BiH
АНУ
БиХ

FXclass

Helixone® - focussing on β_2-microglobulin



The elimination of large molecular weight uraemic solutes during convective renal replacement therapies necessitates a membrane structure that restricts the loss of essential proteins.
Helixone®, the new polysulfone-based membrane of the FX-class of dialysers is the product of nanotechnology-based membrane fabrication procedures (NCS™ - Nano-Controlled Spinning Technology).
With its highly defined pore structure and distribution, Helixone® facilitates the unrestricted removal of solutes in the β_2-microglobulin size range.

Fresenius Medical Care

RADOVI
Odjeljenje medicinskih nauka
Centar za medicinska istraživanja

Izdavač
Akademija nauka i umjetnosti
Bosne i Hercegovine

Recenzenti
Članovi Odjeljenja medicinskih nauka ANUBiH

Saradnici
Ćerić Snježana, Kovačević Rasim i Radić Danica

Tiraž: 200

Štampa
DES

Sarajevo, 2003. Godine

Izlazi godišnje

Na osnovu mišljenja Federalnog ministarstva obrazovanja, nauke, kulture i sporta broj 03-15-1349/03 od 25. 04 2003. godine ova publikacija je oslobođena plaćanja poreza na promet proizvoda i usluga.