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AKADEMIJA NAUKA I UMJETNOSTI BOSNE I HERCEGOVINE
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ACADEMY OF SCIENCES AND ARTS OF BOSNIA AND HERZEGOVINA

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WORKS

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PHENOLIC ACIDS AND ANTIINFLAMATORY ACTIVITY OF CENTAURIUM UMBELLATUM GILIB (CENTAURIUM ERYTHRAEA RAFN.) GENTIANACEAE

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Summary

Centaurium umbellatum Gilib. (sin.*Centaurium erythraea* Rafn.) *Gentianaceae* grows in Asia, Northern Africa and Europe, and it has also been carried to Northern America. It is used in herbal medicine as stomachic agent and amarum. Today, due to its antioxidant characteristics, and its diuretic anti-inflammatory and analgetic properties, it continues to draw the attention of researchers. This plant contains bitter substances (secoiridoids), xanthone derivatives, phenol-carbonic acids and flavonoids.

This study examines the concentration of four phenolic acids in the aboveground part of plant: chlorogenic, caffeic, p-coumaric and ferulic acids. They have been examined by HPLC method. The following content values that were calculated per 100 grams of dried drug have been found:

Acid	Content in mg
Chlorogenic acid	59,66
Caffeic acid	11, 64
p-Coumaric acid	3, 07
Ferulic acid	10, 49

During drug examination on animals (mouse ear model) anti-inflammatory property of the drug aqueous extract has been confirmed. On the basis of the determined concentrations of phenolic acids, it can be stated that phenolic acids are partly responsible for anti-inflammatory effect of the acetone extract of the aboveground part of *Centaurium umbellatum* Gilib *Gentianaceae*.

Key words: *Centaurium erythraea*, phenolic acids, anti-inflammatory activity, inflammation

Introduction

Centaurium umbellatum Gilib sin. (*Centaurium erythraea* Rafn.) *Gentianaceae* grows in Asia, Northern Africa and Europe, and it has also been carried to Northern America (1). It is used in herbal medicine as stomachic agent and amarum. Today, due to its antioxidant characteristics (2), and its diuretic (3), anti-inflammatory and analgetic properties (4,5), it continues to draw the

attention of researchers. The plant contains bitter substances (secoiridoids), xanthone derivatives, phenol-carbonic acids and flavonoids (6,7).

Picture no. 1: Centaurium umbellatum Gilib.
From Flora von Deutschland Österreich und der Schweiz.(Prof. Dr. Otto Wilhelm Thomé) 1885, Gera, Germany

Objective of the Study

Objective of the Study was research of local anti-inflammatory property of *Centaurium umbellatum* Gilib. Gentianaceae and concentration of phenolic acids.

Material and Methods

The samples used (Centaurii herba) were taken from the "Apoteke Sarajevo".

Study of local skin inflammation on the mouse ear model

There are numerous methods mentioned in literature that are used for the research of local anti-inflammatory effects of the substances on skin. Since the purpose of the study is to use Centaurii herba extracts in potential skin inflammation treatment, it has been decided to apply a method of visible inflammation and to apply one-off inflammation treatment to check whether certain herbal extracts have pharmacological effect.

Literature mentions various chemicals applied to healthy skin in various concentrations; it also mentions synthetic substances, alkaloids and Oleum crotonis. The earlier described method was used in this study (8). The mice used in the research were Swiss Albino mice of both gender, 28 ± 4 grams, from the brood of the Pharmacological Institute of the Sarajevo Medical Faculty. Mice were divide in the four groups(3 mice in one group). 3% dissolution of *Oleum crotonis* in acetone, quantity 10µl, was applied to both ears in order to provoke inflammation.

Table 1: Outline of application of extracts and comparative substances on the mice groups that were studied (3% acetone dissolution of *Oleum crotonis* applied to both ears of tested animals)

Group No.	L-ear two hours after application of Oleum crotonis
1	Acetone
2	Hydrocortisone cream 1%
3	*Centarium umbellatum* extract
4	Aspirin 5% in aethanolum

L-Left ear R-Right ear

The following chemicals were used: Acetonum BP 1988 Se 6435501 Lex Portoroz, Croton Oil Sigma, Ethanol, Hydrocortisone 1% cream (Hydroderm) Splabo, Heist Belgium Se 0001102/10 2004, Aspirin, standards of phenolic acids Sigma.

The extract of *Centaurium umbellatum* Gilib sin. (*Centaurium erythraea* Rafn.) *Gentianaceae* used in the research was prepared as dissolution of 1 portion of drug and 5 portions of 70 % acetone in order to study anti-inflammatory effects and concentrations of phenolic acids. Extraction was performed with cold acetone during the period of one hour, with constant stirring. Then the filtrate was made to which dissolving agent was added, up to the measuring mark. This extract was used for further research of free phenolic acids and mouse ear skin application.

HPLC method study of free phenolic acid concentrations

Lichosper column 100, Rp 18,5 µm, 250 mm x 4,6 mm, 5 µm
Temperature column: 30°C
Mobile phase A: water : acetonitrile : phosphoric acid 85% (900:100:4 V/V/V)
Mobile phase B: water : acetonitrile : phosphoric acid 85% (150:850:4 V/V/V).
Gradient Chromatographing Program was performed in a following manner:

Table 2 : Gradient program

T	Mobile Phase A	Mobile Phase B
0	100	0
17	100	0
37	85	15
40	30	70
42	30	70
43	100	0

Flow of mobile phase in column was 1,2 ml/min.
Detection, US detector, wave length 320 nm; Injection volume 20 µm

Preparation of Standards for HPLC Analysis

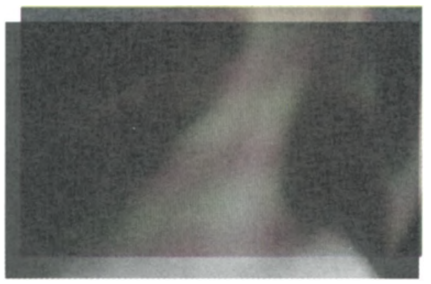
Standards of caffeic, ferulic, p-coumaric and chlorogenic acids are prepared in the following manner: 10 mg of phenolic acid is stirred into 100 ml methanol.

Calculation

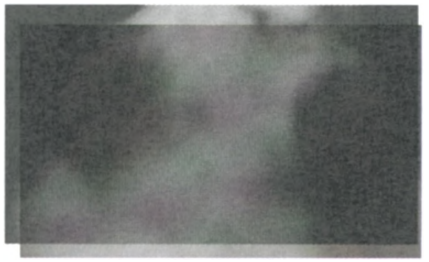
Content of phenolic acids was calculated on next way:
phenolic acid (mg) = average surface of sample chromatogram x 10 / surface of individually tested acid / 5

Results

Group 1
Twenty minutes upon application of 10 µm 3% *Oleum crotonis* in acetone, scratching of right ear was noticed; ear reddened and became more raised. After two hours, ear redness and swelling were noticed, blood vessels were clearly visible; and after six hours the difference between L and R ears became obvious. After 24 hours, irritation of left ear was not noticeable. The edge of the right ear was dark red, cracked, noticeably thickened and blurred. The left ear was more transparent, without visible swelling, while blood vessels were noticeable. After 48 hours, the difference between left and right ears became even more obvious.



picture no. 2: Left and right ears two hours after application of *Oleum crotonis* (group 1)



picture no. 3 : Left and right ears four hours after application of *Oleum crotonis* (group 1)

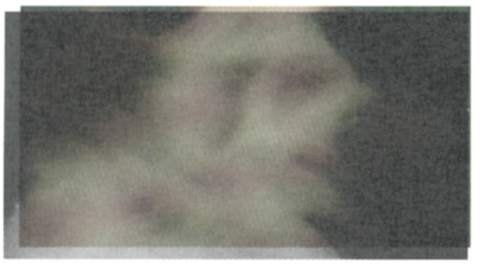
Group 2

Generally, after application of 10 µl 3% *Oleum crotonis* in acetone, scratching and redness of outer ear part was noticed with all groups. Two hours after application, redness was very visible, ears got blurred and more raised, with visible blood vessels.

Four hours after application of 10 mg of hydrocortisone 1% cream, mild anti-inflamantory effect on the left ear was noticed.

After 24 hours, there was a noticeable difference between right and left ears. Left ear was less red and significantly thinner than the right ear, without dark edge and cracks, and was more transparent. Right ear was red to dark red, thickened, blurry, with very visible blood vessels.

After 48 hours, the difference between right and left ears increased, and haematomata were noticed on the right ear.



picture 4 : Left and right ears four hours after application of hydrocortisone cream (group 2)

picture 5: Left and right ears 24 hours twenty four hours after application hydrocortisone cream (group 2)

Group 3

In this group, with one mouse, there was visible difference between ears, four hours after extracts application. With other two mice, the difference between left and right ears was insignificant. After 24 hours, huge difference between treated (lighter, with somewhat visible blood vessels) and non-treated ear was noticed. Non-treated ear was red with more visible blood vessels. After 72 hours, the difference in appearance increased.

picture 6: Left and right ears four hours after application of Centaurii herba extract (group 3)

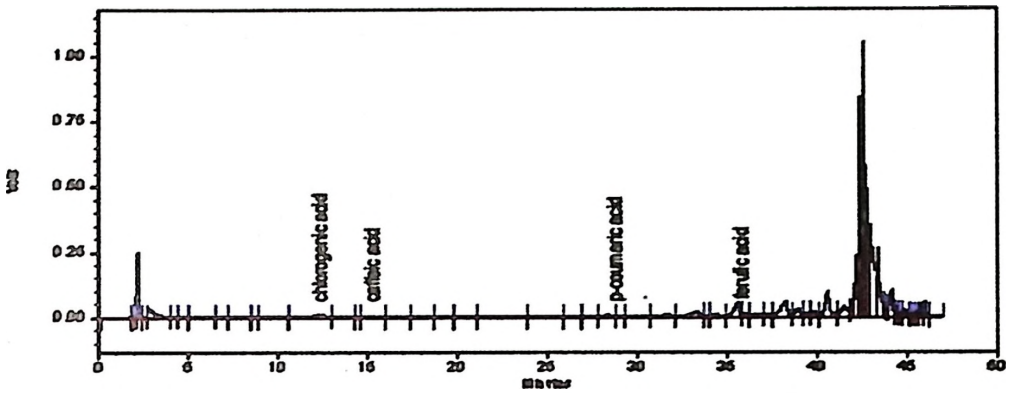
Group 4

Four hours after application, right ear reddened and swelled, and had a visible bloody edge. Left ear was red, but did not swell. With all three mice, there was a significant difference noticed after 24 hours.

picture 7 : Left and right ears four hours after application of aspirin in ethanol

Results of phenolic acids research

On picture 8, there is a standard chromatogram made in the course of the research of phenolic acids concentrations in the sample.



Picture 8 Acetone extract chromatogram made during research

Table 3: values of examined phenolic acids (mg) per 100 g of dry substance

Phenolic acid	Rt	Surface of "area under the curve" standard	Surface of "area under the curve" sample	Concentration (mg) in 100 g
Chlorogenic acid	12,425	20991	626184	59,66
Caffeic acid	15,342	43081	250831	11,64
p-Coumaric acid	28,863	61858	94989	3,07
Ferulic acid	35,898	44490	233429	10,49

Discussion

In this study, the subject of HPLC method research were concentrations of four phenolic acids that may partly participate in the anti-inflammatory activity of *Centaurium umbellatum*. In the case of the mouse ear model, *Centaurium umbellatum* extract has stronger anti-inflammatory effect than aspirin dissolved in aethanolum, while its anti-inflammatory effect is weaker than that of hydrocortisone cream.

Berkan and others (5) also studied anti-inflammatory effects of *Centaurium umbellatum* extract, however there was no correlation made with the substances responsible for such pharmacological activity.

Conclusion

Extract of herbal drug of *Centaurium umbellatum* (*Centaurii herba*) in acetone shows mild local anti-inflammatory effect. The content of phenolic acids found in the drug are as follows: chlorogenic acid 59,66 mg, caffeic acid 11, 64, p-coumaric acid 3,07 mg and ferulic acid 10,49 mg per 100 g of dried herb (table 3).

On the basis of phenolic acids concentrations found and research performed on mouse ear model, it can be presumed that phenolic acids are partly responsible for anti-inflammatory effect of the extract of the aboveground part of *Centaurium umbellatum* Gilib. *Gentianaceae.*

Sažetak

Centaurium umbellatum Gilib. (sin.*Centaurium erythraea* Rafn.) *Gentianaceae* rasprostranjena je u Aziji, Sjevernoj Africi i Evropi, a prenesena je i u Sjevernu Ameriku. Koristi se u narodnoj medicini kao stomahik i amarum. Danas pobuđuje i dalje interes istraživača zbog antioksidativnih osobina, kao i diuretičnog antiinflamatornog i analgetičkog djelovanja.

Biljka sadrži gorke materije (sekoiridoidi), derivate ksantona, fenil karbonske kiseline (fenolne kiseline), flavonoide.

U ovom radu ispitan je sadržaj četiri fenolne kiseline prisutne u nadzemnom dijelu biljke i to: hlorogenska kiselina, kafena kiselina, p-kumarinska kiselina i ferulna kiselina. Ispitivanje fenolnih kiselina obavljeno HPLC metodom. Nađene su slijedeće vrijednosti računato na 100 g osušene droge:

Fenolna kiselina	Sadržaj u mg
Hlrogenska kiselina	59,66
Kafena kiselina	11,64
p- Kumarinska kiselina	3,07
Ferulna kiselina	10,49

U ispitivanjima na životinjama (model uške miša) potvrđena je antiinflamatorna aktivnost vodenog ekstrakta droge. Na osnovu nađenih količina fenolnih kiselina može se reći da da su fenolne kiseline, odgovorne za dio antiinflamatornog djelovanja acetonskog ekstrakta nadzemnog dijela biljke *Centaurium umbellatum*. Gilib. *Gentianaceae.*

Ključne riječi: *Centaurium erythraea*, fenolne kiseline, inflamacija

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HEMODYNAMIC AND RESPIRATORY RESPONSES OF DOGS TO HEMORRHAGE AFTER TREATMENT WITH DIHYDROERGOTOXINE AND NALOXONE

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Abstract

The endogenous opiate receptor antagonist (naloxone, NAL) and alpha-adrenergic receptor antagonist (dihydroergotoxine, DHETX) were infused separately or simultaneously in dogs to determine their effects in hemorrhagic shock. Mean arterial pressure, heart rate, electrocardiogram, respiratory rate, hematocrit and plasma protein concentration were measured during sustained posthemorrhagic hypotension (180 min, 40 mmHg) in 22 dogs. Animals were divided into four groups: DHETX-treated (n=6), NAL-treated (n=5), DHETX+NAL-treated (n=5), and SAL (saline)-treated (control group, n=6). The treatment was performed before bleeding. After 3 hours of posthemorrhagic hypotension, all shed blood was returned to the dogs, and animals passed through a postretrasfusion period for 60 min. The animals which survived experimental procedure were observed in next 24 hours. Under the present experimental conditions, prophylactic administration of DHETX had better effects on preservation of parameters mesaured and survival of dogs than NAL, while DHETX+NAL treatment had the worst effects (no one dog survived).

Key words: hemorrhagic shock, dogs, opiate receptor blockade, alpha adrenergic receptor blockade

Introduction

Endogenous opiates might play a role in altering cardiovascular hemodynamics during hypovolemic shock (1), and modulate the hemodynamic instability, neuroendocrine, and cytokine responses to hemorrhagic shock (2). Furthermore, a role of the opioid peptides in cardiovascular responses to several shock and trauma paradigms has also been suggested based on the cardiotonic and improved hemodynamic responses to treatments with naloxone (1, 3, 4). Although several studies failed to demonstrate improved cardiovascular status in hemorrhaged animals treated with naloxone (5, 6), including failure to demonstrate improved survival, it is still commonly believed that activation of endogenous opioid system plays a depressor and hence a detrimental role in cardiorespiratory recovery after bleeding (7).

Previous experimental results show that naloxone improved the hemodynamic and biochemical state of cats in hemorrhagic shock (3). Vargish et al. (8) reported a significant positive inotropic effect in dogs after naloxone infusion during hemorrhagic shock. Gu et al. (9) showed that the opiate antagonist, naloxone, significantly potentiated the inotropic effect of infused epinephrine in the canine isolated heart/lung. We have previously reported that the alpha adrenergic antagonist phenoxybenzamine is benefitial in canine hemorrhagic shock (10, 11, 12). The present study was designed to investigate a possible link between effects of naloxone, an opiate receptor antagonist, and dihydroergotoxine, an alpha adrenergic receptor antagonist, given separately or simultaneously during posthemorrhagic hypotension in anesthetized dogs, more exactly, to investigate whether naloxone and dihydroergotoxine could improve cardiovascular and respiratry function in dogs exposed to prolonged poshemorrhagic hypotension.

Material and Methods

A total of 22 mongrel dogs (10-25 kg) of both sexes were anesthetized with chloralose (0.1 g/kg) intravenously and intubated with endotracheal tubes. Additional chloralose was given whenever necessary to maintain anesthesia. Femoral arteries and veins of either side were cannulated with polyethylene catheters filled with a 0.9% saline solution containing heparin. Right femoral vessels were used for bleeding (artery) and for a drugs and saline infusion (vein). Left blood vessels were used for mean arterial pressure (MAP) measurement (artery) with Statham P23 pressure transducer and for blood sampling (vein). The electrodes are attached to the legs for electrocardiogram (ECG) lead II registration. The pneumograph was placed around the chest and connected to a volumetric pressure transducer for respiratory rate (RR) measurement. All parameters (MAP, ECG, RR), including heart rate (HR) automaticaly obtained from ECG, were continuously recorded on a Grass 7D polygraph.

Following the surgical preparation, heparin (500 units/kg) was given, and animals were allowed to stabilize for approximately 30 min. Control values were recorded thereafter and their mean was taken as baseline value (100%); the blood samples were drawn for determination of hematocrit (microhematocrit technique) and plasma protein concentration (Biuret-method), too.

After procedure described above, experimental animals have been introduced in the state of hemorrhagic shock by the bleeding during 15 minutes until the fall of MAP to the level of 40 mm Hg. This level of posthemorrhagic hypotension was

maintained 180 minutes by giving back or removing blood as necessary (Fig 1., period from 0 to 180 min). At the end of hypotensive period all shed blood, which remained in reservoir, was retransfused. The animals were observed in the next 60 minutes of postretransfusion period, catheters were removed, the wounds were closed, and the animals were returned to their cages. Survival was determined at 24 hours.

Four groups of hemorrhaged dogs were studied as follows: DHETX (n=6) (dihydroergotoxine treated, bolus 0.1 mg/kg in 8 ml 0.9% saline + 0.1 mg/kg/h in 12 ml 0.9% saline during 30 min infusion); NAL (n=5) (naloxone treated, bolus 2 mg/kg in 8 ml saline + 2 mg/kg/h in 12 ml saline during 30 min infusion); DHETX+NAL (n=5) (DHETX and NAL treated, each in same dose and way as separately); and SAL (n=6) (control group, 0.9% NaCl treated, volume and way as in first 3 groups). DHETX, NAL (separately or simultaneously) and vehicle (0.9% NaCl) were given before hemorrhage.

Two-milliliter blood samples were collected at -15, 0, 30, 60, 120, 180, and 240 min for analysis of hematocrit and plasma protein concentration. Statistical analysis was performed by Student's t-test. The level of statistical significance was assessed at $P<0.05$.

Results

Table 1. shows the values of bleedout volumes in the moment of decrease of MAP to 40 mm Hg in all experimental groups. Observing all volume of blood in ml or expressed in ml/kg of body weight, we can notice a significant differences between treated groups. The bleedout volume until hypotension of 40 mmHg is smallest in control group, but is the biggest in NAL-treated group.

Table 1.

BLEEDING VOLUME TO MAP=40mmHg				
Groups:	n	Average of body weight (kg)	Total blood volume (ml)	Blood loss per kg body weight (ml/kg)
SAL	6	17,0	174	10,27
DHETX	6	13,9	382	27,48
NALOXONE	5	12,5	536	42,88
DHETX+NAL	5	14,8	476	32,16

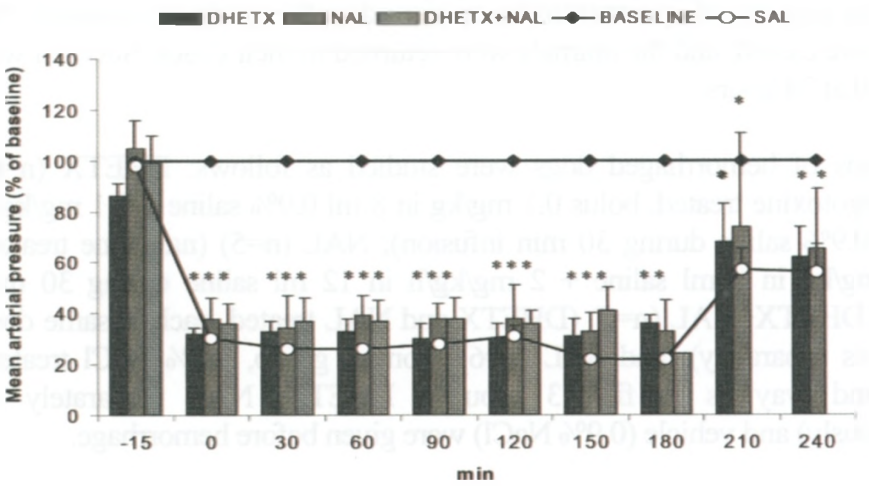

Fig. 1. Changes in mean arterial pressure (% of baseline) in hemorrhaged dogs treated with dihydroergotoxine (DHETX), naloxone (NAL), dihydroergotoxine+naloxone (DHETX+NAL) and saline (SAL). Values are mean ± SEM. Significant differences in each group: *P<0.05 compared with baseline value (100%). Significant differences between different groups at same time point (P<0.05): **a** = DHETX vs. SAL; **b** = NAL vs. SAL; **c** = DHETX+NAL vs. SAL; **d** = DHETX vs. NAL; **e** = DHETX vs. DHETX+NAL; **f** = NAL vs. DHETX+NAL.

Hemorrhage induced sharp decreases in MAP (Fig. 1.). Changes of MAP in terms of percentage regarding the values before bleeding (100%, baseline) in all groups are significant (P<0.001) during the hypotensive period. However, the differences between groups have not been significant during the hypotensive period, what is reasonable considering the used method. The rastauration of MAP after retransfusion is the best in NAL- treated group (74% of baseline), but in DHETX-treated group 68% and in control group 57% of baseline. In DHETX+NAL-treated group none animals survived the complete experimental procedure.

This improved blood pressure profile was not due to a difference in severity of the shock protocol between the treated and untreated shock groups. The bleedout volumes until MAP was reduced to 40 mmHg were 10.27 ml/kg for control dogs; 27.48 ml/kg for DHETX-treated dogs; 42.88 ml/kg for NAL-treated dogs; and 32.16 ml/kg for DHETX+NAL-teated dogs (Table 1). The increased bleedout volumes experienced by the treated shock dogs would be expected to

produce a more severe shock state resulting in a lower postretransfusion MAP, however, the opposite occurred (except for DHETX+NAL-treated dogs).

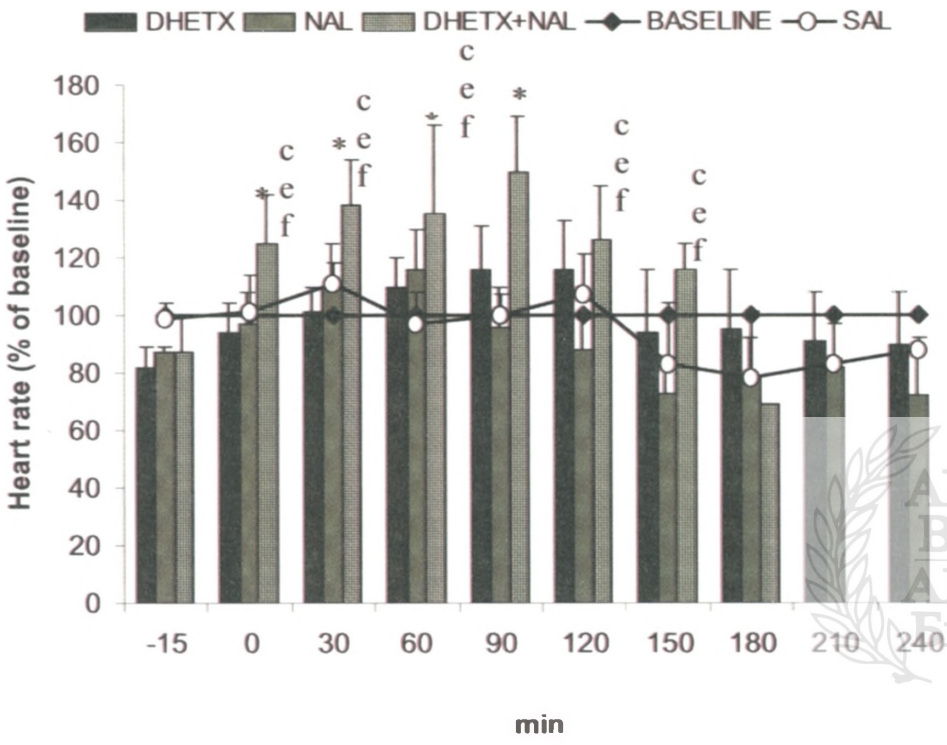

Fig. 2. Changes in heart rate (% of baseline) in hemorrhaged dogs. Values are mean ± SEM. Groups and symbols are the same as in Figure 1.

Figure 2. shows changes of HR during the experimental procedure. After the hemorrhage, HR increased in dogs of all groups regarding the baseline values. During the hypotensive period (until 90 minutes) the increase of HR is only in DHETX+NAL-treated group higher than prehemorrhagic value. After the initial increase we noticed a slow tendency of decrease of HR in dogs of control, DHETX-treated and NAL-treated groups which is more intensive from 120 to 240 min. During all hypotensive period (from 0 to 150 min) HR at DHETX+NAL-treated dogs was significantly higher than in other three groups.

Figure 3. illustrates the representative ECG during prolonged posthemorrhagic hypotension in four experimental groups. Beside the DHETX-treated group, in

all groups a significant changes in ECG appeared. In NAL-treated group and in DHETX+NAL-treated group those changes are more visible than in the control group. Tachycardia is present in all experimental groups, but the elevation of ST-segment is a characteristics only in NAL-treated and DHETX+NAL-treated groups, but the height of R-wave is decreased significantly. In all groups the significant shortening of PQ-interval was observed. In DHETX +NAL-treated group the characteristic pictures of ECG disappears gradually, and in terminal phase of experiment we can not notice PQRST-complexes. In NAL-treated group changes of ECG appear already during infusion of NAL, what means before the hemorrhage. In DHETX-treated dogs recorded electrocardiograms are extremly good, except the tachycardia mentioned above, we can not notice any other changes.

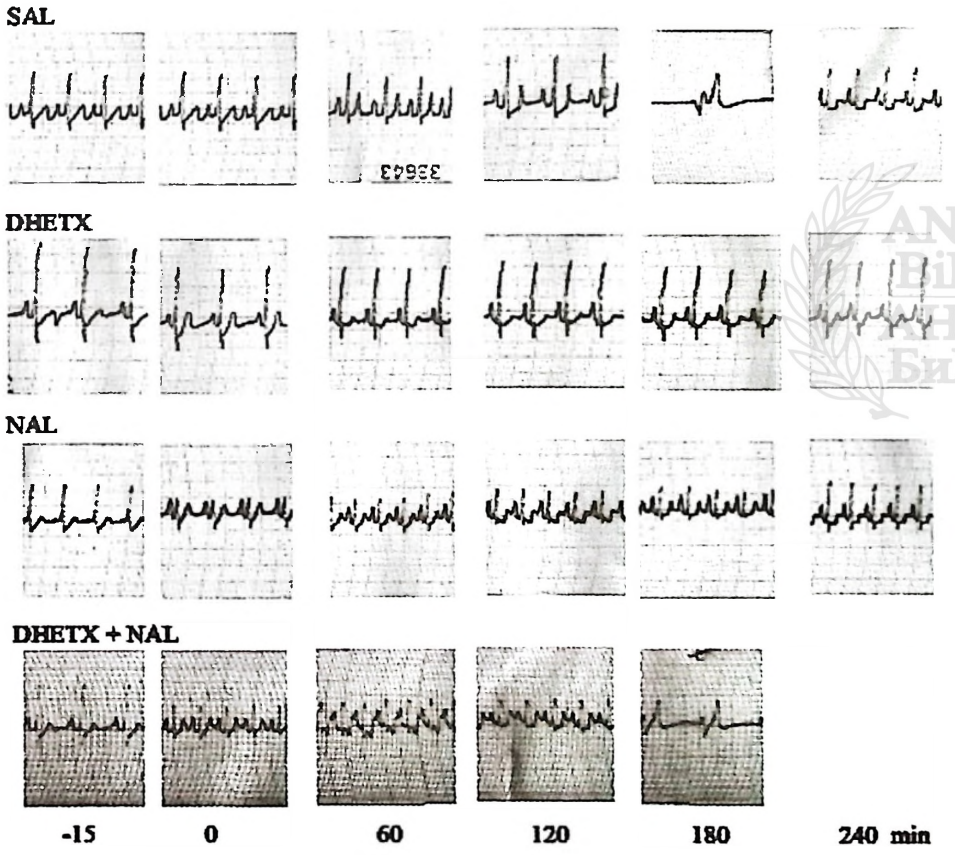

Fig. 3. This series of representative electrocardiograms shows the effects of naloxone and dihydroergotoxine given separately or simultaneously during posthemorrhagic hypotension in anesthetized dogs.

Changes in values of respiratory rate (RR) are presented in Fig 4. In NAL-treated group there are the biggest aberrations regarding the basal values. This could be absolutely disturbed breathing, where the normal phases of breathing is disappearing, i.e., instead of normal breathing it appear panting. Changes are significant comparing with other three groups. The most stable breathing is recorded in DHETX- treated group.

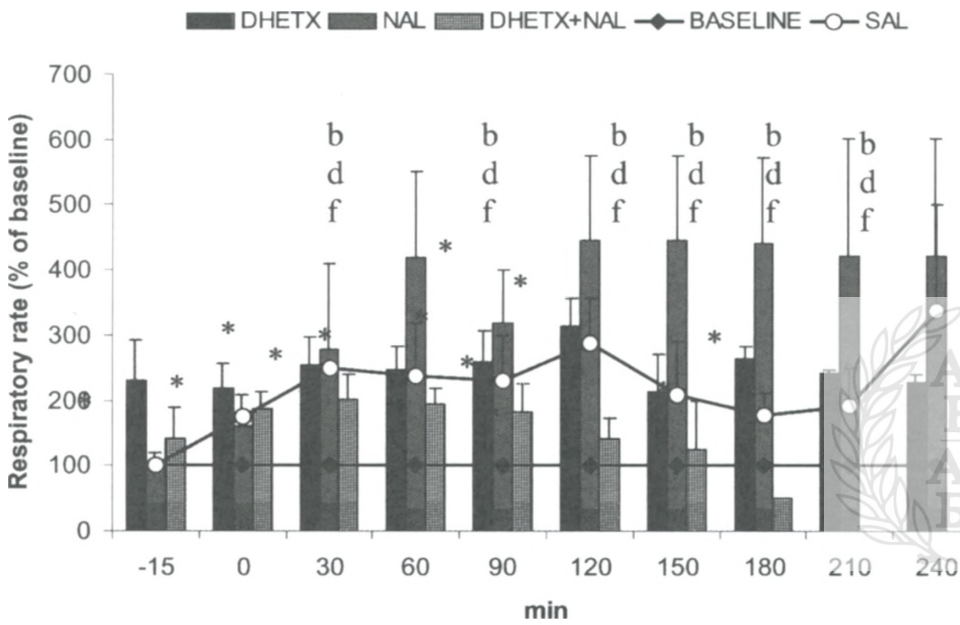

Fig. 4. Changes in respiratory rate (% of baseline) in hemorrhaged dogs. Values are mean ± SEM. Groups and symbols are the same as in Figure 1.

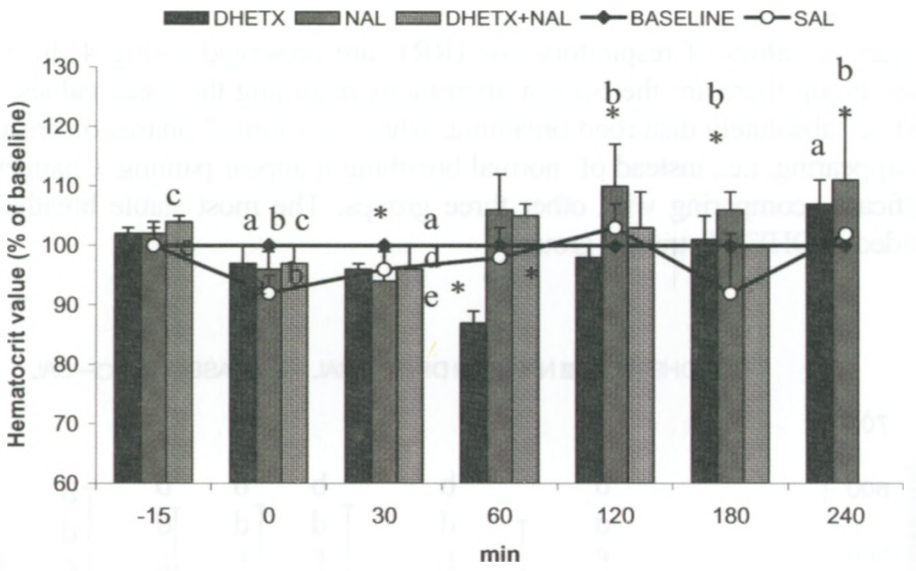

Fig. 5. Changes in hematocrit (% of baseline) in hemorrhaged dogs. Values are mean ± SEM. Groups and symbols are the same as in Figure 1.

Immediately after the hemorrhage and fall of MAP to 40 mmHg (0 min) and in 30 minutes, the hematocrit values (Ht) in dogs of all four groups are lower regarding the baseline values (Fig 5.). The fall of Ht in DHETX-treated dogs continue also in 60 minutes of hypotensive period, while in other groups, Ht shows a slow tendency of increase until the end of experimental procedure.

Figure 6. summarizes the decreases in plasma proteine concentrations during the course of the 240-min experiment for the four experimental groups of dogs. By testing of differencies between groups we established that only in NAL-treated group the values of proteinemia are significantly different as compared to other groups, especially in later phase of experimental procedure.

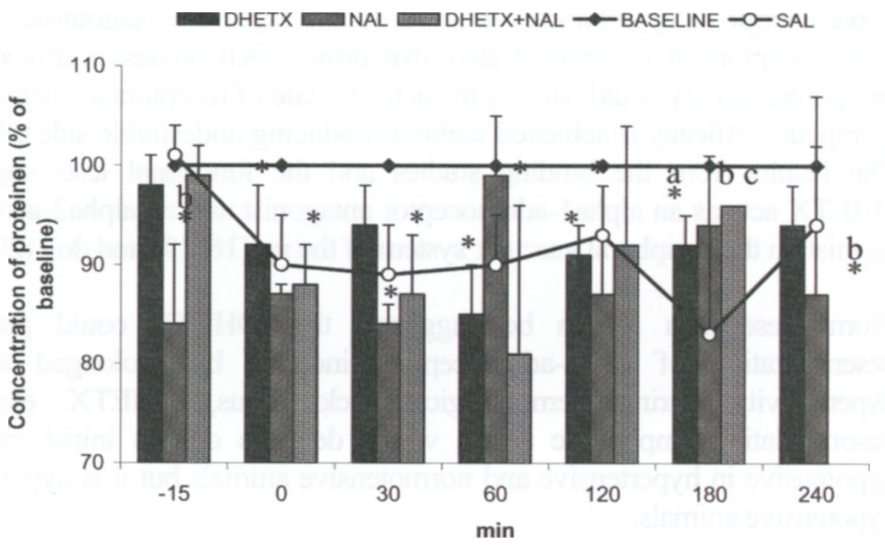

Fig. 6. Changes in plasma proteine concentration (% of baseline) in hemorrhaged dogs. Values are mean ± SEM. Groups and symbols are the same as in Figure 1.

Discussion

This study was carried out to investigate whether naloxone (NAL), an opiate receptor antagonist, and dihydroergotoxine (DHETX), an alpha adrenergic receptor antagonist, could improve cardiovascular and respiratory function in dogs exposed to prolonged posthemorrhagic hypotension.

It is well known that the bleedout provokes an strong activation of opioide (1-7) and adrenergic systems (10-12). Three hours long experimental posthemorrhagic hypotension of around 40 mm Hg is lethal for 70-90% of animals regardless transfusion or retransfusion (12, 13). Our previous data (11, 12) have showed that phenoxybenzamine (alpha adrenergic antagonist) in hemorrhagic shock in dogs delay the appearance of irreversible disturbances, improve cardiovascular functions, liver and pancreatic blood flow, and finally, increase the percentage of survival as compared to untreated animals. In available litterature we did not find data about what kind of effects DHETX has in hemorrhagic shock. Investigating hemodynamic effects of different ergot alcaloids, Sušič et all (14) are established marked effect of DHETX on cardiovascular system with clear antihypertensive effects. It was proved that DHETX possess a high afinity for alfa-2 adrenoceptors which it antagonize competitively (15, 16).

Several studies have shown that DHETX can interfere with at least three types of receptor (15-19): alpha-adrenergic, 5-hydroxytryptaminergic and dopaminergic receptors and exercises a double agonistic/antagonistic activity on these receptors. It is reported also, that drug which possesses mixed agonist-antagonist activity could balance the activity state of receptors in such a way that therapeutic efficacy is achieved without producing undesirable side effects (19). The results from the binding studies and the functional tests suggest that DHETX acts as an alpha1-adrenoceptor antagonist and an alpha2-adrenoceptor agonist on the peripheral vascular system of the rat (16, 17) and dog (20).

From these data it can be suggested that DHETX could prevent the desensitization of alpha-adrenoceptors induced by prolonged adrenergic hyperactivity during hemorrhagic shock. Thus, DHETX exercises a vasoregulating amphoteric action which depends on the initial tonus: it is hypotensive in hypertensive and normotensive animals but it is hypertensive in hypotensive animals.

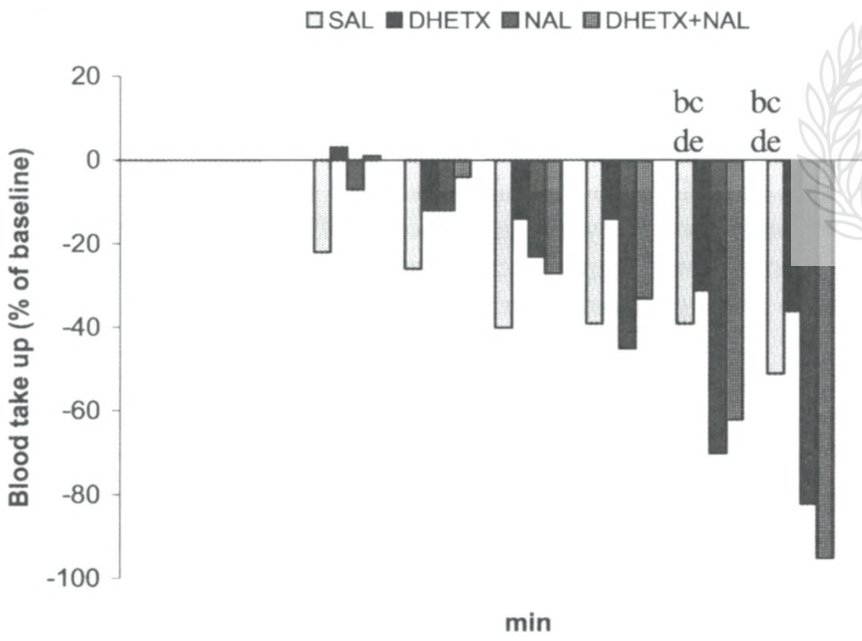

Fig. 7. Changes of volume spontaneously returned blood from reservoir into circulation (% of volume recorded after hemorrhage of dogs to 40 mmHg of MAP). Groups and symbols are the same as in Figure 1.

The results of our work shows that DHETX given before the hemorrhage, has a favourable effects in sense of more stable reactions of cardiovascular and respiratory system during hemorrhagic shock procedure (Fig 1-4.). It is well known that the blockade of alpha adrenoceptors brings to relaxation of postcapillary sphincters and decrease of hydrostatic pressure in capillaries (10) what results in decrease of intravascular fluid loss. The dynamics of changes of Ht (Fig 5.) and proteinemia (Fig 6.) point at this. The dynamics of blood return in circulation from the reservoir (take-up) with aim to maintain the controled hypotension at level of 40 mmHg also confirm those facts (Fig 7.). DHETX-treated group is the most stable in balancing of spontaneous blood return from the reservoir in blood circulation; to the end of hypotensive period (180 min) it was spontaneously returned around 35% of blood which was recorded after the hemorrhage of dogs to 40 mmHg of MAP (Table 1.). In control and NAL-treated groups spontaneous blood return from reservoir in circulation starts immediately after the finish of bleedout and continue during the whole hypotensive period, and because of that only small quantity of blood remain for retransfusion. The least blood in reservoir at the end of hypotensive period remained in group of DHETX+NAL-treated dogs, only 5% from the initial hemorrhaged volume.

The number of survived animals is the biggest in group of DHETX-treated dogs (4/6, 66%), in NAL-treated group 3/5, 60%), in control group (2/6, 33%), but in DHETX+NAL-treated group no one animal survived (Table 2.).

Table 2.

SURVIVAL IN HEMORRHAGIC GROUPS								
Groups:	n	Hypotensive period			Postretransfusive period			% of survival
		60 min	120min	180min	60min	120min	48 hours	
SAL	6	5	5	3	2	2	2	33,33
DHETX	6	6	5	4	4	4	4	66,66
NALOXONE	5	4	4	4	3	3	3	60,00
DHETX+NAL	5	5	3	1	0	0	0	0,00

Results about the activity of naloxone in circulatory shock are contradictory. In the literature are more data about the favourable influence of NAL on the improvement of cardiovascular hemodynamics and survival of state of shock (1, 3, 4, 8, 13) than data which confirm opposite effects (5, 6). In our experiments three of five dogs survived what is much better than results in untreated group.

Respiratory rate in NAL-treated group increased from 60 min more than 4-fold regarding to the baseline value (Fig 4.). It is known that opioids inhibit the peripheral chemoreceptors by the μ opioid receptors, while activity of respiratory center is inhibited by μ and δ receptors (21). Since NAL has a high affinity to μ receptors, it blocks the central and peripheral opoid activity (22). In this way respiratory center is deblockated and acts »without brake» that leads to enormous increasing of RR in NAL-treated dogs, probably potentiated by effects of peripheral hypoxia presented in a shock.

According to effects of NAL on RR noted above, the low increase of RR in DHETX+NAL-treated group is unexpected. We suppose that favorable effects of DHETX on cardiovascular parameters dominate in this group, by which the following effects are achieved: better blood supplying of CNS, more adequately information receiving of central and peripheral chemoreceptors from periphery, as well as substantial deceleration of respiratory center activity.

The hemodynamic component to the protective effect of naloxone may involve a modest positive inotropic effect, and maybe, potentiated the affect of epinephrine at beta-adrenoceptor or beyond (9). The maintenance of blood pressure after retransfusion in NAL-treated dogs sustains a normal tissue perfusion pressure neccessary for normal blood flow. In these conditions it is showed that NAL-treated shock cats exhibited lower circulating amino nitrogen concentrations and plasma cathepsin D and myiocardial depressant factor (MDF) activities than shock cats receiving saline (3). The metabolic component appears to involve the stabilization of lysosomal membranes and the prevention of proteolysis, possibly by nonspecific effects preventing the formation of MDF as well as having direct value in the maintenance of normal circulatory function. These possibly nonspecific actions of naloxone may act in concert with its well-known specific opiate antagonism to protect shock animals by a dual mechanism.

DHETX+NAL treatment had the worst effects on hemodynamic and respiratory stability and survival of hemorrhaged dogs (no one dog survived). We assumed that DHETX excluded the favorable effect of NAL on heart function, while NAL excluded the protective effects of DHETX on peripheral blood vessels.

Conclusion

Under the present experimental conditions, prophylactic administration of DHETX had better effects on preservation of parameters mesaured and survival of dogs (66%) than NAL (60%), while DHETX+NAL treatment had the worst effects (no one dog survived).

Apstrakt

Nalokson (NAL), antagonista endogenih opijatnih receptora i dihidroergotoksin (DHETX), antagonista alfa-adrenergičnih receptora su aplicirani pojedinačno ili istovremeno psima u cilju određivanja njihovih efekata u hemoragijskom šoku. Srednji arterijski pritisak, frekvenca rada srca i respiracije, hematokritska vrijednost i koncentracija proteina u plazmi su mjereni tokom tročasovne posthemoragijske hipotenzije kod 22 psa. Životinje su bile podjeljene u četiri grupe: DHETX-tretirane (n=6), NAL-tretirane (n=5), DHETX+NAL-tretirane (n=5) i NaCl-tretirane (n=6, kontrolna grupa). Aplikacija supstancija je izvršena prije iskrvarenja. Nakon tročasovne posthemoragijske hipotenzije cjelokupna preostala količina iskrvarene krvi je vraćena psima, i životinje su držane još jedan sat u postretransfuzionom periodu. Preživljavanje pasa je praćeno još sljedećih 24 sata. Ustanovljeno je da u ovim eksperimentalnim uslovima profilaktično davanje DHETX-a pokazuje veći zaštitni efekat u očuvanju praćenih parametara i preživljavanja pasa u odnosu na NAL, dok je tretman sa DHETX+NAL imao najgore efekte (ni jedan pas nije preživio).

Ključne riječi: hemoragijski šok, psi, blokada opijatnih receptora, blokada alfa-adrenergičnih receptora

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LAND HOSPITAL-LANDESSPTAL AND VAKUF'S (MUSLIM CHARITABLE) HOSPITALS IN SARAJEVO ON THE END OF XIX CENTURY

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Abstract

The authors presented work of Muslim Charitable Hospital in Sarajevo, which was established in the year of 1866., and the reason why Lands Hospital (Landespital) was also established in the same city. They presented the latter and the first five years of its functioning too. They also presented six tables and nine figures, which undoubtfully proved that Land Hospital was not built for domestic inhabitants, already for medical purposes of occupational troops, and all those who were coming with them. But domestic inhabitants very soon occupied almost all beds in new hospital, because the Hospital was established and allotted and sanitary occasions in Bosnia and Herzegovina were bad. Irrespective of many community hospitals which were not finished and ended, and with the Muslim's Charitable Hospital all problems were not solved, because of its small capacity and arising of necessities of inhabitants

Key words: Vakufs hospital - Sarajevo – Landesspital, Sarajevo – Hospitals

Introduction

The first hospital in Sarajevo city was founded in the year 1866., and it was established from Muslim's Charittable Fund, named Ghazi Husrefbey's Vakuf, and from those times it was called Muslim's Charittable Hospital, Vakufska Bolnica. It was founded by Topal Sherif Osman pasha, who was last turkish governor of Bosnia. Several months later, was founded turkish military hospital, which was functioning till Serbian Agression on Bosnia nowdays, like Out-Patient Department of Military Hospital. In the year 1884. Muslim's Charittable Hospital was restorated and reorganised, and it was gained image of open hospital. The third medical hospital was nowdays Clinical Center of University of Sarajevo It was founded in the year 1892. and it was named Landesspital. From this hospital arosed the Clinical Center of Sarajevo University 1992., normally it was made modern and reestablished by several times.

Muslim's Charittable Hospital

The first consultant of this Hospital was naturalised Hungarian, and latter was Dr. Carlo Bayer from Bohemia from Hradec Kralove. In Turkish

Military Hospital were two of turkish military surgeons Dr. Nouri and Dr. Jamal. The man nowdays can be astonished, but four of domestic students were send to study medicine, two of them in Instambul (Bosniacs) and two of them (Serbs) to Vienna. Bosniacs were finished their medical studies and Serbs were not, they became buissnismen. Two Bosniacs, who qualified, were Dr. Haji Medmed Samii Serbic and Dr. Zarif Skender. Dr. Haji Mehmed Samii Serbic was immidiately went to Tuzla and he worked as a general practicioner there for the rest of his life, but Dr Zarif Skender worked in the Hospital very short time and soon he died from tuberculsosis.

Till the year 1883. was in the whole country five community hospitals, and from 1884. till 1892. number of community hospitals were augmented, and one could find twelwe. Minister Kallay anounced 1892. established regional hospitals. In the sane time this Muslim's Charittable Hospital was converted in the Insane Asylium, with the opening of the new hospital. In the meantime, number of community hospitals were rising permanently, and in year 1901./02. whole Bosnia and Hercegovina had 24 hostpitals.

During of occupation of Bosnia by Austro-Hungarian troops one could find two former hospitals only. Muslim's Charittable Hospital had 40 beds in the very begining, and in this Hospital were treated all patients irrespective of their religion, free of charge. Specially were admitted olicemen, prisoners, whores, and patients with the syphilis. Latter The Chief Doctor, Consulatant (primarius, how he was called) performed first brain operation in this hospital. Actually he made three operations on the brain, in the tretmanet od depressive facture and epilepsy because bone fragemnt was in the brain in all patients. All patients were cured and were without the fits. It happened in the year 1891. only nine years after the first brain operation (Mac Ewen operation of brain apscess) Till year 1878. the Hospital was well established, because almost all patients were from domestic origin From this year all civilians were very hardly accepted in Military Hospital. Latter situation was harder. Because of that and because of larger number of foreing workers who came wih Austro-Hungarian troops because of economic reasons, started asking for acceptance of the new hospital. Normally this situation auggmented expences in the Muslim's Charitable Hospital (the sum was 1.600 crowns), and that was the reason that City Magistrate took over expences from Ghazi Husrefbeys Vakuf. It happened by the decision of Regional

Goverment. In the year 1882, expences for running the Hospital was 37.428 crowns.

Number of patients in Muslim Charittable Hospital were 245 in the year 1882. it was 715 in the year 1888. On the whole for 12 years were 10.981. patients. Results of hospital treatment were good. Up to 90% of all patients leaved the Hospital like cured. Mortalty rate was 5 or 6 per cent, depending of the period.

As necessity for the new hospital aroused, it was established July 1st. 1894. It sterted functioning with 236 beds, and in the year l9o1, it has 325 beds. Minister of the finance of Local Goverment, von Kalay ordered that new hospital have to be very modern and equiped by very sofisticated instruemnts end machines in those times.

Tradition in Sarajevo is to have two hospitals, started very early, one could see that is was started when Muslim'sCharittable Hospitalwas founded. Thast tradition is lasting and nowdays. One is former Landesspital nowdays called Clinical Center of Sarajevo University and second is former Military Hospital, nowdays called State Hospital.

Landesspital

So it started functioning the biggest medical enployment in Bosnia and Herzegovina. It had four departments: Deparment for Surgery and Ophtalmology, Deparment for Medical Diseases, Department for Dermatovenerlogy and Deparment for Gynecology and Obstetrition. Four chiefs of these Departments made Managing Council and they were appointed by the Ministery of the Health. One of four guys was General Manager of the whole hospital, firstly elected and secondly appointed by Ministery of the Health, only for two years. Actually, the members of Managing Council were also, a man who performed autopsyes called prosector, and the Chief of the Pharmacy, but they could not be elected or appointed to be General Manager of the Hospital. The duty of the General Manager was to make a supervision on the Management, coordination of the Deparments, contact with the Goverment, supervision on admistration of the Hospital, and other paper work. He had two asistents for this busines, one for Hospital income and the other for different financial bussines. Everything of it was in limits of budget which was promoted by the Goverment and Ministery of the Health. Hospital pharmacy was in charge of one pharmacologist and kitchen was in charge of one female

cook. Both had special manager who was responsable to the general menager only.

All technical problems, widening of the hospital and inner runing the Hospital was under one technician from Technical Department of Local Goverment. Mutual questions, specially medical, were solving on the conferenecs of all four Chiefs of Department, sometime excluding the Pharmacist and Prosector. Chief of Departmnts were representativs of their Deparments and medically quite independent. Chief of the Surgery was Dr Joseph Preindelsberger, who performed mostly urology. Chief of Medical Department was Dr. Geza Koerbler, and Chief of Dermatovenerology was in this time very famous Dr.Lopolod Gueck, who was author, in the same time, of first medical dictionary of bosnian medical language.Chief of Ginecological and Obstetritional Department was Dr. Otto Weiss.

Dr. Otto Weiss was the first General President of the Colleghium. He was elected by his colleges and appointed by the Ministery of the Health and Goverment in June 1894, Dr. Geza Koebler was General Manager of the Hospital. He was elected and confirmed two times more, in the years of 1898. and 1900. Pharmacy was runned by Max Teich and he was member of Colleghium but he was bot the right to be elected and appointed to be General Manager or President of the Colleghium. It is very interesting that Dr. Joseph Preindelsberger was not permited entering in the new operation theatre Dr. Carlo Bayer, who performed even three operations on the brain in Muslim's Charitable Hospital with the explanation that he was neurologist, not surgeon, but both were general practicioners. With it he stopped developing of the neurorsurgery which was developing in the Sarajevo firstly before in other lands which vere inhabited by Southern Slavs. It was only eleven years after operation of Mac Ewen on the brain abscess. Neurosurgery was developed in Sarajevo after the Second World War. See picture.

In this Hospital was attended everybody irrespective of the religion, although it was very important who belongs to the different nation. This could be seen on the table 1.

Table. 1: Belongings of patients to the different nations

Belongins	**Male**		**Female**	
	1895.	**1900.**	**1895.**	**1900.**
Bosniacs	19,41%	21,07%	4,82%	9,67%
Ortodox (Serbs)	26,63%	25,89%	17,71%	22,83%
Catholic (Croats, Austrians,Hungarians and others)	46,27%	44,65%	67,71%	59,12%
Protestants (Austrians, Hungarians and others)	1,19%	1,19%	3,05%	1,75%
Jews	4,50%	7,16%	6,73%	6,55%

It is visible whose was the Hospital and whome it was built for. Bosniacs at those times was most numerous nation and consisted more than 5o % inhabitants and, after them Otrtodox (Serbs) was about 33 %, Roman Catholic (Croats, Austrians, Hungarians and others) was only 17%.

Table. 2: Results of being in the Hospital

	Males	Females
Cured	91,08 %	91,80%
Not cured	3,46 %	3,34 %
Died	5,46 %	5,33 %

Even to-day may be praised some hospital for these results.

Table 3.: Location of the different patients

From Sarajevo	12.468.	or 56,57%
From Sarajevo area	2.750	12,48%
From some area from Bosnia	6.472	29,4o%
From Austria or Hungaria	293	1,33%
From other lands	49	0,22%

Table 3. should be compared with table 1, and it is visible that inhabitants of Sarajevo, although were the most neumerous in the Hospital, and specially of Bosnia were not so participated in the Hospiatal.

In the yaer 1900, one could in with the cognition about very successfull work in the Landesspital, so it happened that the number of the patients augmented which comes from the other parts of Austro-Hungarian Monarchy. This could be vsisible on the table 4.

Table 4.: The patient comes from

Year	Bosnia	Austria	Croatia	Others
1895.	54,94%	20,10%	21,81%	3,11%
1900.	58,61%	17,76%	20,35%	2,71%

It is, also, evident that domestic inhabitants more and more occupied beds in the Hospital, and it is so confirmed that beds in the some hospital were given by necessities and not by intentions. On the table five one could see the frequency of the patients according to diagnoses. It is on the medical aspect the most interesting thing it could be visible that pathology of the inhabitants was changed, but not very. We could see it nowadays some diagnoses, except chronic infections like tuberculosis and syphilis, and parasites, which are not visible in Western and North Europe

Table 5.: Diagnoses

Venerial diseases	17,52 %
Diseases of the stomach and inestestine	9,29
Diesases of the blood	9,21
Diseases of the sexual organs	8,96%
Disease of the skin	8,19 %
Injuries	6,77 %
Diseases of respiratorry system	6,30 %
Discarges without diganosis	6,24 %
Deseases of eyes	4,47 %
Tuberculosis	4,43 %
Deseases of peripheral nervous sysem	4,33 %
Desesase of urinary system	3,08 %
Deseases of bones	2,83 %
Deseases of circulatory system	2,30 %
Parazites	1,33 %
Deseases of joints	0,51 %
Suidcides	0,33 %
Deseases of musculi	0,08 %

It is very interseting tables 6. and 7. for medicals doctors because from those Tables, one could see number if the operations and number of deliveries. Number of deliveries showed that domicile inhabitants took over the Hospital beds .

Table 6.: Number of operations

Year	surgical	gynecological
1894.	264	29
1895.	458	68
1896.	514	56
1897.	725	81
1898.	885	106
1899.	1024	86
1900.	1232	156
Total	**5102**	**578**

Table 7.: Number of deliveries

Year	Number
1894.	8
1895.	64
1896.	75
1897.	94
1898.	91
1899.	115

Conclusions

1.Muslim's Charittable Hospital and Landesspital in Sarajevo in the first year their existence justified their establishment, and domicile inhabitants took over more and more hospital beds.

2. Landesspital was buildt for austro-hungarian soldiers and those who comes with them. In the meantime this Hospital become General Peoples Hospital treating all the domicile inhabitants, who took over the Hospital

3. It was have to be, because Muslim' s Charitable Hospital was very small and it was not able to treat everybody who needed it or wished it. Because it was te main reason for establishing new hospital

Abstrakt

Zemaljskla bolnica i vakufska bolnica u sarajevu na kraju 19.vijeka

Autori prikazuju rad Vakufske bolnice u Sarajevu, osnovane 1866. godine o kojoj ima malo medicinskih podataka i razloge osnivanja Zemaljske bolnice u Sarajevu, Landesspitala, te njen rad u prvih pet godina od početka njenoga funkcioniranja. Poslije iznošenja tabela, koje to nesumnjivo i dokazuju, autori su došli do zaključka, da Landesspital nije doduše gradjen za potrebe domaćega stanovništva, nego za potrebe austrougarske vojske i svih onih koji su došli zajedno s njom, ali da je domaće stanovništvo ubrzo preuzelo primat u broju zauzetih postelja i zauzelo gotovo sve krevete u toj bolnici. To se je i moralo dogoditi, jer je bolnica gradjena namjenski i zrdavstvene prilike u Bosni i Hercegovini, untoč velikoga broja općinskih bolnica nisu riješene stvaranjem vakufske bolnice zbog njenoga malehnoga kapaciteta i naraslih zdravstvenih potreba stanovništva.

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Addition: Pictures:

Fig. 1. Landesspital when it was started

Fig 2. Muslim's Charitable Hospital when it was started.

Fig. 3.Muslim's Charitable Hospital, Tgreating the burn of the calf.

Fig. 4.Landesspital inside.

Fig. 5. Landesspital. Kitchen.

Fig. 6. Landesspital. Pharmacy

Fig. 7. Landesspital. Distribution of the food

Fig. 8. Muslim's Charittable Hospital. Out - patient room.

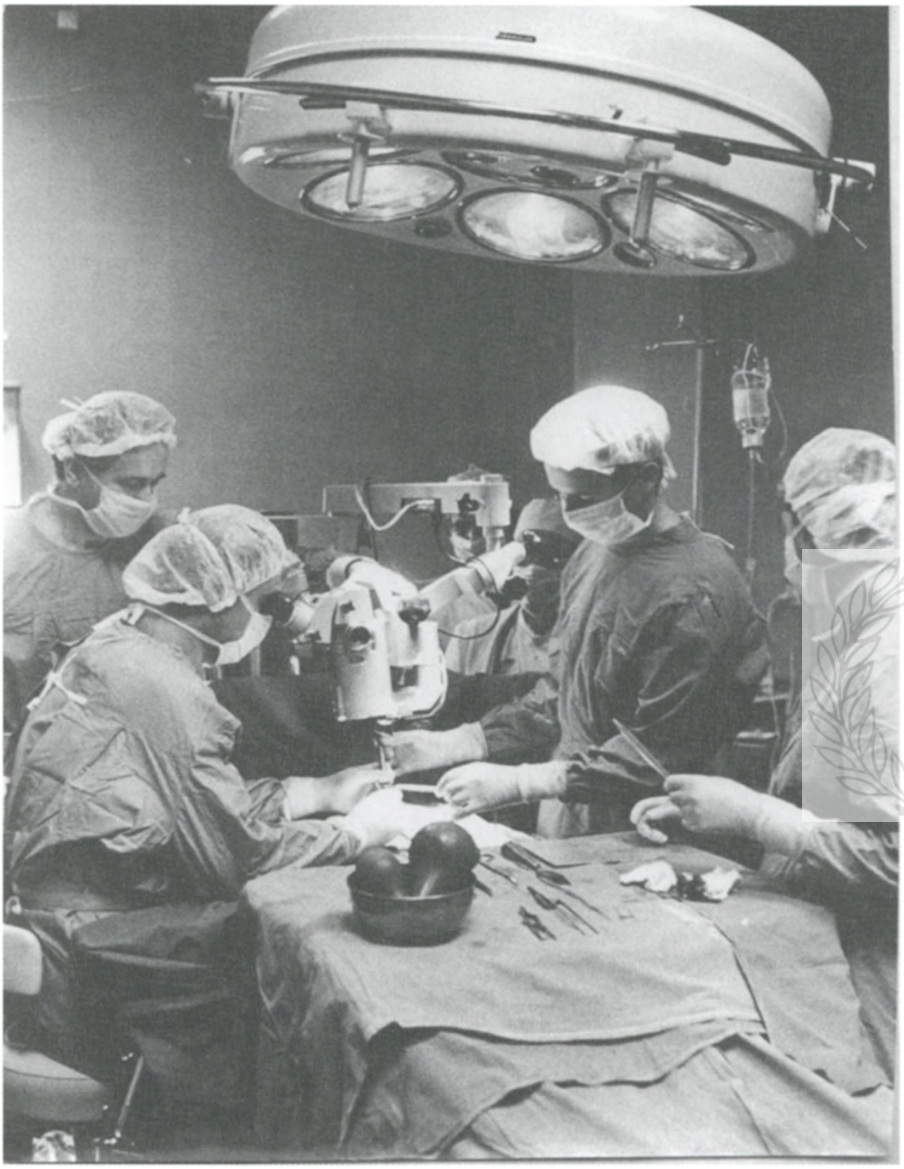

Fig.9. First microneurosurgical operation on peripherial nerve in Sarajevo, made in Neurosurgical Departmentof Cinical Center which was follower of the Landesspital made in 1968, May 29.

THE EFFECTS OF EARLY INSTITUTIONAL REARING ON MENTAL HEALTH OF CHILDREN AGED 8 TO 12 YEARS

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Abstract
The aim of my study was to investigate the consequences of early institutional rearing on the mental health of a sample of children aged 8-12 years. This study was conducted between the 15th May and the 15th June 2003 in two institutions in Bosnia and Herzegovina. These institutions were chosen because they share the same care model. A sample of 30 children completed structured questionnaires, which had been translated from English. These were the Child Behavior Check List-form for parents, TRF- for teachers and YSRF for youngster. I also used my own socio-demographic questionnaire, which measured other characteristics of the sample. The control group was 60 children who attended the elementary schools in Tuzla. Our research confirmed that early deprivation in an institution has a negative effect on behavior in every day's life. The lack of parental authority and family protection, together with suffered personal losses among close relatives, contribute to trauma of institutionalized children and diminish their invulnerability. Institutionalized children lean towards apathy, which can be a sign of emotional stress they are submitted to and point towards significant sources of deprivation in every day's life. Mental health problems are much more represented among children in institutions. Our research confirmed that young children from institutions are a vulnerable group of people and emphasizes the need for equalization of the Bosnian care model to the European standards.
Key words: institutional rearing, mental health

Introduction

In many European countries 3-4% of children below 18 years of age are entrusted to social care services for a certain period of time (1). In Bosnia & Herzegovina the number ranges between 3000 to 3500 children entrusted. There are 1130 living in orphanages, 120 in the institutions for children with special needs, and 2200 in foster homes (2).

While abandoning or death of either parent are most common reasons children are entrusted to the institutions, particular medical and/or social reasons (disease in family, psychosis or mental retardation of parents, negligence or child maltreatment, poverty, and distressed families) are not uncommon.

Physical and mental health of children, living in disrupted conditions, is endangered, and therefore orphanage presents safe environment in which

a child can continue to grow and develop outside the influence of its biological family.

In such cases institutionalization may contribute to psychical recovery and enable the child to develop to its full biological potential. The outcome is affected by the age by which a child has been institutionalized, circumstances under which the child was admitted and the duration of the institutionalization, genetic factors in addition to the quality of accommodation (continuity and sensitivity of care).

Institutional or foster placement are primarily run by professionals in field of social welfare, however due to the fact that this age group is very vulnerable it is critical to have a multidisciplinary approach (2,3).

Physical, cognitive and socioemotional development of a child in an institutions differs from development of a child living in a family (4, 5). Besides the separation (4), institutionalization is characterized by other negative circumstances: the number of care-takers/nurses versus the number of children below the optimal, work shifts of staff and poor stimulation (5), possibility of failure to diagnose serious medical conditions due to staff fluctuation (3). Besides these factors there are risk factors, such as dysfunctional of the biological family, trauma, fatal exposition to substance abuse and inheritance of vulnerabilities that are influential to the level of behavioural morbidity amongst the children without parents (1). Familiarization with the normal psychological development and research on institutionalized children's emotional, social and behavioural problems led many western states to change their public opinion on how to take care of the institutionalized children. Child's characteristics and the age became important parameters while choosing an optimal solution for his or her care taking. The adequate foster family secures continuity in personalized care and presents most accepted care-taking solution for children below the age of 5 (3). Clinical trials show that older children, and adolescents, requiring alternative care due to family circumstances, do not adapt well with the foster family, therefore institutionalization is optimal solution for them (3).

The purpose of this research was to test the impact of early institutionalization on the mental health of children ages 8 to 12.

Place of Study, Subjects And Methods

Place of Study

The research was conducted at two orphanages located in Bosnia and Herzegovina. The subjects live in "orphanage families" numbering 8 to 12 children of different ages and sex in which older children, to an extent, take care of the younger children depicting the model of development of a traditional Bosnian family. One mentor (care-taker) is present for each "orphanage family" during the day, while there is only one mentor for all during the night. The number of care-takers/nurses versus the number of children was close in both institutions and below the optimal at the time of surveying.

Subjects

The Group A was made up of 30 institutionalized children ages 8 to 12. There were an equal number of males and females. Fifteen children were below the age of 3 and the other fifteen were below the age of seven at the time of institutionalization. Fifty-six percent (17) of children had brothers or sisters at the same environment.

The control Group B was made up of 60 children ages 8 to 12 who lived with their biological families. The subjects were randomly selected from five elementary schools in Tuzla.

The sample distribution of both groups according to sex and age is shown in Table 1.

Table 1: The sample distribution of both groups according to sex and age

Gender	Age		Group A		Group B	
	Years	$\bar{x}\pm SD$	N	%	N	%
Female	8-10	8.75 ±0.69	11	36.6	21	35.0
	11-12	10.52 ±0.51	4	13.3	8	13.3
Male	8-10	8.36 ±0.48	5	16.6	11	18.3
	11-12	10.75 ±0.70	10	33.3	20	33.3
Total	8-12	90	30	33.3	60	66.7

Methods

This research is quantitative, transversal and descriptive. The research lasted from 15th of May 2003 to 15th of June 2003. Parents, care-takers, teachers and sampled children were informed about the research project, its goals, and their consent to participate in the research through a letter. Children attending school with special needs were excluded from this research. Parents, teachers and students agreed to be surveyed. The survey was anonymous and they were filled out in the privacy of ones homes. The first author personally monitored surveying in the orphanages.

Survey questionnaires

Socio-demographic survey adopted for either group was used. The survey inquired for basic demographic information, social situation, family and other important life events, such as death of immediate and extended family members, and hard diseases within the family.

Standardized questionnaires were used to determine mental health (6,7,8) – Child Behaviour Check List -for Ages 4-18 (CBCL) (6), Teacher's Report Form for Ages 4 -18 (TRF) (7), Youth Self-Report Form for Ages 11-18 (YSRF) (8).

The CBCL was designed to be filling out by biological parents and parent surrogates (6), and consists of 118 behavioral problems sorted into internal and external problems. Internal problems consist of withdrawn behavior, somatic complaints and anxious/depressed behavior, while external problems consist of delinquency and aggressive behavior. In orphanages mentors (care-takers) filled out the survey regarding the children that were familiar to them for at least 6 months. CBCL and YSRF are compatible questionnaires with minor wording differences due to the subject difference (6,8). Many questions coincide with the questions from the TRF. Even though correlation between the answers may be low, it does not necessary denote unreliable subjects, but different aspects of child functioning (6,7,8).

Teachers, or other adults from school environment who knew the student by at least two months filled out TRF. The advantage of gathering reports on student's behavior from teachers is avoidance of family's dynamic influence (7).

Statistics

Epi Info version 6 was used for survey design, data entry and statistics, while SPSS was used for evaluation of standardized questionnaires. Graphics were done using Microsoft Excel. Descriptive statistics model was used to analyze data (mean ± standard deviation). To test the significance of the difference between the samples, χ^2 test was used. Statistical hypothesis has been tested at the level of significance $\alpha=0.05$; the difference between samples is considered significant only if $p < 0.05$.

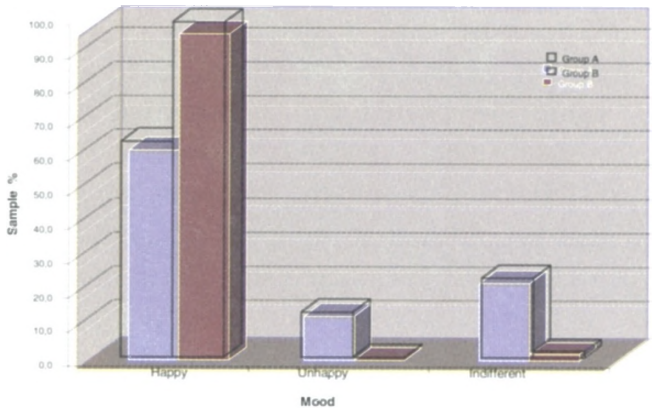
Results

There has been total of 90 surveyed children, 30 (33%) of which live in orphanage (Group A) and 60 (66.7%) of which live with their biological families (Group B). Children who belong to Group A have suffered more family losses due to illness, disabilities and deaths compared with Group B (Table 2).

Table 2: Family losses due to disease, disability, and death of immediate and extended family members

	Disease and disability				Death in family				Total	
	Close Relatives		Extended family		Close relatives		Extended family			
	n	%	n	%	n	%	n	%	n	%
Group A (N=30)	6	20.0	5	16.7	6	29.0	5	16.7	22	73.3
Group B (N=60)	8	13.3	12	20.0	3	5.0	15	25.0	38	63.3
Statistic Significance	p<0.05		p>0.05		p<0.05		p>0.05		p>0.05	

Children from Group A have had significantly more ill and disabled ones among their close relatives ($p < 0.05$) as well as more death cases ($p<0.05$) compared to children from Group B which have experienced more illnesses, disabilities and death cases that had happened to their extended family. The subject's dominating mood is charted (Picture 1).



Picture 1: Subjects' mood

At the moment of the examinations, there were 19 (63.3%) children in the Group A that felt well, 7 (23.3%) indifferent children and 4 (13.3%) unhappy children. In the B Group, 59 (98.3%) children felt happy, whereas only 1 (1.7%) child was indifferent. A significant difference was found between these two examined groups ($p<0.05$).

CBCL questionnaire has been filled out by 30 mentors (care-takers) (Group A) and by 60 parents (Group B). The questionnaire measured internal, external and total problems that children experienced (Table 3).

Table 3: Behavioral problems of children based on care-takers and parental report

Behavioral problems (CBCL problem scale)	**T score ($\bar{X}$ ±SD)**		**Significance**
	Care-Takers (Group A, N=30)	**Parents (Group B, N=60)**	
Internal	52.77±10.01	45.52±8.97	$p<0.05$
Withdrawn	57.03±8.56	51.20±3.11	$p<0.05$
Somatic complaints	53.77±5.90	53.30±6.01	$p>0.05$
Anxious/Depressed	55.80±6.23	50.45±4.33	$p<0.05$
External	52.33±10.36	44.35±8.89	$p<0.05$
Aggressive behavior	54.93±7.76	50.83±7.33	$p<0.05$
Delinquent behavior	60.03±8.94	52.28±5.50	$p<0.05$
Total	54.83±9.92	47.22±9.75	$p<0.05$

A simple variants analysis has shown statistically significant difference ($p<0.05$) between Groups A and B at all the problems scales measured by CBCL, except at the scale of somatic symptoms.

The presence of the problems from the internal, external and total group of problems has been shown in Table 4, and it is based on teachers' report.

Table 4 : Behavioral problems of children based on teachers' report

Behavioral problems (TRF problem scale)	T score ($\bar{X}$ ±SD)		Significance
	Group A (N=30)	Group B(N=60)	
Internal	58.21±12.12	51.69±9.92	p<0.05
Withdrawn	58.96±10.42	54.61±7.97	p>0.05
Somatic complaints	53.86±6.63	52.74±6.19	p>0.05
Anxious/depressed	60.64±9.71	55.35±6.10	p<0.05
External	57.46±9.48	54.13±10.11	p>0.05
Aggressive behavior	57.82±7.41	56.28±7.99	p>0.05
Delinquent behavior	60.18±9.55	56.51±7.72	p>0.05
Total	61.75±12.15	56.69±10.30	p<0.05

Report on teachers' questioner shows that there are significantly more anxious/ depressed, internal and total problems in Group A. Even though withdrawn, somatic symptoms, delinquency and aggressive behavior as well as external problems have been somewhat more expressed in Group A, there has not been any statistically significant difference (p>0.05) compared to Group B.

YSRF has been filled out by 10 children from Group A and 13 children from Group B. Internal, external and total problems are shown in Table 5, and they are based on self assessment of the children.

Table 5: Behavioral problems of children based on their own assessment

Behavioral problems (YSRF problem scale)	T score ($\bar{X}$ ±SD)		Significance
	Group A (N=30)	Group B (N=60)	
Internal	54.60±7.88	43.00±8.62	p<0.05
Withdrawn	54.20±7.37	50.77±2.49	p>0.05
Somatic complaints	54.70±5.73	52.69±5.37	p>0.05
Anxious/depressed	58.50±7,41	51.31±2.06	p<0.05
External	51.60±8.35	35.85±10.39	p<0.05
Aggressive behavior	54.40±6.67	50.15±0.55	p<0.05
Delinquent behavior	54.80±6.58	51.31±3.50	p>0.05
Total	57.70±7.12	40.08±8.15	p<0.05

The mean T scores for internal problems measured by YSRF is higher for Group A. Statistically significant difference ($p<0.05$), accordingly, has been found with the anxious/ depressed and internal problems. Even though, delinquent behavior has been expressed more within the Group A, statistically significant difference ($p>0.05$) has not been found between the examined groups in terms of this problem. Aggressive behavior, external and total problems have been more fully expressed with the Group A's examinees and this difference has been statistically significant ($p<0.05$)

Discussion

The sample of 30 children, either sex, ages 8 through 12; living in two orphanages in Bosnia & Herzegovina were surveyed in order to test the effects of early institutionalization on mental health. Group A had significantly more diseased and disabled immediate family members, in addition to higher death cases involving immediate family. Group B had higher numbers of diseased and disabled extended family members, and higher death cases involving extended family members. Besides high family loses children from Group A have less information about or contact with their extended family, as compared to children from Group B, adding more traumas to children from Group A.

Similar studies researched positive and negative effects of institutionalization, but only in children who were released from the institution after a period of time (3). Even though there was heterogeneity a big percentage showed very serious psychosocial unconformity. It has been observed that the overall outcome depends on the length of institutionalization, and that males are more vulnerable compared to females. Males have shown to develop more deficiency even after a short period of time. Primary problem for male adolescents who once lived in an orphanage is the law, while primary problem for female adolescents is non-marital teenage pregnancy (3). Some residents may develop mental problems during adolescence or later in life, and may require psychiatric treatment (3).

63% from Group A were happy, and 36.7% were unhappy and indifferent. The numbers are not consistent with basic characteristics of school-age children regarding positive attitude towards future (9). Children in Group A are leaning towards apathy, which can be emotional sign of stress they are exposed to. Institutionalization and separation of school-age children from their relatives creates intensive and long-term reactions that can be signalized through depression, emotional frigidness, or anger (10).

UNICEF's qualitative study (2) shows that every child without parental guidance carries a heavy burden of its own past and faces stigmatism and limitations in every aspect of life. This indicates a need for psycho pedagogical support for this group of children.

According to the statistics from CBCL, there is a significant difference between Groups A and B on the scale of withdrawn, anxious/depressed behavior, aggressive behavior, delinquency, internal, external and total problems. The only problem without significant difference was somatic complaints. Wolkind and Rutter (11) found similar results by observing 2% of children ages 11-12 who spent some time in institutions from the age of 5.

The overall range of psychiatric dysfunctions was high. Gender was a significant factor; males had more behavioral dysfunctions; mostly conductive. This research clearly showed asperity in differencing between effects of institutionalization from others, such as reasons why was the person institutionalized and what happened after the person was released. Notable difference between the control group and institutionalized group was the quality of family life, respectively family disharmony.
The results of this research are consistent with other studies conducted in large orphanages which showed 50-60% of dysfunctions amongst the children according to care-takers and teachers (3).

CBCL was used by Verhulst and associates in one large study in Holland (12,13). The sample had 2148 internationally adopted children ages 10-15, who spent at least few days up to 10 years in orphanages. The adopter parents reported more behavioural problems in comparison to the control group, even though the difference was not significant due to lack of males ages 12-15 (23% males in comparison to 100% males in controlled group). Their sample shows that children institutionalized after the age of two were more problematic then others (12,13). Thanks to the CBCL, Verhulst and associates (12,13) noticed psychiatric dysfunctions prevalence rate at 28%; which was higher in comparison to the general population.

Problems relating mental health are considerably higher present in institutionalized children alarmingly indicating a need for psycho-pedagogical support.

Group A has considerably more anxious/depressed, internal and total problems according to teachers' assessment. Goldfarbov's study (14) had

similar results showing that early institutionalization has different long-term effects, such as anxiousness, scariness, lack of sentiment, and acceptance that will remain evident even after the child was adopted.

There is a compatibility between care-takers' and teachers' assessment relating anxious/depressed behaviour, internal and total problems. Delinquent and aggressive behaviour is not reported by teachers, unlike care-takers do; meaning delinquent and aggressive behaviour is not significantly high at school.

However, both groups, according to their self-perception, statistically differed in opinions about problems of anxious/depressed behavior, aggressive behavior, internal, external and total problems. These results can be explained by the high-risk environment surrounding children throughout their development process. Tizard and Hodges (15) noticed that is almost impossible to create a satisfactory accommodation for small children. A well organized institution secures a good amount of stimulation and can improve cognitive development, but deficit in social development still remains.

All three surveys showed similar results on anxious/depressed behavior, aggressive behavior, internal problems, and total problems. Much higher aggressive behavior was reported by youth and care-takers, while teachers are not considerably aware of this behavior at schools. This indicates aggressive behavior (conductive deformity) of children within institutions due to lack of adults supervising and individual attention. Unless aggressive behavior is suppressed at an early age, it may contribute to delinquent behavior later on (16). According to the literature (16), if problematic behavior becomes common in different aspects (at school, home, playground), there is a bigger chance that it will continue in future.

Poor psychological adjustment found in one third of those who were institutionalized for a certain period of time in the age of 0-7 years, could also be found in the study of Mapstone (17) who followed a sample of 340 children out of the large sample of 15,000 from the British National Study of Child Development. One third of all of those surveyed functioned three times more poorly than the general population. This difference increased significantly until the age of 11. The National Study of Child Development (18) showed that the children who have been adopted from an institution showed little difference to their control group living with biological parents in the age group from 0-7 years, while their adjustment seemed to have worsened until the age of 11. This is taking into account their improved living situation. Maughan and Pickles (19)

continued to follow this group into their adolescence and later. Significant worsening between the age 7 and 11 does not seem to continue beyond this age. The most significant disturbance present in adopted children appeared at the age 11 and vanished soon afterwards. This period has been portrayed as a period of great vulnerability in connection with identity issues (9).

Results of this study show that institutionalized children, in many areas, are functioning worse than children from the general population, which is in accordance with other researches by Tizard and Hodges (15). Their research indicates that institutionalzied children ages 8 behave differently at school than children raised in a family enviroment. Despite of their results Tizard and Hodges (15) note that it is early to tell any long-term effects in children ages 8 due to their early childhood experience.

Conclusion

The lack of the parental care at an early age has a negative impact on the every day behaviour of institutionalized children. Loss of a close relative adds to even higher degree of trauma. The children without parental care represent specially vulnerable, sensitive and risky group that demands multidisciplinary research and intervention. They lean more towards apathy that can represent emotional stress indicator to whom they were exposed to and point to substantial sources of deprivation in every day life. Their depression, expressed indifference or extensive anger can be reactions to institutionalization and separation from relatives. The stress level with older adolescents can be increased even with a fact that patrons of state residential institutions remain a burden to the society until age of maturity (18) when they must leave the highly protected and protective environment and face the realities of independent life.

The results from this research should help better the quality of health care for children raised in institutions, taking over necessary measures for overcoming of recognized problems and prevention of behaviour disorders in puberty and adolescent age.

Apstrakt
EFEKTI RANOG INSTITUCIONALNOG ODGAJANJA NA MENTALNO ZDRAVLJE DJECE UZRASTA OD 8 DO 12 GODINA

Cilj studije bio je utvrditi posljedice ranog institucionalnog odgajanja na mentalno zdravlje djece. Ispitano je 30-ero djece uzrasta od 8 do 12 godina. Istraživanje je sprovedeno u periodu od 15. maja do 15. juna 2003. u dvije institucije u Bosni i Hercegovini. Obje institucije su organizovane na istom principu dom-porodica. Iz studije su isključena djeca koja pohadjaju školu za djecu sa specijalnim potrebama. Ispitivani uzorak od 30–ero djece ispunjavao je standardizirane upitnike koji su prevedni sa engleskog jezika: Upitnik za procjenu dječijeg ponašanja za uzrast od 4 do 18 godina - forma za roditelje (Child Behavior Check List - CBCL), Upitnik za procjenu dječijeg ponašanja za uzrast od 4 do18 godina - forma za učitelje i nastavnike (Teachers' Report Form -TRF) i Upitnik za mlade za uzrast od 11-18 godina (Youth Self Report Form - YSRF). U istraživanju je korišten i vlastiti sociodemografski upitnik koji je mjerio druge karakteristike uzorka. Kontrolnu grupu sačinjavalo je 60-oro djece istog uzrasta iz nekoliko osnovnih škola u Tuzli. Istraživanjem je potvrdjeno da rano lišavanje u instituciji negativno utiče na ponašanje osobe u svakodnevnom životu. Nedostatak roditeljskog autoriteta i zaštite i porodične povezanosti, uz pretrpljene lične gubitke medju bliskim srodnicima, doprinose traumatizaciji institucionalizovane djece i smanjenju njihove otpornosti. Institucionalizovana djeca naginju apatiji koja može predstavljati emocionalni pokazatelj stresa kojem su djeca izložena i ukazivati na znatne izvore deprivacije u svakodnevnom životu. Problemi iz oblasti mentalnog zdravlja su značajnije prisutni medju institucionalizovanom djecom. Sve navedeno ukazuje na vulnerabilnost navedene populacije i potrebu približavanja bosanskog modela njihovog zbrinjavanja evropskim standardima.

Ključne riječi : institucionalno odgajanje, mentalno zdravlje

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ROKITANSKY SYNDROME

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Abstract: Mayer-Rokitansky sindrome belongs to the group of heterogenous disorders of female urogenital organs. The main component of this syndrome is vaginal aplasia or vaginal agenesis followed by rudimented uterus. For this occasion we have chosen this syndrome between a large group of malformations of women's urogenital tract considering that this year whole world is celebrating two hundred years since the birth of Carl Freiherr Von Rokitansky. We are showing this syndrome and following modificiated David's operation of neovagina formation

Key words: Mayer-Rokitansky syndrome, vaginal aplasia, neovagina

ROKITANSKY, CARL, FREIHERR VON the founder of the Vienna school of pathological anatomy, pathologist, philosopher and politician, born on the 19th of February 1804 at Koniggratz in Bohemia. He studied medicine at Prague and at Vienna, graduating at the latter place in 1828. Soon afterwards he became the assistant to Johann Wagner, the professor of pathological anatomy, and succeeded him in 1834 as prosector, being at the same time made extraordinary professor, ten years later (1844) he reached the rank of full professor. To his duties as a teacher he added in 1847 the onerous office of medico-legal anatomist to the city, and from 1863 he filled an influential office in the ministry of education and public worship, wherein he had to advise on all routine matters of medical teaching, including patronage. A seat in the upper house of the Reichsrath rewarded his public labours in 1867, and on his retirement from all his offices in 1874 he was made a commander of the Order of Leopold. He joined the Imperial Academy of Sciences as a member in 1848, and became its president in 1869. He was president also of the medical society of the Austrian capital and an honorary member of many foreign societies. On his retirement at the age of seventy his colleagues celebrated the occasion by a function in the aula of the university, where his bust was unveiled.

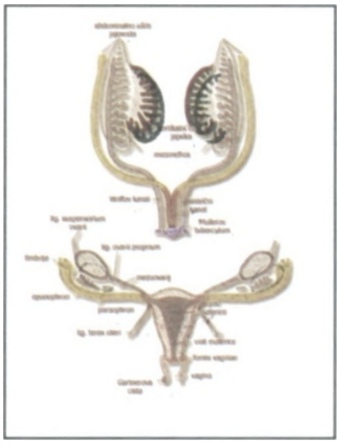
Malformations of women's urogenital tract are quite common with incidence of 1-3%. These malformations are compatible with life but consequences of them are usually infertility or primary sterility.

Between a large group of these malformations we have chosen this syndrome considering that whole world this year is celebrating two hundred years since birth (1804 AD) of Carl Freiherr Von Rokitansky

Rokitansky syndrome or Mayer-Rokitansky syndrome (MRS) is quite rare abnormality of female urogenital tract with estimated incidence of 1:4,000-5,000 live female births. Basically it is characterised by normal external genitalia , an absent vagina, absent or rudimentary uterus, and normal fallopian tubes and ovaries. Although it etiology is not still clear there is no doubt that it is a result of some embryological defect, probably the disturbances in fusion of the Wolffian and Müllerian ducts. Embryologists define the MRS as a spectrum of Müllerian anomalies, including vaginal agenesis with or without renal anomalies, in genotypic and phenotypic female subjects with normal endocrine status.

Main characteristics of syndrome are:

- vaginal aplasia
- uterine aplasia or rudimented uterus
- amenorrhoea
- Morphological and functional development of ovaries with normal ovarian cycles and normal blood levels of estrogen and progesterone which means normal secondary sex characteristics.

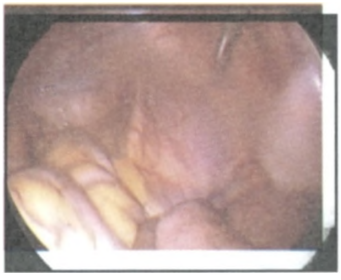


This syndrome was first described by Mayer in 1829. He described partial and complete duplications of vagina in 4 stillborns associated with multiple skeletal, cardiac, facial and urological abnormalities. Rokitansky in 1838 described 19 adult autopsy cases of uterovaginal agenesis including 3 cases in which unilateral renal agenesis was noted. In 1910 Küstner described several cases with similar genital anatomy and observed that skeletal and renal anomalies were common. It is now generally accepted to name this syndrome as Mayer-Rokitansky syndrome, although there are still lots of authors who include the other names in it.

The addition of names of other authors who have contributed to knowledge and the range of accompanying anomalies to this eponymous title is excessively cumbersome and to be discouraged.

Embryologically, normal development of Müllerian duct depends on previous normal development of Wolffian duct. A defect of the Wolffian duct foreshadows a defect at similar level in the Müllerian duct. However, although the Wolffian duct may be normal, the Müllerian duct that develops later may be intrinsically defective. It may be lacking totally, or may arrest at some point of its intrinsic development, migration, fusion or canalisation, therefore resulting in a wide spectrum of reproductive tract anomalies including imperforate hymen, vaginal septa, vaginal atresia, or complete Müllerian aplasia.

The Müllerian duct (MD, ductus taramesonephricus) develops independent of the coelomic epithellium above the mesonephros. This part of the duct gives rise to the infundibulum of the uterine tube with its fimbriated ostium abdominale. The part of the duct which lies along the mesonephros as far as its caudal pole makes a contribution to the ampula and less often the isthmus. In the area of mesonephros the MD fuses with the Wolffian duct (WD, ductus mesonephricus). The WD gives rise to ampula and the isthmus. Below the caudal pole of the mesonephros, as well as beyond the attachment point of the inguinal ligament of the mesonephros, the later round ligament of the uterus, the MD develops as an outgrowth of the WD and no longer as an independent structure. The Mayer-Rokitansky syndrome is, in its formal genesis, a non-fusion of the MD with WD. This explains the fact that in classic cases of MRS, the Fallopian tube with a very small part of the cornu uteri extends only as far as the connection with the round ligament of uterus.



The urologic components of this syndrome that are eventually present belong to disturbances of Wolffian structures, namely because of complete lack of a Wolffian duct, or because of inhibition, debilitation or duplication of the ureteral bud. Therefore we can find a broad spectrum

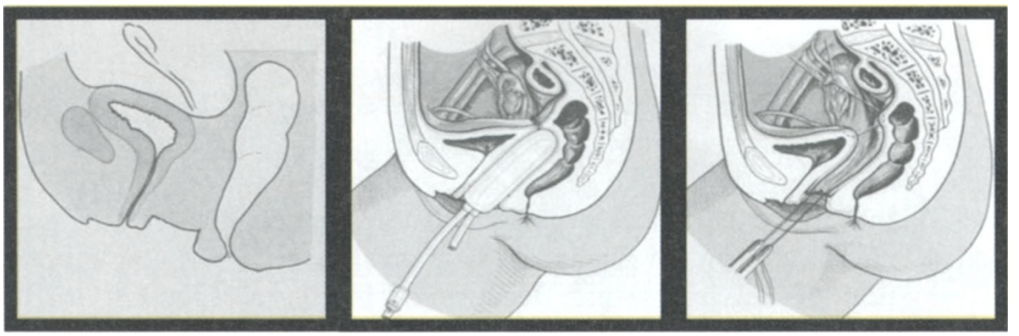
of symmetric and asymmetric urological anomalies such as absent kidney, pelvic kidney, absent or duplicated ureters, blocks and stenoses in urinary system, vesicoureteral reflux etc. On this picture we have showing a case of pelvic kidney that we found during explorative laparoscopy.

This syndrome does not appear to be genetic in origin, and possibility of genetic transmission seems unlikely. There are some hypotheses about the etiology of this syndrome based on potential deficiency of gestagen and/or estrogen receptors, mainly in explaining various forms of rudimentary vagina.

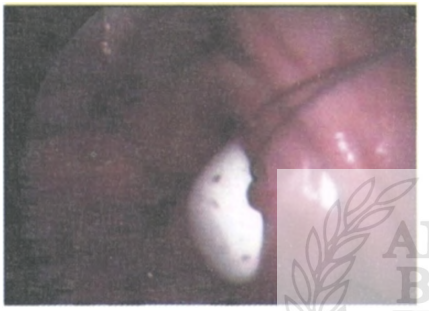
Syndrome is usually diagnosed during puberty when it is showed as amenorrhoea or hymenal atresia. After the cognition of vaginal and uterine aplasia, issues about further possibilities of sexual life and fertility give rise, mainly in women with stabile relationships, especially if married.
For this occasion we are showing modification version of David's neovagina operation. Due to forty-five years of clinical and surgical experience during which it has been diagnosed thirty seven of the cases of this syndrome and operated twenty three of them. We have chosen David-s neovaginal surgical procedure using pelvic peritoneum as a epithelial coverage for tunnelisated neovaginal canal because this operation in our experience was the most successful one.

Before the operation our technicians create a plastic mold of typical phallic dimensions that is going to be inserted in future vagina. The mold is properly sterilizations, and prepared for the operation. The patient undergone general anesthesia and in dorsal litothomy position urinary permanent (Foley) catether was inserted . At the same time the rest of the surgery team performed the standard procedure for laparoscopic surgery. Under laparoscopic guidance we observed rudimentary uterus, and two healthy, and functionally active ovaries with signs of ovulation on the right ovary.

Carefully we place the large bore 18-gauge needle trough the urethrovesicorectal space till the reached. Following the needle we peritoneum that was visualised laparoscopically was dissect the space



bilaterally, taking permanent care to avoid injuries of adjacent organs.



The peritoneum under the rudiment of uterus is opened, clamped and fixated with four resorbable surgical sutures. The canal of neovagina is dilatated with at first one and later by two fingers, and after all it was tunelisated by mold. The sutures are clamped and pulled through the neovaginal canal by surgical instruments. The mold is inserted into neovagina by traction of the peritoneum and by pressure to mold itself. The peritoneum above the mold and under the uterine rudiment is obliterated by resorbable surgical sutures, resulting in closure of peritoneal cavity. The peritoneum pulled trough neovaginal canal is fixated to vestibular mucosa of initial incision. The mold is fixated to vaginal vestibule, and left *in situ* for 10 days. Postoperative course was normal. The patient is dismissed at 15 postoperative day and advice to insert and pull out the mold several times daily. She is encouraged to start her sex life as soon as she finds it acceptable.

Patient returns three months later on a control visit. Neovagina is usually without stenoses. She usually describes her initial sexual experiences as satisfactory. The troubles that appeared were connected with her infertility. A few years ago that was big problem because there were no IVF facilities but now this is not the case. There are a few possibilities as IVF and surrogate mothers etc.

Discussion

The diagnosis of MRS can be made summarising the results of physical examination, and of different other diagnostic procedures.

Each patient that experiences primary amenorrhoea connected with some kind of vaginal abnormality should do hormonal analyses and sonographic examination of pelvic organs. Because of high incidence of urinary abnormalities associated with each patient should do urologic examination, expecially IVP and cystoscopy. If there are possibilities chromosome evaluation and buccal smear should be obtained, to exclude rare syndrome of testicular feminisation.

The high-resolution transabdominal real-time ultrasonography provides additional possibility to visualise precise details of pelvic anatomy, including visualisation of Müllerian structures, kidneys, bladder, septa in internal genital organs, ovarian cysts etc. Ultrasound is expecially useful to visualise distended obstructed segments of the genital or urinary tract. For the diagnosis of MRC clinical experience showed that trans-abdominal sonography rarely provides adequate images that could be of practical use. The trans-rectal ultrasonography provides much better images that correspond perfectly with the real anatomic situation. Today it is used not only as a diagnostic procedure, but also as a part of intraoperative equipment that is helping the surgeon to have a better visualisation of surgical route.

Magnetic resonance imaging is now gaining wide acceptance in imaging congenital abnormalities of genital tract. It is especially useful in paediatric gynaecology for evaluation of paediatric uterovaginal abnormalities, but it is still not widely accessible, nor included in routine preoperative evaluations.

From the time of Mayer and Rokitansky till today the therapy of MRS is evolving, and changing its route. There is a wide spectrum of different surgical and non-surgical methods to solve this problem, and none of it yet did not show itself as perfect.

Non-surgical methods as Frank-s mold therapy, "Interfemoral intercourse" or bicycle seat stool-therapy could be applied to women with high self-confidence who have stabile emotional relations with their partners and strong feelings of self-acceptance, or do not want any surgical correction

The rate of such patients is to our experience not too high. The results of these "pressure" methods are doubtful.

Surgical procedures applied to MRS are different. All of them have the same goal- to create a new vagina in urethroretrovesical space, on its physiological site. All of them have the same difficulties too: how to prevent closure and stenoses of this artificial vagina that is lacking in its original musculature, tonus, inervation and vascularisation, how to ensure adequate lubrication for the intercourse and to prevent dyspareunia.

The aim of vaginoplasty should be creation without excessive morbidity of a neovagina that will be satisfying in appearance, function and feeling. The multitude of methods described in literature indicates the fact that an ideal approach has not yet been found. We conclude that beside non-surgical, the peritoneum pull-through technique, and perhaps skin grafts are the methods of choice. In cases where immediate vaginal reconstruction after oncological surgery is indicated, myocutaneous flaps are preferred. Only in cases in which other methods have failed should recto-sigmoid transplantation be considered.

After the successful neovaginal formation there is still a question of female infertility that is the reason of many marital crushes. Such issues can be solved by IVF and ET procedures with gestational carriers. The specific medical and legal issues involved in facilitating genetic offspring in these instances must be considered. These include the initial matching of the genetic parents with the gestational carrier, cycle synchronisation for in vitro fertilization and embryo transfer, anatomic difficulties of oocyte retrieval, birth certificate documentation, and current legal status of gestational carrier.

Abstrakt:

Mayer-Rokitansky sindrom pripada grupi heterogenih poremećaja ženskog urogenitalnog trakta. Glavna komponentna sindroma je vaginalna aplazija ili ageneza koju prati postojanje rudimentirane materice. Za ovu priliku mi smo odabrali ovaj sindrom iz velike grupe malformacija urogenitalno trakta imajući u vidu da ove godine cijeli svijet slavi dvije stotine godina od rođenja Carl Freiherr Von Rokitansky-a. Ovdje ćemo prikazati ovaj sindrom te modifikovanu Davidovu operaciju formiranja neovagine.

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PROGNOSTIC FACTORS IN NON-SMALL CELL LUNG CARCINOMA

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Summary

In recent years, a group of new prognostic factors have been added to the list of well-known clinical prognostic factors of non-small cell lung cancer (NSCLC). Among these are: mutation in K-*ras* oncogene, abnormal p53 proteins as result mutations on p53 tumor suppressor gene, the presence of N-CAM expression as measured by Mab immunostaining, elevated serum levels of neuron specific enolase (NSE) etc.

Today in B&H, these biomarkers not yet fine their place in every day clinical praxis. That is reason why we cannot speak about their prognostic significance on our clinical material.

Our positive prognostic factors that are significant for the treatment of patients with NSCLC are:

Stage I – IIIa, performance status <2 (ECOG), and age <60 – for resectable NSCLC and Stage IIIb, performance status <2 (ECOG), age <70 and loss body mass up to 5.5kg – for local advance NSCLC. Elevate of serum level of LDH and leucocytosis in this stage of NSCLC is result: extent of disease, metastatic disease and bad final issue.

Key words: **NSCLC (Non-small Cell Lung Cancer), performans status, stage, LDH, leucocytosis.**

Non-Small Cell Lung Carcinoma (NSCLC) today, mainly enclose the non-small cell carcinoma, adenocarcinoma and carcinoma of large cells. Unfortunately, in majority of patients, in the moment of making of diagnosis, disease is in the stage that is loss for surgical treatment. In spite of developing the new chemotherapy agents and strategies of the multimode treatments, the survival rate in the several last decades stay unchanged and very poor. In the big series of unselected patients, the five years survival is between 4 and 7%, regardless of which kind the treatment was made[1, 2]. Between the patients from the Clinic of Lung Diseases and TB "Podhrastovi" the five years survival was 2.1%. A relatively small group patient has been diagnosed in the stage of surgically acceptable disease. But and in the cases of patients who can consider as potentially cured, after radical surgical treatment, only one third of them will be on live after 5 years from surgery. On the other side, even at the patients which tumors similar like surgically respectable, majority of them already has disseminated metastases. The important differentiations were evidence between tumors with and without squamous differentiation[3], but and within every

subgroup of NSCLC there is significant variability in the view of clinical behave[4].

In the treatment of NSCLC, there would be very important identification of reliable predictors of survival and effects on chemotherapy. It would be make easier and better identification of patients who could have benefit from additional treatment with chemotherapy. On the other side it could help knowledge for omission chemotherapy at the patients who have minimally chances for benefit.

In generally, prognostic factors of NSCLC could be divided in prognostic factors before the treatment and prognostic factors that are in connection with therapy of locally advanced NSCLC.

The problem that we will elaborate here are pretreatment prognostic factors.
They could be classified in four great groups:
Pretreatment clinical prognostic factors for NSCLC,
Standard biological factors,
Pathological factors and
Molecular - biological factors.

Today, in routinely work in Bosnia and Herzegovina, in estimating of prognosis of NSCLC we mainly using clinical factors and in limited number of cases biological and pathological factors. In this article during the evaluation of each factor we will present and our own results.

Patients and methods

Our own experiences are based on elaboration of 67 patients with NSCLC, diagnosed and treated on Clinic of Lung Diseases and TB "Podhrastovi", and less number of them on Clinic for Thoracic Surgery and Institute for Oncology of Clinical Center University of Sarajevo. Histology examination was made on Institute for Pathology of Medical Faculty University of Sarajevo.

In estimated group, by sex, there were 50 male and 17 female patients (index = 3:1), age 58.4±12.6 (range 26 – 74).

By histology type in 48 cases there were small-cell carcinoma, 18 cases with adenocarcinoma and 1 case was carcinoma of large cells.

By stage of disease there was:

- Loco-regional disease	(St. I – IIIa)	10 pts = 15%
- Locally advanced disease	(St. IIIb)	43 pts = 64%
- Metastatic disease	(St. IV)	14 pts = 21%

Results

Clinical pretreatment prognostic factors for NSCLC

The most important prognostic factors are showed on table 1.

Table 1: Prognostic factors for NSCLC

TNM stage
Performance status
Sex
Age
Loss on weight
Increase of level of LDH (Lactate dehydrogenises)
Histology type

TNM stages – Expansion of Disease

TNM stages giving the answer on question: "Where is disease in body, on which places?" Today, there is too much polemics about prognosis of NSCLC. But all of them are agree about fact that earlier stages of NSCLC providing a longer survival and better prognosis.

This fact corroborates all studies worked on this theme up to now. On the basis a more studies, the Britons[5] were worked a table of 5-year survival patients with NSCLC, by stages of this disease, and which is very similar with some from the other develop countries (table 2).

Performance status (PS)

Together with the stage of expansion of tumor, performance status is very important prognostic factor. The poor PS providing a high risk for death and without progression of disease. In study of Movsas et all[6] the patients with low PS (Karnofski index = 50-70%) were treated with chemo-radio therapy and they had the lowest mean survival (7.8 months) and the lowest a quality time of survival (6.7 months).

Table 2: Staging by groups and prognosis of NSCLC in Great Britain and patients of Clinic for Lung Diseases and TB – 2000.

Stages	TNM subgroup	5-year survival in %	1 year %	2 year %	3 year %
	Carcinoma In situ				
IA	T1 N0 M0	61			
IB	T2 N0 M0	38			
IIA	T1 N1 M0	34			
IIB	T2 N1 M0	24	27	90	70	60
	T3 N0 M0	22			
IIIA	T3 N1 M0	9			
	T1 N2 M0	13			
	T2 N2 M0	13			
	T3 N2 M0				
IIIB	T4 N0 M0				
	T4 N1 M0	7			
	T4 N2 M0				
	T1 N3 M0		5	67.4	30.3	11.6
	T2 N3 M0				
	T3 N3 M0	3			
	T4 N3 M0				
IV	anyT & any N M1	1	7.14	-	-	

The PS has the goal to give the answer on question how disease influencing on vital daily activity of patients, with the aim of definition of real treatment and prognosis. There are two ways of definition of PS (Karnofski index and ECOG table of PS which is presented in table 3).

Table 3: ECOG Performance status

Grade	
0	Full activity, without restrictions (as before disease)
1	Restricted fisical activity, but able for ambulatory treatment, patient is able for easier work (easy home work, work in office and similary)
2	Ambulatory patient which is able to take care about himself , but he/she is not able for any work activity. He/she spending more than 50% time out of bed.
3	Patient is able only for restricted care about himself. He/she spending in chair or in bed more than 50% time per day.
4	Fully inactivity. He/she is not able to take care about himself/herself. Patient is fully band for the bed or chair.
5	Death

Fig 1: Relation of overall survival and PS for st. I-IIIa
PS on patients Clinic of Lung Diseases and TB

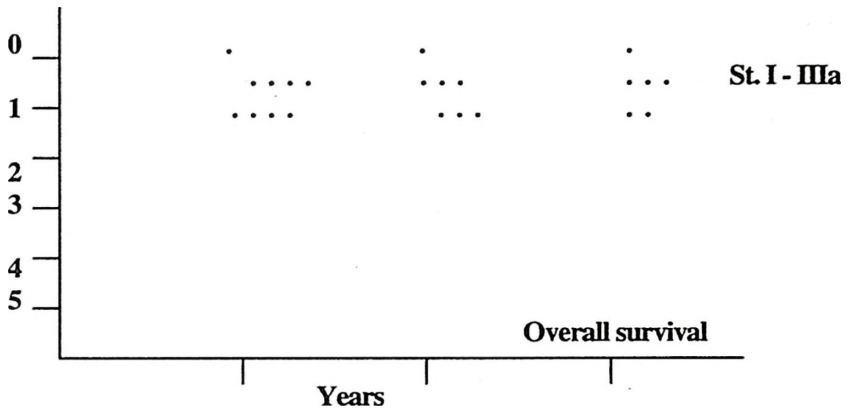
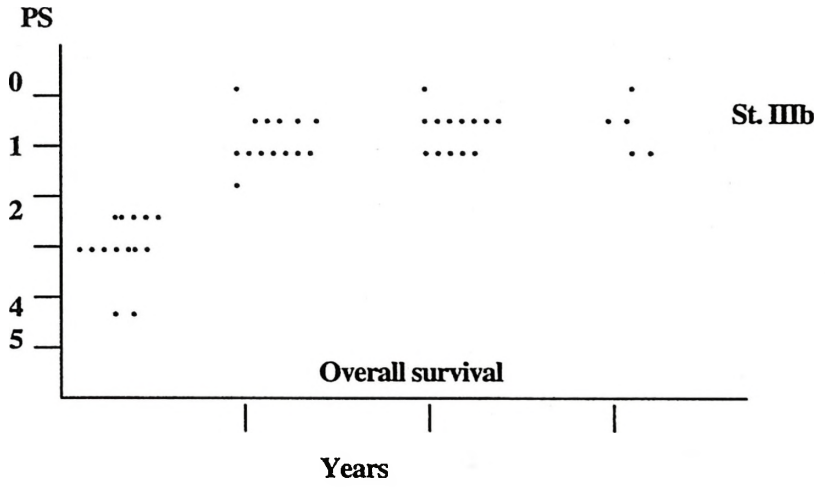
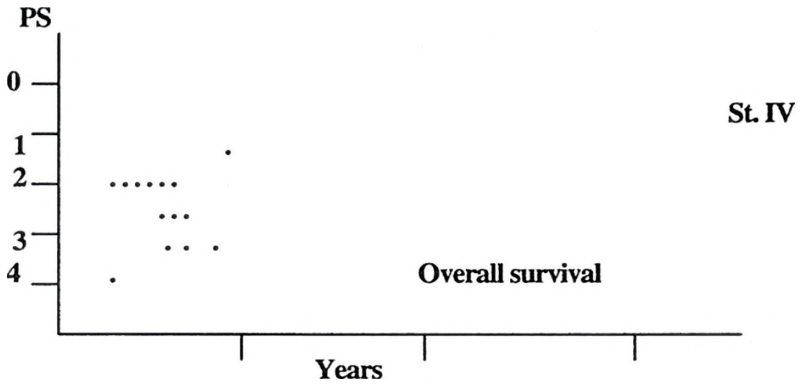


Fig 2: Relation of overall survival and PS for st. IIIb
on patients Clinic of Lung Diseases and TB



As it presented on Figs. 2 and 3, our patients with PS<3 (ECOG), had overall survival less than one year.

Fig 3: Relation of overall survival and PS for st. IV
on patients Clinic of Lung Diseases and TB



Sex as an prognostic factor

The International Association for the Study of Lung Cancer (IASCL) indicated sex as a possible prognostic factor[7]. Today, usually this is linkage for the role of female sexual hormones, which is still not completely illuminated. There are two facts that are in relation with female sexual hormones. One is "the protecting role" of female sexual hormones in relation to tobacco smoking and the second is a longer overall survival of female patients with NSCLC. Our observations don't corroborating these facts.

Age as prognostic factor

Some indicated only a minor influence of age as a prognostic factor. The reason for that is co morbidity at the older patients (>70 y). It's cardiovascular diseases, COPD, diseases that disturb renal function, diabetes mellitus, the general fall of functional status, arthritis's and peripheral vascular diseases.

The percentage of survival by stage and age of patients with NSCLC, in our study population is showed on the tables 4, 5 and 6.

Table 4: Percentage of survival by age.
Patients treated surgery only
– Stage I – IIIA

Age	N	Percentage of survival 1 y	2 y	3 y
21 - 31	2	50	50	50
31 – 40	1	100	-	-
41 – 50	3	100	100	66.6
51 – 60	3	100	100	100
61 – 70	1	-	-	-
71 – 80	-	-	-	-

Table 5: Percentage of survival by age
Treated by chemo-radio therapy
– Stage III b

Age	N	Percentage of survival 1 y	2 y	3 y
21 - 31	2	100	-	-
31 – 40	4	50	-	-
41 – 50	8	62.5	37.5	12.5
51 – 60	14	71.5	35.7	14.3

Table 6: Percentage of survival by age.
Treated by chemo-radio therapy
- Stage IV

Age	N	Percentage of survival 1 y	2 y	3 y
21 - 31	-	-	-	-
31 – 40	1	-	-	-
41 – 50	2	-	-	-
51 – 60	5	-	-	-
61 – 70	5	20	-	-
71 – 80	1	-	-	-

As it's showed on tables 4, 5 and 6 in our sample patients with resectable NSCLC, age till 60 is a good prognostic factor. For locally advanced disease, age till 70 with chemo-radio therapy is still a good prognostic factor (two years survival is 40%).

Total weight loss

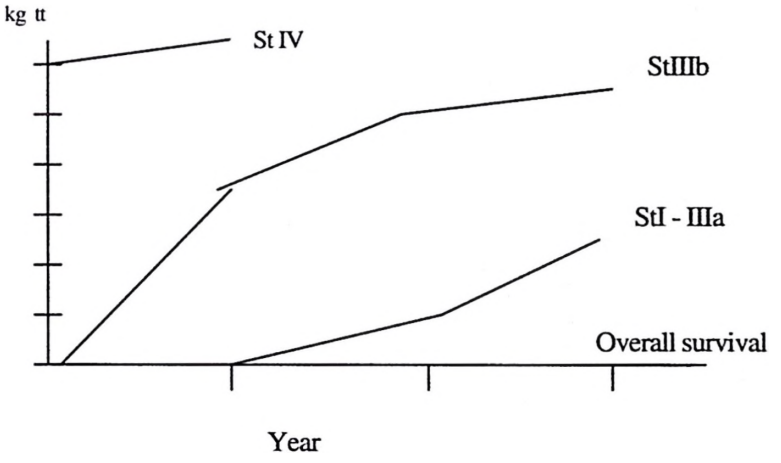
Total weight loss till the final diagnosis also is not neglect prognostic factor. The total weight loss is reflection of general influence of the disease. The most commonly adopted definition of weight loss from 4.53 kg in recent studies is changed with definition that it is loss on weight 5 - 10%, from the weight 6 months before the diagnosis.

Our results (table 5 and figure 4) showing that the total weight loss is proportional to overall survival and stage of disease. The advanced stages of disease are characterized with the bigger median total weight loss.

Table 5: The median weight loss by stages of median loss disease and years of overall survival

Stage	N	1 y	2 y	3 y
I - IIIa	10	0 kg	0.8 kg	3.4 kg
IIIb	43	4.5 kg	5.3 kg	5.5 kg
IV	14	6.8 kg		

Fig. 4: The overall survival and median loss on weight by stages of disease



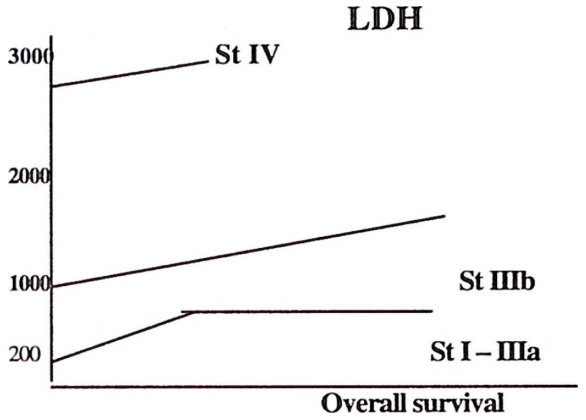
Pretreatment serum LDH

The level of pretreatment serum lactate dehydrogenase (LDH) at the patients with NSCLC seems to reflect the overall tumor burden and may therefore be of major influence on long-term survival for the patients with NSCLC.
Our results are showed on table 6 and figure 5.

Tabela 6: Pretreatment serum LDH and overall survival by stages of disease

Stage	1 y	2 y	3 y
I – IIIa	280±167	275±177	290±192
IIIb	348±162	556±74	682±82
IV	2703±885		

Fig 5: Pretreatment serum LDH and overall survival by stages of disease



Range for serum LDH in our laboratory is 230 – 460 U/L. There is evidence from results given in table 6 and figure 5 that increase of the level serum LDH is reflect of stage of disease (locally advanced and metastatic stage).

Histological types as prognostic factor of NSCLC

In discussion about prognosis of NSCLC, the data about metastasis on the other distant locations are very important. Today, it's known that adenocarcinoma showed a less locally progression and relatively quickly expansion in a brain and the other distant localizations. Different from it, the squamous cell carcinoma showed a poor tendency for distant metastases and more widening in locally advance.

When histology is in function of prognosis, there is very important fact that the growth of lung cancer has exponential character and the time of survival, in the moment of making the diagnosis, is in function of size of tumor, respectively of its double increasing[8]. For small-cell carcinoma, the time of double increase of tumor is about 50 days, for squamous and large cells carcinomas about 100 days, and for adenocarcinomas is about 80 days[9, 10]. In the case of lung cancers, some calculated that the tumor cell with diameter from 10μm producing a node with diameter of 1 cm for about 1 year, and after 30 multiplications. Starting with this fact, extrapolation to backwards, some studies showed that slow growing tumors, as squamous lung carcinomas and adenocarcinomas could be present in lungs of patients 8 – 15 years before the making of diagnosis, and in the cases of small cell carcinoma, 3 years ago[11]. It could be meaning that natural course of lung cancer is characterized with unrecognizing growth, and that is

clinically recognized a short time before the lethal end. Because of that, the clinics has a very short time to do something, and that is reason why the all clinical stages of this disease could be consider as the last stage. It's very disappointing to accept the fact that even on beginning twenty-first century we don't have the efficacy methods for detection of disease in pre-clinical stage.

Biological pretreatment prognostic factors for NSCLC

Leucocytes

Pretherapeutic absolute white blood cell and relative neutrophil counts were considered as abnormal if $> 10x10^3$ cells/μL^{-1} and > 75% of the total white blood cells were present, respectively. Both abnormal white blood cell and neutrophil counts were identified as poor prognostic factors (median survival times of 24 – 32 weeks). Even that, the increased white blood cells and neutrophil counts could be the sign of the infection of lower respiratory tract which is in connection with tumor, what making the prognosis worse.

Standard serum tumor markers

Such substances are tumor-specific and can be produced by one, few, or several types of cancer. Other substances are produced by tumor cells in larger amounts than by normal cells. Occasionally, normal cells release abnormal quantities of their products in response to invasion by cancer cells.

Lung tumor markers fall into several categories, including oncofoetal proteins, structural proteins, enzymes, membrane antigens, peptide and nonpeptide hormones and other tumor-associated antigens. They may play different roles in clinical practice including the assessment of prognosis.

At least three classes of tumor markers have prognostic significance in NSCLC, even about that the opinions of many experts are divided. It is carcinoembryonic antigen (CEA) and two cytokeratin-derived markers: tissue polypeptide antigen (TPA) and Cyfra 21. These tumor markers may predict clinical outcome mainly because their evaluation correlates with the tumor mass and malignant potential of the tumor.

In a recent French study on the prognostic value of six different tumor markers, the analysis was based on multivariate models of survival[13]. It was found that, besides metastases (p=0.017) and CEA125 (p=0.03) were significantly correlated with the outcome of 88 nonsurgical NSCLC patients. Furthermore,

elevated levels of Cyfra 21-1 during the course of disease were also an independent predictor of poor survival. In a recent study on lung cancer prognosis[14] 1296 consecutive patients seen over a 16-yr period were analyzed by Cox regression models. In every multivariate test, TPA emerged as being among the most important predictors of survival. Depending on the combination of variables in the model, TPA proved to be the second most important factor after either stage or performance status, and in front the other important clinical factors, such as the number and type of metastatic sites, the T and N factors, or the weight loss.

Even that, today on global level therefore has not consensus about prognostic role of tumor markers at the NSCLC.

Pathological pretreatment prognostic factors for NSCLC

In a multivariate model of recurrence of the Lung Cancer Study Group based on 392 stage- I NSCLC cases with final pathological review, it was reported that patients with squamous cell tumors had a lower risk of recurrence and tumor-related death[15]. As well as in the Leuven Lung Cancer Group experience on 140 surgically treated patients with IIIA-N2 NSCLC, patients with non-squamous cell tumors had a significantly increased risk of tumor-related death. Regarding relapse patterns, different authors suggested that squamous cell tumors are more prone to locoregional recurrence and adenocarcinomas to distant recurrence[16, 17, 18]

The results received by comparison of average time of survival and pathological type of tumors, from patients of Clinic of Lung Diseases and TB are showed on table 7, 8 and 9.

Table 7: Median time of survival by pathological type of tumor

St. I – IIIa; N=10 (Pts. of Clinic of Lung Diseases and TB)

Pathological type	Squamous	Adenocarcinoma	Large cells carcinoma
No patients	6	3	1
Average time of survival in months	39.3	24.6	10

p= 0.003 (α=0.05)

Table 8: Median time of survival by pathological type of tumor

St. IIIb; N=43 (Pts. of Clinic of Lung Diseases and TB)

Pathological type	Squamous	Adenocarcinoma
No patients	31	12
Average time of survival in months	27.2	20.3

p= 0.009 (α=0.05)

Table 9: Median time of survival by pathological type of tumor

St. IV; N=43 (Pts. of Clinic of Lung Diseases and TB)

Pathological type	Squamous	Adenocarcinoma
No patients	10	3
Average time of survival in months	11	8.2

p= 0.032 (α=0.05)

As it showed in tables 7, 8 and 9, patients with squamous cell carcinoma had statistically significant a longer median time of survival, regardless on stage of disease.

The absence of differentiation and presence of lymphatic and blood vessel invasion are significant elements of aggressively of lung tumors. On the basis of light microscopy, the differences could be make on: good, median, small or none differentiation of lung cancers. Small differentiation is associated with poor survival from NSCLC[19, 20].

But, presence of blood vessel invasion is a better prognostic factor than differentiation of lung tumors. Many studies are evidenced that the presence of blood vessel invasion was associated with decreased survival. In a US study on 289 consecutive stage-I NSCLC patients, vascular invasion proved to be of significant prognostic value both in univariate ($p<0.01$) and multivariate (p $p<0.05$) analyses[19]. A French group reported that venous but not arterial invasion correlated with the T-factor and p-TNM, whereas lymphatic vessel invasion correlated with the N-factor and p-TNM[20]. In their multivariate model on 96 resected NSCLC patients, lymphatic vessel invasion and p-TNM were important predictors for poor disease-free and overall survival.

Discussion

Our financial conditions don't allow us molecular-biological studies concerning determination of diagnostic and prognostic values some cellular molecules

respectively of gene abnormalities. Concerning that, in the western countries today, they making the investigations of the role of angioneogenesis, as well as investigations of prognostic value of activation of K-Ras oncogene, p53, p16 and the other gene abnormalities, and the level of neuron specific enolasis. On this place we will give just explanation of their role in the prognosis of NSCLC.

Molecular-biologic pretreatment prognostic factors

Neoangiogenesis

The observation of increased microvessel density in tumors not only serves as an independent prognostic indicator, but also suggests that antiangiogenic therapy may be an important component of treatment regimes for cancer patients[21]. Many positive regulators, including growth factor receptors, matrix metalloproteinases, and integrins, have been correlated with increased vascularity of tumors and poor prognosis for patient survival. Thus, these mediators may represent ideal targets for antiangiogenic therapy[22]. In tumor samples, neoangiogenesis may be evaluated as vascular density and as expression of angiogenic regulators, and both these methods may provide useful indications from a prognostic point of view[23].

In a series of 407 NSCLC, the number of microvessels was significantly associated with poor prognosis in terms of overall survival[24]. Angiogenesis was quantified as microvessel count and the median value of this series was 20 vessels. In the univariate analysis, patients with larger tumors, more advanced stage, greater degree of regional lymph node involvement, or more vascular tumors experienced reduced overall survival.

Vascular endothelial factor of growth (VEGF) is one of the most important tumor-derived cytokines that contributes to the increased permeability of tumor vasculature, and which shows a mitogenic activity on endothelial cells. A great number of studies demonstrate its influence in lung cancer progression and poor overall survival of the host[25].

K-Ras oncogen activation

In the NSCLC, the family of mutant oncogens is part of Ras family the small G proteins that have influence on cell growth. This including: N-Ras, K-Ras, H-Ras protooncogens and their activity are mediated by guanin-nucleotid changeable factors (GEF), which changes guanozindiphosphate (GDP) in guanozintriphosphate (GTP). Ras family of protoncogens has a role in

transmission the signals of growth from cell surface to nucleus. In the cells this protooncogen is finding in inactivated GDP form. As the answer on linkage of ligands on the cell receptors, Ras family of protooncogens being activate only transient, but in the cases of mutation this protooncogens in only one their amino acid part, they can locked in active position and on that way they beginning the oncogens.

Analysis of the rest of DNA by Ras protooncogen from lung cancer cells of humankind discovered the activating mutations of K-Ras oncogens. In adenocarcinoma its presence in 30%, and someone that investigated these mutations found that it has prognostic significance[26, 27].

In a study of 69 patients with totally resected tumor (adenocarcinoma) the authors found significant decrease of K-Ras mutations in patients who had a longer overall survival, and disease free survival[28]. Mitsumi et al[29] were showed that patients with early and late stages of NSCLC but and positive Ras mutations has a shorter time of survival in comparison whit these without mutations.

Prognostic value of p53 and p16 gene abnormalities

Among several genetic aberrations that have been implicated in lung cancer, alterations in the p53 and p16 tumor-suppressor genes are the most common.

Mutations in the p53 gene usually result in increased steady-state levels of p53, which may play role in carcinogenesis through transdominant mechanisms, perhaps involving oligomerisation between mutant and wild type proteins. During the last 10 years, a large number of studies have evaluated p53 alterations in lung cancer[30]. Some studies have demonstrated that a p53 mutation is associated with poor prognosis of NSCLC[31, 32], while others have reported no significant effect[33], or have even concluded that p53 protein overexpression can be a good prognostic characteristic[34].

Genetically, the p16 gene can be inactivated by point mutation or homozygous deletion, as observed in various human primary tumors, including lung cancer. Recently, Belinsky et al[35] showed, for the first time, that inactivation of p16 gene by aberrant methylation is an early and likely critical event in the development of lung cancer.

In a group of 98 surgically treated stage I-IIIA NSCLC patients, p53 mutations were detected in 46 (47%) cases (point mutations in 8 and promoter hypermethylation in 34). No correlation was found between p53 and p16

abnormalities and various clinicohistological factors, including age, sex, histological type of tumor and TNOM stage.

Survival analysis revealed that both the patients with p53 and p16 abnormalities tended to have poorer prognosis than the patients without p53 (p=0.02) and P16 (p=0.01) abnormalities. In the multivariate analysis, however, when the types of p16 inactivation were analyzed, p16 hypermethylation rather than point mutation was associated with poor prognosis[33].

Evaluation of p53 by yeast functional assay was performed in 42 patients. Twenty-seven of the 42 (64%) NSCLC samples contained mutant p53 in the yeast functional assay with the higher frequency in squamous cell carcinoma (16 of 22 (73%)) than in large cell carcinoma (4 of 7 (57%)) and adenocarcinoma (7 of 13 (54%; p<0.02)). Preliminary prognostic analysis showed that patients scoring positive for yeast test had significantly shorter disease-free survival (median 11 months) than those that scored negative (median 23 months)[36].

Conclusions

On the basis the clinical, biological and pathological pretreatment factors of NSCLC, in everyday praxis, we can say something about the prognosis of the NSCLC. Molecular-biological pretreatment factors are not in the routine praxis. On sample of 67 patients with NSCLC, from Clinic of Lung Diseases and TB, Clinical Center University of Sarajevo, the authors are proved the next:

a) The possibility of survival with today's therapeutic treatment are:
- stage I – IIIA : 1-year 90%, 2 – year 70%, 3 – year 60%;
- stage IIIB : 1-year 67.4%, 2 – year 30.3%, 3 – year 11.6%;
- stage IV : 1-year 7.14%

b) The patients with stage I-IIIA had PS 0 – 1,
- stage IIIB had PS 1 – 4,
- stage IV had PS 2 – 4.

c) Age till 60 is a good prognostic factor for the three years survival for the resectable NSCLC. In the cases patients with locally advanced NSCLC, the age from 70 is a good prognostic factor for the two years survival with the chemo radiotherapy treatment.

d) The more median weight loss is linkage with the higher stage of disease and less survival; weights loss more than 5.5 kilograms suggest for the metastatic stage of disease.
e) We were finding the increase of serum LDH more than its referent values at the locally advanced disease. Increasing of serum LDH more than double referent value is suggesting for the metastatic disease.
f) White blood cells number more than $10x10^6$/ml and percentage of neutrophils more than 75% are poor prognostic factors for NSCLC.
g) Squamous cell carcinoma has a better prognosis concerning the overall survival than adenocarcinoma and large cell cancer regardless on stages of disease.

Accordingly, the positive prognostic factors that are significant for the treatment patients with resectable NSCLC are: stage of disease I – IIIA, PS<2 (ECOG), and age of patients (less than 60).

For locally advanced disease the main prognostic factors are: stage of disease IIIB, PS<2 (ECOG), age of patient (less than 70), weight loss till 5.5kg. High level of serum LDH and increase number of white blood cells in this stage of disease are result of tumor growth and his metastatic widening. We could say that this factors condemning in advance the therapeutic approaches.

Sažetak

U posljednjoj deceniji skupina novijih prognostičkih faktora dodana je listi dobro poznatih kliničkih prognostičkih faktora za nemikrostanični rak pluća (NSCLC). Među novijim izdvajaju se: mutacije u K-*ras* onkogenu, abnormalni p53 proteini kao posljedica mutacija na p53 supresorkom genu, imunohistohemijskim bojenjem dokazano prisustvo adhezivnih
molekula neuralnih stanica kičme (N-CAM), porast serumskog nivoa neuron specifične enolaze (NSE) i slično.

U današnjim uslovima u BiH navedeni biomarkeri još uvijek nisu našli svoje mjesto u svakodnevnoj kliničkoj praksi. To je razlog zašto ne možemo govoriti o njihovom značaju u prognostičkom smislu na našem kliničkom materijalu.

Naši pozitivni prognostički faktori od značaja za dalji tretman pacijenata sa NSCLC još uvijek se odnose na:

stadij bolesti I – IIIa, performans status < 2 (ECOG) i dob pacijenta <60g – za resektabilni NSCLC, te

stadij bolesti IIIb, performans status < 2 (ECOG), dob pacijenta <70g i gubitak na tjelesnoj težini do 5.5kg – za napredovali NSCLC. Porast nivoa serumske LDH i leukocitoza u ovom stadiju bolesti prvenstveno su odraz napretka u rastu tumora, metastaziranja i moglo bi se reći da unaprijed osuđuju terapijski pristup na neuspjeh.

Ključne riječi: NSCLC (nemikrostanični plućni rak), performans status, stadij bolesti, LDH (laktat dehidrogenaza), leukocitoza.

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ADVERSE REACTIONS OF CYCLOSPORINE IN PATIENTS AFTER KIDNEY TRANSPLANTATION

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Abstract

Increased level of cyclosporine in whole blood leads to different severe adverse reactions causing disturbance in function of kidney as well as liver, central nervous sistem, increased blood pressure, gingival hipertrophy. Low doses of cyclosporine lead to HVGR (host versus graft reaction).Cyclosporine level in whole blood does not depend upon dose only. So the level of cyclosporine has to be determined regularly to avoid either severe adverse reactions of the drug or HVGR.

In the present study we analysed cyclosporine levels in 15 patients after kidney transplantation. Cyclosporine levels were determined by fluorescence polarization immuno assay, and the monoclonal whole blood was carried out by analizer ABBOT - TDX. In these patients the cyclosporine level determinants were performed six times during three months. In intervals of 15 days, at the exactly same time, we followed the parameters determining the kidney and liver functions : creatinin, ALT(Alanin aminotranssferasis), AST(Aspartat aminotranssferasis), serum concentraratins of bilirubine, and ultrasound diagnostics of kidney and liver). The results of our study have shown that frequent monitoring of cyclosporine concentrations in whole blood in patients with transplanted kidney is extremly important. It is the way how to maintain the cycosporine levels in whole blood in the recommended interval between 100 and 300 ng/ml. By doing it the adverse drug reactions of cyclosporine in our study were reduced to a minimal level.

Key words:Cyclosporine-Determination-Nephrotoxicity-Hapatotoxicity

Introduction

Comparing drug cyclosporine with other older immunosuppressive drugs we noticed survival graft of skin, heart, kidney, bone marrow , lung, and liver was improved (1,2). Cyclosporine affects immune response which is specifficly aimed against transplantation antigen (3). It acts as immunosuppresive suppressing clonal expansion and functinal activation of lymphocities(4,5) The most serious factor that limits its use is cyclosporine toxicity, first of all nephrotoxicity which is dose dependent(6). Cyclosporine applied in high doses is also causes hepatotoxicity (especially in patients with earlier damaged liver function) (7). One of more adverse drug reactions is gingiva hypertrophy, hirsutismus and side effects of CNS (8).

Monitoring of cyclosporine is important indicator during immunosuppressive cyclosporine therapy.Although cyclosporine is very toxic drug the number of adverse drug reactions may be reduced by successive follow up of drug concentration in blood (9)

Material and methods

In our study we determined cyclosporine levels in blood of kidney transplantation patients. The follow up was carried out in Internal Clinic, partly in Central lab University Clinic Center, and partly in the Department of Pharmacology Tuzla University. The cyclosporine level was carried out six times every 15 days. The cyclosporine determination was performed by FPIA using ABBOT-TDX. As a sample EDTA blood was used ,which was first treated with reagent for hemolisis and precipitation. Afterwards cyclosporine was determined in supernatant. Test sensitivity is 25 ng cyclosporine per 1 ml of blood. Statistic treatment of the results was performed by correlation determination.

Results

The cyclosporine level obtained by application of FPIA manhood were between 45 and 1.000 ng/ml blood with average level 182 ng/ml blood.The creatinine level was between 97 and 977 nmol/L average 190 ng/ml blood.Aspartat aminotranssferasis level was between 115 and 889 nkat/l. Average was 318 nkat/L and the level of alanin aminotransferasis was between 110 and 1699 nkat/L with an average of 365 nkat/L:The results can be seen in the tab. 1. Kidney transplant recipient was between 22 and 65 year old and the average was 36 years. Kidney transplant was between 1 and 12 years old and the average was 5.5 years.

Table 1. The concentrations of cyclosporine in whole blood, serum creatinines, serum AST and ALT in patients after kidney transplantation (X-1),and averages (X-2)).

	(X-1)	(X-2)
Cyclosporine(ng/ml)	45-100	182
Creatinine(nmol/L)	97-977	190
AST(n kat/l)	115-318	318
ALT (n kat/l)	110-1699	365

Discussion

Apart from determination of cyclosporine level in blood we also observed parameters relating to kidney and liver function (serum creatinine and transaminases due to high cyclosporine nephrotoxicity and hepatotoxicity (10). Cyclosporine level should be maintained within referent once between 100 and 300 ng/ml of blood in order to prevent graft rejection on one hand and as well as adverse drug reaction on the other hand (11). We observed low cyclosporine level in patients exposed to infection (12). Higher cyclosporine doses should be given immediately after transplantation because bioavialability is gradually increased during the treatment, so oral doses should be gradually reduced to maintain permanent concentration in blood. By performing frequent cyclosporine monitoring we have reduced nephrotoxicity hepatotoxicity to a minimal level ,what justifies the aim of this work (13,14)

There was no statistic significant linear correlation between the concentration of cyclosporine level in blood and the level of serum creatinine, because we tried to maintain the cyclosporine level within the refemce value in successive observation of the drug level in blood.

There was no statistic significant correlation between cyclosporine level in blood and the AST and ALT levels i.e.The correlation coefficient does not different from 0 and therefore we can prevent hepatotoxicity. Frequent cyclosporine monitoring has its significance for future research to reduce adverse drug reactions to a minimal level in applying this drug(15,16,17,18,19). Other significant effects are related to nephrotoxicity and hepatotoxicity effect in particular.

Conclusion

In regard to expressive nephrotoxicity and hepatotoxicity as well as related great number of possible adverse effects during application of cyclosporine teary, it is required to follow up successive drug concentration in blood. We have proven in this work that monitoring of cyclosporine concentration in blood may reflect efficiency immunosuppressive application.

Apstrakt

Povečani nivo ciklosporina u punoj krvi može voditi u vrlo teške i neželjene poremećaje u funkciji bubrega, jetre, centralnog nervnog sistema, povečani krvni pritisak, gingivalnu hipertrofiju.Niske doze ciklosporina vode u HVGR reakciju, domaćina protiv presada.Ciklosporin vrijednost u punoj krvi nije ovisna samo od doze.U našoj studiji mi smo analizirali vrijednosti ciklosporina u 15 pacijenata nakon bubrežne transplantacije.Vrijednost ciklosporina bile su određivane fluroscetnim polarizacijskim imunoesejom a, primjenjen je monoklonalni test u punoj krvi na aparatu ABBOT-TDX.U ovih pacijenata vrijednosti ciklosporina su određivane 6 puta u trajanju od tri mjeseca.U intrevalima od 15 dana normalno u isto vrijeme, mi smo određivali laboratorijske parametre koji determiniraju funkciju bubrega i jetre : kreatinin, AST, ALT, serumsku koncetraciju bilirubina, ultrazvučni pregled bubrega i jetre.Rezultati u ovoj studiji su pokazali da često rađen monitoring ciklosporina u punoj krvi u pacijenata sa presađenim bubrezima je ektremno važan.To je put da se vrijednost ciklosporina u punoj krvi najbolje održava između 100-300 ng/ml.Kod ovih vrijednosti neželjene reakcije od ciklosporina bile su reducirane na minimalni nivo.

Ključne riječi Ciklosporin-određivanje-nefrotoksičnost-hepatotoksičnost

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THE DOSAGE OF ANTIPSYCHOTIC DRUGS IN THE UNIVERSITY DEPARTMENTS OF PSYCHIATRY IN FEDERATION OF BOSNIA AND HERZEGOVINIA

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Abstract

The aim of this pharmaco - epidemiological study was to establish which antipsychotic are currently in use and determine in what daily dosages these drugs are given to patients in three Psychiatric departments in the Federation of Bosnia and Herzegovina (Sarajevo, Tuzla, Mostar).

Majority of patients received classic antipsychotic medications. In Sarajevo most frequently administered was promazine, orally and parenteral, for total of 38 patients or 28, 6% of Sarajevo sample. In Tuzla most frequently administered antipsychotics were haloperidol and thioridazine for 20 and 21 patients, which are 19, 2%, and 20, 2% of total number of patients in Tuzla. In Mostar the leading antipsychotic is haloperidol, orally administered, and with which 29 patients were treated. This represent 31, 2% of total number of patients in Mostar.

Clozapine was applied in all three centres but in very small percentage of cases.

Treatment with daily doses antipsychotics in the Department of Psychiatry in University Clinical Centres in Federation of Bosnia and Herzegovina are mainly in accordance with international standards. Only problem is area of simultaneous use of multiple antipsychotics, both in acute and chronic cases, and frequent use of antiparkinsonics in chronic cases. Because of disseminated results, were considered that the continuous medical education will lead to improvement of treatment quality for the patients with schizophrenia.

Key words: antipsychotics, schizophrenia.

Introduction

Treatment of schizophrenia consists a combination of psychosocial therapy and antipsychotic drugs. Drug treatment should de individualized for each patient with close monitoring. Negative symptoms of schizophrenia tend to respond less well to drug therapy than do positive symptoms (1)

It is well known that low-potency antipsychotic are more sedative and they have strong antimuscarinic and antiadrenergic effects, but they cause less acute extrapyramidal symptoms than the high-potency agents with a reversed pattern of adverse-effects. Sedative and muscarinic effects usually diminish with continued use, although sedative effects may be useful for behavioral control in acute phase of illness (2).

Antipsychotic is classified in three groups:

- First group antipsychotic is sedative, mild antimuscarinc and extrapyramidal adverse effects. Type of antipsychotic used in this study were: chlorpromazine, methotrimeprazine and promazine;
- Second group are slightly sedative effect, strong antimuscarinic effects, and lower extrapyramidal adverse effects than antipsychotic first and third group (pericyazine, pipothiazine, thioridazine);
- Third group are weak sedative effect, slightly muscarinic effect, and very strong adverse effects (fluphenazine, perphenazine, prochlorperazine, etc)

Other antipsychotic which belong phenotiazine derives, have likeness with antipsychotic of third group. Type of antipsychotic we used were: butyrophenones derives (haloperidol, droperidol, trifluperidol) and derivatives thioxanthenes (chlorprotixene, chlopenthixol and flupenthixol).

Atypical antipsychotic sulpiride and clozapine are not included in this classification.

Antipsychotic do block receptor sites for noradrenalin (NA) and dopamine (DA). Antipsychotic that blocked more NA receptors is sedative, and antipsychotic which blocked more DA receptors is including in controlling hallucinations and delusions. Atypical antipsychotic is involved in blockade of serotonin receptors (5-HT). Usually antipsychotic increased excitability hypothalamic centers (3).

In Federation of Bosnia and Herzegovina are accepted modern attitudes in the treatment of psychotic patients. These attitudes imply treatment within the community, closing of the big hospitals and preference of out-patients clinics for the treatment of the patients. In this sense one question must be posed, and that is to what extent is accepted and applied modern pharmacological treatments of schizophrenia. These modern, contemporary treatments imply use of new antipsychotic drugs, because in the area of psychopharmacology there are dramatic changes in the world, in a sense that classic antipsychotic are in the retreat and currently new atypical antipsychotic drugs are in use, which have been shown as far more efficient and safer for the patients.
The aim of this study was to establish which antipsychotic are currently in use and determine in what daily dosages these drugs are given to patients.

Patients and methods

Sample was made of all patients admitted to University Hospitals in the Federation of Bosnia and Herzegovina (Sarajevo, Tuzla, Mostar), questionnaire was applied It consists: demographic data, questions from general criteria for schizophrenia according to ICD-10, antipsychotic, route of administration, dose and time of administration, data for other drugs that were taken in a same day.

Data were evaluated with statistical methods. Mean values for all registered results of individual tests were calculated. Statistical methods used in this study were: the analysis of variance, significant differences between mean values and tests for significant correlation between tests used in the study. Results are presented in text, tables and figures.

Results

Haloperidol daily doses

Table 1. Haloperidol - daily doses

	N (number of patients)	Median of daily doses	Std. Deviation	Std. Error	95% CI (Confidence Interval for Mean)		Min.	Max.
					Lower Bound	Upper Bound		
Sarajevo	16	6,3750	4,92443	1,23111	3,7510	8,9990	1,00	15,00
Tuzla	20	8,1500	3,83577	0,85771	6,3548	9,9452	3,00	15,00
Mostar	29	18,1379	15,01362	2,78796	12,4271	23,8488	2,00	75,00
Total	65	12,1692	11,75807	1,45841	9,2557	15,0827	1,00	75,00

The highest average daily dose of haloperidol were administered in Mostar 18,13mg, while similar daily doses are applied in Sarajevo and Tuzla 6, 37 mg and 8, 15 mg. (Table 1 and Chart 1).

Chart 1. Haloperidol- daily doses

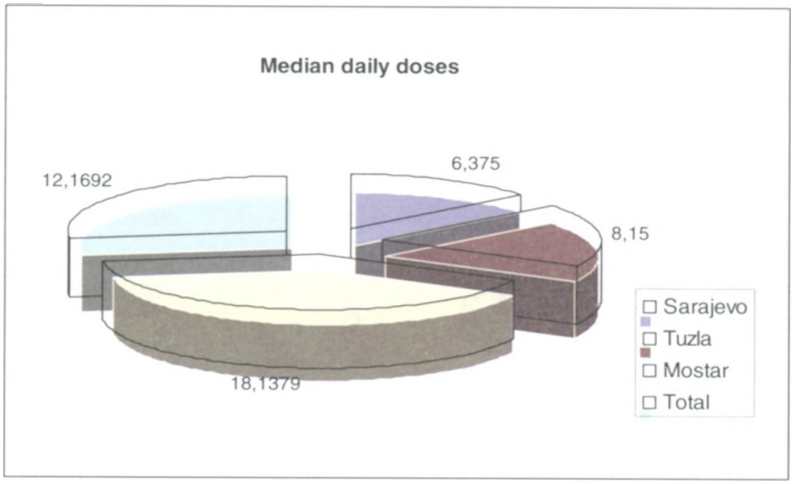

Table 2. Haloperidol – daily doses (ANOVA)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1893,390	2	946, 695	8,440	**0,001**
Within Groups	6954,748	62	112,173		
Total	8848,138	64			

There is a significant statistical difference between the centers by application by haloperidol daily doses applied at the **0,001** level (table 2).

Promazine daily doses

Table 3. Promazine daily doses

	N (number of patients)	Median daily doses	Std. Deviation	Std. Deviation	95% CI (Confidence Interval for Mean)		Min.	Max.
					Lower Bound	Upper Bound		
Sarajevo	26	211,5385	81,61825	16,00666	178, 5721	244,5048	100,00	400,00
Tuzla	11	197,7273	124,22487	37,45521	114, 2719	281,1827	75,00	450,00
Mostar	12	141,6667	100,18921	28,92214	78,0095	205, 3239	50,00	300,00
Total	49	191,3265	99,15673	14,16525	162,8454	219,8077	50,00	450,00

Average daily dose of promazine was 191,32mg, while the lowest daily dose is applied in Mostar 141, 66 mg, and highest in Sarajevo 211,538 mg (table 3).

Table 4. Promazine- daily doses (ANOVA)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	40665,465	2	20332,733	2,169	,126
Within Groups	431273,310	46	9375,507		
Total	471938,776	48			

A significant statistical difference was not noticed between the tested centers regarding the applied daily promazine doses (table 4).

Clozapine daily doses

Table 5. Clozapine – daily doses

	N (number of patients)	Median daily doses	Std. Deviation	Std. Deviation	95% CI (Confidence Interval for Mean)		Min	Max
					Lower Bound	Upper Bound		
Sarajevo	3	266,6667	57,73503	33,33333	123,2449	410,0884	200,00	300,00
Tuzla	11	240,9091	125,63475	37,88030	156,5065	325,3117	75,00	450,00
Mostar	4	168,7500	94,37293	47,18647	18,5816	318,9184	75,00	300,00
Total	**18**	229,1667	111,55650	26,29412	173,6909	284,6424	75,00	450,00

Average daily dose of clozapine was 229, 16 mg, while the lowest daily dose is applied in Mostar 168, 75 mg, and highest in Sarajevo 266, 66 mg.
Within the total sample minimal daily dose of clozapine is 75 mg, and maximal daily dose is 450 mg (table 5).

Table 6. Clozapine- daily doses (ANOVA)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20.336,174	2	10.168,087	0,798	0,469
Within Groups	191.226,326	15	12.748,422		
Total	211.562,500	17			

A significant statistical difference was not noticed between the tested centers regarding the applied daily clozapine doses (table 6).

Discussion

In the USA conducted a comparative research regarding the applied antipsychotic in the period from 1989. until 1993. During the 1993 antipsychotic were prescribed to 299 patients (42%) from a total of 709 hospitalized patients. The treatment begins usually within the first 24 hours, while the hospitalization lasted around eighteen days. Highly potent antipsychotic were used 2, 4 times more frequently than the low potent; 13% of patients were treated with clozapine. Average daily doses of the applied antipsychotic equivalent to daily doses of chlorpromazine were 305 mg; and the maximal doses were 32% higher. Doses of highly potent antipsychotic (fluphenazine and haloperidol) are only 22%-33% above the mean values (4).
The doses in the United States are approximately double as the doses in the Europe (200-300 mg/day). Some of the side effects of clozapine, such as: convulsions, confusion and sexual dysfunction, are related to dose and blood levels. But, increase of body mass was not dependant on dose. Latest reports show that optimal blood levels of clozapine should be between 200 and 250 µg/ml, although a lot of patients had a good response even on lower concentrations (5).

The contemporary researches provided a strong evidences about the efficiency of second generation antipsychotic in the treatment of schizophrenia, and clearly indicated that cause much less extrapyramidal side effects (EPS) than the traditional medications (6). Also there are evidences that these medications have less potency to cause tardive dyskinesia (TD) than the first generation antipsychotic, and can be useful in the treatment of preexisting TD. In general patients tolerate much better these medications than older antipsychotic, with few important exceptions, including the risk of agranulocytosis in clozapine use and potential to increase body mass by many medications from this group. Due to their superior safety in terms of neurological side effects, it is considered that the second generation antipsychotic should be available as the first choice of treatment in schizophrenia, and preferred in first episode patients (7).
Conducted a study in the public hospitals in Hong Kong with obtained data about the treatment with antipsychotic medications in relation to the dose of antipsychotic, combined use of multiple antipsychotic, administration in divided daily doses and coadministration of antipsychotic with the medication for Parkinson's disease. Sample was consisted of 957 schizophrenic patients randomly selected. A census was made, and the questionnaire was consisted of items about demographic and clinical data, as well as description of all

medications that patients received on the day of census. Results of study indicates that the average dose of antipsychotic was 854 +/- 759 (mean: 600; range 0 - 4450) mg CPZeq. More than two thirds of patients received multiple medications simultaneously, while less than 20% take their medication in divided daily doses. Antiparkinsonics was used in 69, 6% of patients (8).

Apstrakt

Cilj ove farmako-epidemiološke studije je bio da se ispitaju doze neuroleptika, koji se aktuelno primjenjuju u tretmanu psihotičnih bolesnika u tri psihijatrijske klinike univerziteta u Federaciji Bosne i Hercegovine (Sarajevo, Tuzla i Mostar). Ispitani bolesnici pretežno su konzumirali klasične (tipične) antipsihotike. U Sarajevu 38 bolesnika (28,6% od ukupnog broja pacijenata) su koristili promazin, peroralno i peranteralno. U Tuzli najviše primjenjivani antipsihotici su haloperidol (20 ili 19,2%) i tioridazin (21 ili 20,2%), dok je u Mostaru najčešće propisivan haloperidol (29 ili 31,2%).

Klozapin je administriran u sva tri centra kod veoma malog broja pacijenata. Dnevne doze antipsihotika u svim centrima Federacije Bosne i Hercegovine su u granicama medjunarodnih standarda. Razlike se mogu primijetiti samo u slučajevima, kada se primijenjuje više lijekova i kada se koriste antiparkinsonici kod bolesnika na dozama održavanja.

Ključne riječi: antipshotici, shizofrenija.

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OPTIMIZATION OF MULTIPLEX RT-PCR FOR LEUKEMIA TYPING AND SUBTYPING

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Abstract
Molecular typization and subtypization of leukemia by RT-PCR, is important due to monitoring of minimal residual disease (MRD), during and after treatement of patients with acute leukemia. In our experiments of PCR optimization, we used BIO-RAD procedure for multiplex RT-PCR screening and split-out PCR for leukemia subtypization. This method is adapted or optimized for Perkin Elmer Gene Amp 9600 thermal cycler and Qiagen HotStar Taq DNA polymerase as well.According to manufacturer instructions, using of some other PCR engines require optimization of corresponding PCR parameters. We used Perkin Elmer 2400 thermal cycler and Hot Star Taq DNA polymerase. Total RNA was extracted from whole blood specimens, obtained from Clinics of hematology-KCU Sarajevo, taken from patients with acute leukemia, transferred to cDNA, and amplificated in PE 2400. Detection of PCR products is performed by 1,5% agarose gel electrophoresis. Obtained optimal PCR parameters were: annealing temperature - 65°C, number of cycles for both, first round PCR amplification and nested PCR amplification was 30, final concetration of MgCl in reaction mixture was 3,28 mM. Instead recommended amount of cDNA (5 µl) and Hot Star Taq DNA polymerase (0,4 µl) we used amounts of 10 µl and 1 µl
By using of this optimized PCR protocol we detected genetic aberration inv(16)(p13; q22) and by using of split out PCR, subtype G(192 bp electrophoretic band)(CBFB / MYH11 fusion genes).
Key words: leukemia, typization/subtypization, fusion gene transcripts, multiplex RT-PCR screening, split-out multiplex PCR.

Introduction

Detection of fusion gene transcripts, as specific markers and prognostic factors of different leukemia types/subtypes, is very useful approach in MRD (minimal residual disease) monitoring of patients with acute leukemia, during and after chemotherapy treatment. The sensitivity of this PCR analysis of break-point fusion regions method together with other two methods (flow cytometric immunophenotyping and PCR analysis of patient-specific functional regions of rearranged Ig and T cell receptor genes), is at least 10^{-3} (one leukemic cell between 10^3 normal cells).

Up to now, it was detected nine well defined chromosome aberrations, caused by fusion of different genes in the cases of acute leukaemia (Palisquardet al., 1998; Rabbits, 1994.).

- t(1:19)(q23;p13)	E2A/PBX1 fusion genes
- t(4;11)(q21;q23)	MLL/AF4
- t(8;21)(q22;q22)	AML1/ETO
- t(9;22)(q34;q11)	BCR/ABL (two types)
- t(12;21)(p13;q22)	TEL/AML1
- t(15;17)(q22;q21)	PKL/RARA
- inv(16)(p13;q22)	CBFB/MYH11
- del(1p32)	SIL/TAL1

All of these above mentioned genes, involved in production of fusion gene transcripts, are detected in the period from 1993 to 1996. According to these data, experts in the field of molecular biology, were able to designed PCR primers for their amplification and detection. The firm BIO-RAD designed own protocol for qualitative multiplex reverse transcription – polymerase chain reaction (RT-PCR), adapted for screening of 28 different gene translocations and detection for more than 80 breakpoints or mRNA splice variants, as specific markers of particular leukemia types/subtypes. This procedure is also optimized for PE 9600 thermal cycle and using of some other engine require corresponding PCR parameters optimization (Bio-Rad handbook, 2004.; Armitage,2004.; Cox and Sinclair, 1997.).

In our experiments of PCR optimization we used PE 2400 thermal cycler and recommended HotStar Taq DNA polymerase.

Material and Methods

HotStarTaq DNA polymerase(Qiagen)– has been developed to provide hot-start PCR for purpose of higher specificity, minimizing of non-specific amplification products, primer-dimers formation and contamination risks associated with conventional hot-start PCR methods. It is actually modified form of the recombination 94 kDa Taq polymerase, in inactivate state, with no activity at ambient temperature. Using of this enzyme in multiplex PCR, such as BIO-RAD multiplex RT-PCR, prevents the formation of misprimed products and primer-dimers at low temperatures, high PCR specificity and increasing of specific PCR products yield (Qiagen HotStarTaq DNA polymerase handbook, 2002.).

RNA extraction – During 1 to 2 hours after taking of whole blood specimens, from Clinics of haematology - KCU Sarajevo, total RNA was extracted by using of QIAamp RNA Blood Mini Kit (Qiagen cat. no. 52304). The lysis procedures of eritrocytes and leucocytes by using of EL and RLT buffers, is performed by incubation at 4°C and centrifugation at 2000 rpm. The pellet is resuspended in 500 µl EL buffer and than in 350 µl RLT buffer, as well. Disollved pellet is transferred into Qiagen shrederer spin columns (pink color) and centrifugated at 14000 rpm for two minutes. These spin columns were discarded and lysate from collection tubes were transferred into new uncolored spin columns with specific membrane for RNA addition. After centrifugation at 14000 rpm for three minutes, added RNA was resolved in 50 µl Rnase free water and store at - 20°C to next using. In this conditions, extracted RNA remain stability for more than one year(QIAamp RNA Blood Mini handbook, 1999.).

cDNA synthesis – Each specimen must have negative control (10 µl DEPC treated water and 4 µl cDNA mix). In the process of reverse transcription, both 0,2 ml PCR tubes (specimen and negative control) contained 11 µl cDNA synthesis mix (5xMMLV-RT buffer, 100 mM DTT, 10 mM dNTPs mix and MMLV reverse transcriptase), 10 µl extracted RNA and 4 µl BIO-RAD cDNA primer mix(total volume 25 µl). After incubation at 37°C for 45 min., reverse transcriptase was inactivated by incubation at 95°C for 5 min.cDNA was stored at -20°C (Cross et al., 1994.).

PCR amplification – For both, BIO-RAD RT-PCR screening and split-out PCR typing/ subtyping of leukemia, the same cycling programmes for first round PCR amplification and Nested PCR amplification were recommended and optimized for PE 9600 thermal cycle:

First round PCR amplification

15 min.	95°C		x1
30 sec.	95°C		
30 sec.	58°C		x25
1,5 min.		72°	
hold		4°C	x1

Nested PCR amplification

15 min.	95°C	x1
30 sec.	95°C	
30 sec.	58°C	x20
1,5 min.	72°C	
10 min	72°C	x1
hold	4°C	

According to standards of PCR optimisation, we modified annealing temperatures and number of cycles from these programs. We also modified $MgCl_2$ molarity, amount of cDNA and HotStar Taq DNA polymerase.

Detection of PCR products by agarose gel electrophoresis

PCR product are separated by electrophoresis in 1,5% agarose gel, at least 10 centimeters long, in 1xTBE buffer. The gel containing 0,5µg/ml of ethidium bromide. We added in the gel 14 µl of specimen (loading buffer and PCR products). After electrophoresis, the gels were examined by UV transilluminator.

Results and Discussion

After optimization of multiplex RT-PCR (BIO RAD), (adapted to thermal cycle PE 9600),by using of our PE 2400 PCR engine we obtained optimal results after analysis of electrophoretic bands by UV transilluminator under following conditions:

- annealing temperature – 65 C°
- number of cycles - 30 (for first PCR amplification and nested PCR amplification)
- $MgCl_2$ – final molarity 3,28 mM.
- 1 µl HotStar Taq DNA polymerase, instead standard amount of 0,4 µl per PCR reaction
- 10 µl cDNA, instead amount of 5 µl in first PCR master mix (10 x PCR buffer,$MgCl_2$,dNTPS mix, Hot Star Taq DNA polymerase and RNAse free water)

An internal control fragments of 911 bp, must be visible in all electrophoretic specimen lines. It means good integrity of RNA sample and the presence of PCR inhibitors.

One specimen showed genetic aberration inv (16) (p13; q22) by RT- PCR screening and often using of split- out M6 PCR primers (M6A, M6B, M6C, M6D and M6E), we detected 192 bp electrophoretic band, which means subtype CBFB/MYH11 – G.

An deletion on 16 chromosome was initially reported in 1982.This pericentric inversion is associated whit acute leukemia. There are 10 subtypes (A,B,C,D,E,F,G,H,I,J) or variants of CBFB/MYH11 fusion genes, which are generally associated with relatively good prognosis (Van Dongen et al., 1999; Liu et al., 1995; Claxton et al., 1994.).

Multiplex PCR is a demanding technique that requires extensive optimization of Taq polymerase, amount $MgCl_2$, additional reagents and PCR primers.

The cycling parameters such as annealing temperatures and number amplification cycles need to be changed. Hot Star Taq DNA polymerase has been developed to provide hot-star PCR for higher specify. It is activated by initial step (15 min. at 95 C°)(Cross et al., 1994.).

In our optimization experiments we used combination of this enzyme and corresponding PCR buffer (Qiagen). On this way, we obtained optimal results without any contamination, missprimed products and non-specific amplification products, as well.

By using of BIO-RAD multiplex RT-PCR for typing / subtyping leukemia, it is possible to detect new elektrophoretic bands as a result of new specific genes translocation.

For further characterisation of this genetic aberration is necessary to perform investigation by DNA sequencing (Springall et al., 1998.; Van der Reijden et al.,1995.).

Apstrakt: Molekularna tipizacija i subtipizacija leukemije korištenjem multiplex RT-PRC metoda, vezana je zbog praćenja minimuma rezidualne bolesti tokom i nakon tretmana pacijenata sa akutnom leukemijom. U ovom radu prezentirani su rezultati optimizacije ovoga metoda (proizvođač firma BIORD) dizajniranog za screening (RT-PCR) i subtipizaciju leukemije (split-out RT-PCR). Naime, ova procedura je prilagođena za PrkinEmlerGeneAmp 9600 PCR aparat i Qiagen HotStarTaq DNA polimerazu, te korištenje

drugih PCR aparata podrazumjeva optimizaciju procedure po osnovu odgovarajućih parametara, a u cilju dobijanja optimalnih rezultata. Za PCR amplifikaciju korišten je PE 2400 PCR aparat, kao i neophodna HotStarTaq DNA polimeraza. Ukupna RNA ekstrahovana je iz uzoraka cijele krvi dostavljenih od stručnjaka Klinike za hematologiju KCU – Sarajevo, uzete od pacijenata oboljelih od akutne leukemije. Nakon transfera RNA u cDNA, amplifikacije u PE 2400 pomoću odgovarajućeg seta primera (multiplex PCR), detekcije elektroforetskih separiranih amplificiranih PCR produkata u 1,5% agaroznom gelu sa etidium bromidom, optimalni rezultati su dobijeni korištenjem sljedećih optimizacijskih parametara :

- annealing temperature 65°C
- broj amplifikacijskih ciklusa za prvu i nested PCR amplifikaciju bio je 30
- finalni molaritet $MgCl_2$ – 3,28mM
- umjesto standardnih količina cDNA (5µl) i HotStarTaq DNA polimeraze (0,4µl), optimalni rezultati su dobijeni korištenjem 10 µl cDNA i 1 µl enzima.

Korištenjem ovakvih PCR parametara , detektovana je inv (16) hromosomska aberacija, a subtipiziranjem sa split-out PCR-om određen je subtip G (192 bp ele-kroforetska traka) što je upućivalo na CBFB/MYH1 fuziju gena.

Ključne riječi: leukemija, tipizacija/subtipizacija, fuzionisani genski transkripti multiplex RT-PCR screening i split-out RT-PCR.

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MALASSEZIA YEASTS AS COMMENSALS ON HUMAN SKIN

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Summary

Yeasts of the genus Malassezia are known to be members of the skin microflora of humans and other warm-blooded animas. Under the influence of predisposing factors they become pathogenic and are associated with several skin disorders and even systemic infections. The taxonomy of these lipophilic yeasts has recently been modified and includes seven species referred as *Malassezia*. The aim of this study was to analyze the prevalence of *Malassezia* species in normal skin and assess the distribution of the species according to patient sex and age. Forty subjects with clinically healthy skin were studied. The samples were obtained by scraping the skin surface of the scalp of all subjects and then incubated on modified Dixon agar. The yeasts isolated were identified by their morphological and physiological properties according to Guillot *et al* method. *M.sympodialis* was the predominant species on trunk skin and *M.restricta* on scalp skin. We found no differences between isolated species and the sex and age of the individuals.

Key words: *Malassezia*, species, healthy skin

Introduction

Genus *Malassezia* was created by Baillon in 1889 and has never been properly classified (1).

By means of molecular methods the genus was taxonomically revised and enlarged to seven species – in addition to *M.furfur, M.pachydermatis, and M.sympodialis,* four new taxa have been described, namely *M.slooffiae, M.globosa, M.obtusa,* and *M.restricta* (2-4). *M.pachydermatis* is the only non lipid-dependent species. It is considered zoophilic, because it is mainly isolated from animals (5), whereas the remaining six species are obligatory lipophilic and found primarily in humans (6).

The presence of *Malassezia* species in healthy human skin had been detected as early as the second half of 19th century (7). The frequency and density of colonization in healthy individuals is related to the age and to the activity of the sebaceous glands in the area studied (8). It has been demonstrated that *Malassezia* yeasts inhabit various body sites including scalp, forehead, nose, shoulders, abdomen, lower axillae, groin and forearm. Roberts reported that 97% of clinically healthy people carry fungus on the scalp and 92% on their trunk (9). However, under the influence of predisposing factors they become

pathogenic and are associated with several diseases such as pityriasis versicolor, folliculitis, seborrheic dermatitis, confluent and reticulate papillomatosis, and even systemic infections (10).

The aim of this study was to analyze the prevalence of *Malassezia* species from clinically normal skin of the scalp and trunk and to examine if the range of species varies with patients sex and age.

Patients And Methods

Patients

This prospective study was conducted at the Department of Dermatovenerology, University Clinical Centre, Sarajevo, Bosnia and Herzegovina, during the period from April till December 2001. Forty individuals with clinically healthy skin (20 women and 20 men; age range, 13-76 years) were included in the study.

Samples

All samples consisted of scales and scrapings from the upper and middle part of trunk and from scalps in all participants. Collected samples were divided into two portions – one for microscopic examination and the other for culture.

Microscopic examination of the samples was performed after the treatment with lactophenol solution.

The samples for culture were inoculated on modified Dixon agar consisting of 3.6% malt extract, 0.6% mycological peptone, 2.0% desiccated ox bile (Sigma Chemical Co. Ltd, UK), 1% Tween 40, 0.2% glycerol, 0.2% oleic acid, 0.05% chloramphenicol, 0.05% cycloheximide, and 1.2% agar pH 6.0. The medium was always used within one week of preparation and the cultures were incubated at 32°C for seven days.

Identification of Malassezia yeasts

Malassezia species were identified according to the scheme established by Guillot *et al* (11), and their macroscopic and microscopic features and physiological properties were recorded. The macroscopic features of the predominant colonies included their shape, size, color consistency, and the characteristics of the medium around them. Microscopic features of the yeast cells in cultures were described after lactophenol staining and included the predominant morphology, size, and budding base of the yeasts. To assess the physiological properties of the yeasts, catalase reaction was used. A drop of

hydrogen peroxide (30% solution) was added to a culture smear on a glass slide. The production of gas bubbles, indicative of release of oxygen, was considered a positive reaction.

Utilization of Tween compounds was done according to the test originally described by Guillot *et al* (11) and later modified by Gupta *et al* (12, 13).

Statistics

Chi-squared test with Yates' correction for a small sample size was carried out to determine the statistical significance of differences in proportions. We used a statistical software package Minitab 13.0. Significance level was set at $p<0.05$.

Results

Direct Microscopy

Direct microscopic examination of scales from trunk showed the presence of yeast cells in 25 patients (62,5%), but short filaments were observed in only one (2,5%). Spherical and large yeast cells with a narrow budding base were found in 9 patients (22,5%). Smaller yeast cells, oval or cylindrical in shape, were seen in remaining 20 cases (50%). In 11 slides (27,5%) neither yeast cells nor hyphae were observed (Table 1).

In the scales from healthy scalp skin, yeast cells were observed in 21 patients (53%), whereas the remaining 19 cases (47%) were negative. Short filaments were seen in only one patient. Oval and cylindrical yeast cells dominated; they were found in 14 cases (35%). Spherical yeasts were recorded in 7 slides (18%).

No significant statistical differences were found in the direct microscopic findings from healthy trunk and scalp skin (Table 1).

	HEALTHY TRUNK SKIN			HEALTHY SCALP SKIN		
	F	M	Σ	F	M	Σ
Filaments	1	0	1	1	0	1
Yeast cells	11	13	24	11	9	20
Negative	8	7	15	8	11	19
Total	20	20	40	20	20	40
F= female; M=male; Σ= total;						

Table 1. Direct microscopic examination of scales from trunk and scalp of normal subjects

Cultures

Malassezia yeasts were found in 29 (72,5 %) samples taken from healthy trunk skin. The most frequently isolated species was *M.sympodialis* found in 12 (30 %) patients, followed by *M.globosa* (22,5 %), *M.furfur* (17,5%) and *M.restricta* (2,5 %). The percentage of negative cultures was 27,5 %.

The results of culture obtained from healthy scalp skin were positive for *Malassezia* yeasts in 26 (65%) cases. The predominant species was *M.restricta,* found in 12 (30%) patients and the prevalence of other species was 17,5% for *M.globosa,* 10% for *M.sympodialis,* 5% for *M.slooffiae,* and 2,5% for *M.furfur.* The remaining 14 (35%) cultures showed no growth of the colonies (Table 2.).

Statistically significant differences were found in the distribution of the species isolated from healthy trunk and scalp skin – *M.sympodialis* was more frequent in the healthy trunk skin cultures (respective ratio 3.0), whereas *M.restricta* was more commonly positive in healthy scalp skin cultures (ratio 12.0) (Table 2).

	HEALTHY TRUNK SKIN			HEALTHY SCALP SKIN		
	F	M	Σ	F	M	Σ
M. globosa	2	7	9	3	4	7
M. sympodialis	7	5	12	1	3	4
M. furfur	4	3	7	1	0	1
M. obtusa	0	0	0	0	0	0
M. slooffiae	0	0	0	0	2	2
M. restricta	1	0	1	8	4	12
M. pachydermatis	0	0	0	0	0	0
Negative	6	5	11	7	7	14
Total	20	20	40	20	20	40
F= female; M=male; Σ= total;						

Table 2. *Malassezia* species obtained from trunk and scalp skin of normal subject

Distribution of Species Isolated from Healthy Trunk Skin According to Relevant Parameters

Sex

The same number were woman and man (50%).

No statistically significant differences were found between sexes in the species isolated (Fig. 1).

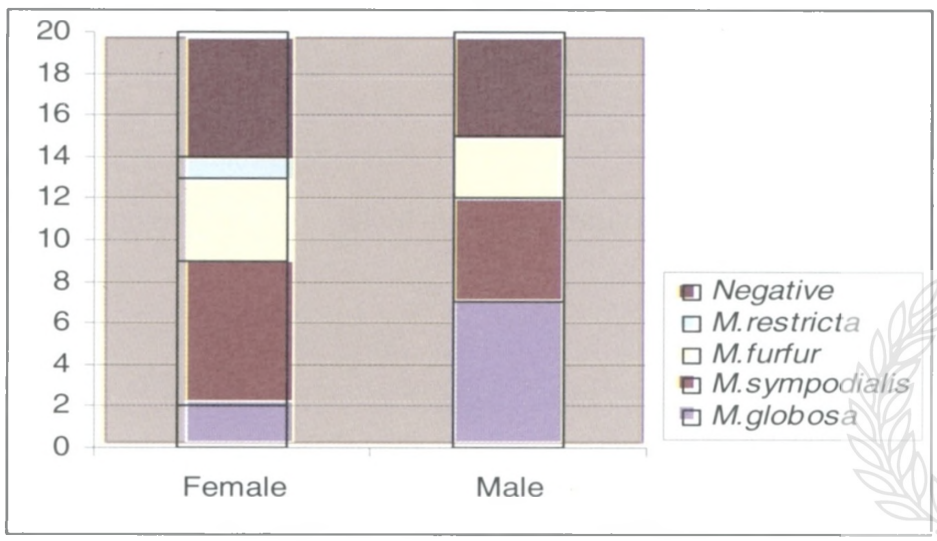

Figure 1. *Malassezia* species distribution from healthy trunk skin according to patient sex.

Age

According to age, patients were divided into five groups, as follows: ≤15 (n=3; 7,5%), 16-30 (n=10; 25%), 31-45 (n=9; 22,5%), 46-60 (n=8; 20%), and ≥61 years of age (n=10; 25%).

No statistically significant differences were found in the species isolated in these five groups (Fig. 2).

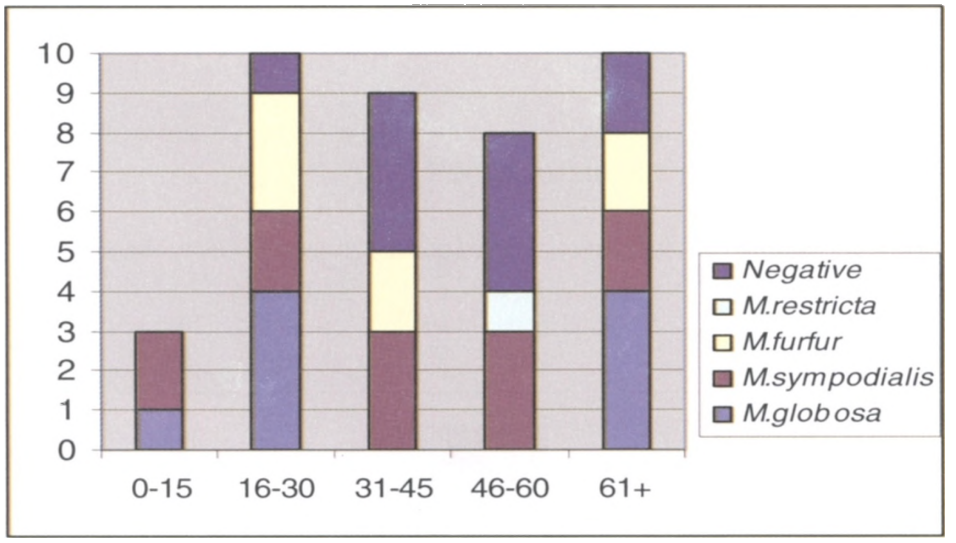

Figure 2. *Malassezia* species distribution from healthy trunk skin according to patient age.

Discussion

A number of investigators have conducted studies of *Malassezia* colonization of healthy skin (9, 10, 14, 15). Such studies have demonstrated that these yeasts are unique among the fungal kingdom as the only species to form part of normal human cutaneous commensal flora. Since the description of the new species some studies have focused on their distribution on normal skin.

We found that the predominant species on normal trunk skin was *M.sympodialis* isolated in 30% of cases. This species emerges as the predominant species on healthy skin, especially on the trunk, where it can be recovered in great numbers in more than 60% of individuals (16, 17). *M.globosa* is less common species found in 22,5% of healthy individuals. This species is reported to be a causative agent of pityriasis versicolor, found in filamentous form in the scales from this skin disorder (13, 16). Also in Russia, Arzumanian found *M.sympodialis* to be the most common species on the skin of 32 individuals, whereas *M.globosa* encountered much less frequently (18). *M.furfur* was isolated in 17,5% and this species is found to be less common inhabitant of healthy skin (10, 13, 15).

In contrast to healthy trunk skin, *M.sympodialis* was recovered less frequently from the scalp skin of same subjects, whereas *M.restricta* was the commonest

species. This species is isolated regularly from the scalp and face of patients with seborrheic dermatitis and normal individuals (10, 12). Aspiroz *et al.* in Zaragoza also found *M.restricta* to be particularly associated with scalp and *M.sympodialis* with the back, whereas *M.globosa* was evenly distributed on the scalp, forehead and trunk (19). The investigators in Canada also found that *M.sympodialis* was the commonest species in 20 healthy control subjects. *M.globosa* was equally likely to be recovered from the scalp and forehead and trunk, but less so from the arms and legs, and *M. restricta* and *M.slooffiae* were recovered more freq gently from the scalp and forehead than from the lower body (12). In our study *M.slooffiae* was found on scalp skin in only two subjects.

M.obtusa and *M.pachydermatis* were not recovered from any of our samples either from trunk or from healthy scalp skin. However, *M.obtusa* is considered to be very rare and only infrequently isolated from the cases of pityriasis versicolor, atopic dermatitis, and seborrheic dermatitis (12). *M.pachydermatis* is confirmed to be clearly adapted to animals, although it has been involved in some systemic human infection (8). The presence of this species on human skin is rare and transient, occurring possibly by transmission from animal pets and environmental sources (20, 21).

A correlation between the prevalence of *Malassezia* species and the age and sex of the subjects has been observed with a low recovery in infants and the moreover occurring at puberty but without differences among the woman and man (15). In our study all *Malassezia* species were identified equally in both sexes and according to the age.

Conclusion

Our results suggest that *M.sympodialis* is the most frequent isolated species from healthy trunk skin, whereas from scalp skin it is determined to be *M.restricta*. *M.globosa* and *M.furfur* are found to be less frequently isolated species. We found no differences between isolated species and the sex and age of the individuals.

Apstrakt: Kvasnice iz roda *Malassezia* čine dio normalne mikroflore kože ljudi i toplokrvnih životinja. Pod utjecajem predisponirajućih faktora postaju patogene i udružene sa nekim kožnim oboljenjima, pa čak i sistemnim infekcijama. Taksonomija ovih lipofilnih kvasnica nedavno je modifikovana, tako da obuhvata sedam specijesa, označenih kao *Malassezia*. Cilj ovog istraživanja jeste utvrditi prevalencu *Malassezia* specijesa na normalnoj koži i utvrditi njihovu distribuciju u odnosu na spol i dob pacijenata. Ispitivano je četrdeset pacijenata sa klinički zdravom kožom. Uzorci kod svih ispitanika su dobiveni struganjem površnog dijela kože sa gornjeg i središnjeg dijela trupa i sa vlasišta, a potom inkubirani na modificirani Dixon agar. Izolirane kvasnice identifikovane su na osnovu njihovih morfoloških i fizioloških osobenosti prema metodi Guillota i suradnika. Nađeno je da je *M.sympodialis* najdominantnija vrsta izolirana sa kože trupa, a *M.restricta* sa kože vlasišta. Nije nađena razlika u izoliranim specijesima u odnosu na spol i dob pacijenata.

Ključne riječi: *Malassezia*, specijesi, zdrava koža

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REPORTS:
International Symposium:

GENETICS IN PSYCHIATRY
DUBROVNIK, August 28th – 30th, 2004

WORDS OF WELCOME

Loga Slobodan, MD, PhD, Corespodent member of ANUBiH

Dear colleagues, dear friends, Ladies and Gentlemen!
I am delighted to welcome all those of you who have chosen accepted our invitation to be part of this Symposium "Genetic in Psychiatry".
It is my great privilege to greet all of you in behalf of Academy of Sciences and Arts of Bosnia and Herzegovina.
The Department of Medical sciences of ANUBH, more precisely, the Board for Neurological and psychiatric investigation, took over the responsibility for organising first scientific conference in Dubrovnik about very important, and up-to-date topic – genetics in Psychiatry. There is a growing need in our society for good experts in the field of genetics in psychiatry. As a matter of fact, ANUBIH is a member of IUC in Dubrovnik and this event represents our first joint activity. We are very grateful to ANUBiH, especially its president Božidar Matić for that decision and support that he gave us in organising this conference.

I am very thankful for all lectures, respected people, who have found time to come here and give very important lectures during this conference. We are especially happy for the fact that our partner at this conference is prof. Maier, the head of Department of Psychiatry, University of Bonn. Greetings for prof. Mayer.

We are also thankful to all participants for their coming to this conference.

We hope that you will find time, in the course of our not too condensed program, for visiting Dubrovnik, one of most beautiful places in the world. I hope that your stay in Dubrovnik will be a pleasant one.

Together with my colleagues in Organising committee I warmly welcome you and wish you all a lot of success.

INTRODUCTION TO SYMPOSIUM "GENETICS IN PSYCHIATRY"

Slobodan Loga
Correspondent member of Academy of Sciences and Arts of Bosnia and Herzegovina

The field of genetics is very huge and if we try to describe it in short: mapping the human genome, cloning animals, and identifying new disease genes. For many of us who are in every day psychiatric practice, the problem is putting this information into context of our work and understanding what recent genetic findings really mean in terms of mental health. To help answer these questions, we have organized this symposium primarily to help our understanding how advances in genetics can help in the treatment of psychiatric diseases.

With the human genome decoded, researchers in field of psychiatric genetics have the task through the newly discovered genes in search of those that lead to disease. The field of psychiatric genetics has become one of the most interesting areas of psychiatric research. These efforts we hope will change the way how we see diseases and their treatment in psychiatry.

The Human Genome Project recently announced that they had sequenced all of the human chromosomes — about three billion bases long. In itself, this is a huge achievement, but knowing the sequence doesn't tell us what the 30,000 to 50,000 genes actually do in the body. Their next step is to figure out what the genes do, and what role these genes may play in disease.

Complex genetic conditions, such as diabetes, heart disease, most common cancers, autoimmune conditions, and psychiatric conditions are result from the interplay of environment, lifestyle, and the effects of many genes. They may be present in more than one family member but don't follow the characteristic inheritance pattern seen with Mendelian conditions.

Familiar, twin, adoption and linkage studies represent the usual tools for assessing the possible role of genetics in mental disease. While there is no doubt that schizophrenia, mood disorders and autism are characterized by a genetic component no linkage study has been successful up to date, apart, probably, the case of autism. It is also evident that no major genes are responsible for these psychiatric diseases: thus, quantitative trait loci analyses might prove fruitful in future research to track the role of different genes contributing to the outcome of different psychopathologies. The main problem, however, is the difficulty of arrying out quantitative analyses since the today's diagnostic tools do not allow a quantitative approach to these phenotypes.

There now exists growing evidence of candidate gene sites for a variety of psychiatric disorders ranging from schizophrenia to reading disability.

Genetic researches in the field of psychiatry try to find the impact on:

- diagnostic
- risks assessment
- prevention and treatment strategies

We are taking family medical history for patients is our everyday task. This helps us to tell if there are genetic factors that may influence a patient's health. But a family history does not provide us with the complete picture of a person's risk. Some people may be at risk for an early-onset disease, as schizophrenia for example, even if they do not have a family history of that disease. Also, we know from many cases that not every person with a strong family history of a disease will inherit the risk factors.

We are now just at beginning to be able to screen people for genetic risk factors present in their family (Tab. 1). In the future, it may be possible for psychiatrist to screen their patients for many genetic-based risks. We hope that individual risk assessments will be created for each our patient based on that patient's set of genes.

In case when we know which genes are involved in a disease, it would be possible develop a test to screen people who are at risk and also start looking for a cure. A diagnostic test by itself will not cure the disease, but it can help identify high-risk people who may require more intensive screening or preventative action. Knowing what genes cause a given disease can also help us understand what goes wrong in that disease, which can help drive the search for drugs that counteract the problem.

Knowing personal risk of potentional patients makes it possible to decrease their chance developing the disease through lifestyle changes, and preventative medical treatments. We know in field of mental health that risk for almost any condition is a function of both our genes and our environment. While we can't change genes, we can apply our knowledge of family medical history to predict risk for specific problems. This, in turn, allows us to focus on the things we can change — diet, lifestyle, screening, treatment — to ensure a long, healthy life.

TABLE 1. *Recurrence risks for psychiatric disorders (1)*

	Life prevalence	Risk to first degree relatives of affected individuals
Schizophrenia	1.0 -1.9%	4.4 -13.8%
Bipolar Affective disorders	0.8 -1.1	4.0 – 9.0%
Severe Unipolar Depression	4%**b** 8%**c**	9%**b** 18%**c**
Panic disorder	1.5 -3.5%	15% -24.7%
Alcohol dependence	14%**b** 3%**c**	27%**b** 5%**c**

b risk to males **c** risk to females

Despite the progress in molecular genetics, the genes responsible for the development of bipolar disorder (BPD) and schizophrenia have not yet been identified. This failure can be attributed to an ambiguous phenotypic description and several variations in the genetic transmission of these diseases. There is a growing consensus that an endophenotype approach may be utilized to overcome the difficulties regarding the phenotypic description and facilitate the identification of the susceptibility or protective genes. The endophenotypes which can be defined as subclinical vulnerability markers may assist in the identification of the genetic underpinnings of psychiatric disorders regardless of the disease status.

Twin studies have demonstrated that the rate of autism is much higher in identical than in non-identical twins of individuals with autism. This is taken to support the opinion of a strong genetic contribution of autism. Siblings of the children with autism have a much increased risk of themselves having autism. About one in twenty of full siblings of individuals diagnosed as suffering from autism have autism (in general population: one in thousand). This is a high risk

given that genetic stoppage occurs in autism. Genetic stoppage means that families who have a child with a severe disorder – such as autism – tend to have fewer children than do those who have normal children (2).

Genetic regulation of immune response showed that it is apparent that genes on at least three chromosomes can contribute to antigen recognition by T cells. Three highly polymorphic gene families are likely to play a role in any autoimmune disease of the nervous system: human leukocyte antigen (HLA) genes, T-cell receptor genes, and immunoglobulin genes (3).

Preliminary results of research into the pharmacogenetics of psychotropic drug response suggests that specific genes may influence phenotypes associated with psychotropic drug administration. These results require further validation.

Genetic advances pose new and unique ethical dimensions to the familiar issues of privacy, confidentiality, access to and justice in health care, and informed health decisions. This means ensuring privacy and confidentiality of all genetic information.

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INTERNATIONAL SYMPOSIUM GENETICS IN PSYCHIATRY

Organized by
Academy of Sciences and Arts of Bosnia and Herzegovina
And
The Department of Psychiatry
University of Bonn
IUC Dubrovnik, August 28th – 30th 2004

CONCLUSIONS

The Symposium on "Genetics in Psychiatry" highlighted the currently rapid progress in unraveling the molecular and genetic basis of major psychiatric disorders and their treatment. Less than two years before the first three susceptibility genes for schizophrenia (dysbindin, neuregulin 1, and G72/30 together with DAAO) were discovered by systematic search including linkage and association studies, and confirmed, the first susceptibility gene for bipolar disorder for bipolar disorder also emerged, most interestingly, a gene which also presents as susceptibility gene for schizophrenia. Neither of the implicated genes or any of its products have been previously postulated to be implicated in the etiology of psychoses. Another conclusion is that both disorders are polygenic with multiple modulate effect genes but without a major gene. Thus this success story demonstrates that our knowledge on the up till now widely unknown pathophysiology of these disorders can rapidly expand in fully unexpected directions. The understanding of action of effective pharmacologic treatment will also greatly increase.

The plenary lectures by Wolfgang Maier and Marcela Rietschel at the Symposium illustrated these innovative developments in an historical context. It became apparent that the relationship between at-risk haplotypes of susceptibility genes and the targeted disorders are substantially more complex than expected. Apparently, neurobiological correlates of the disorder (like hippocampal volume reduction) and candidate core symptoms (like persecutory delusions) are more directly related to the predisposing genetic variations than the clinical diagnostic entities as defined by DSM IV and/or ICD 10. It was also indicated that the bottleneck for further progress is in the availability of informative samples.

The Symposium focused on the contribution of research groups form Bosnia and Herzegovina, Bulgaria, Croatia and Slovenia to this exciting development: Berberović, the leading quantitative geneticist from Sarajevo together with Bojan Šošić, demonstrated the strong genetic heritability of the complex

disorder, schizophrenia by reference to biometrical multifactor models, which have been greatly confirmed by the recent molecular genetic progress.

Radka Kaneva from Sofia demonstrated the utility of isolate populations for finding disease genes in bipolar disorder. She collected multiple Gipsy families with multiple affected and performed a genome wide linkage analysis with very strong linkage signals which are revealing substantial agreement with other genome wide scans. The major advantage of her approach is the homogenous population, genetic background, which might accelerate the identification of not yet uncovered susceptibility genes.

Population genetics in Bosnia and Herzegovina was the topic of a very carefully conducted study by Pojskić and Kapur from Sarajevo who demonstrated that the geographical variation of genetic markers is even stronger than the interethnic variation in Bosnia and Herzegovina. This result allows to consider the genetically influenced disorders in Bosnian population independent of ethnic background.

The ambitious recruitment program by Oruč in Sarajevo focusing on trio-families of bipolar disorder can therefore rely on a demonstrated homogenous population genetic background. This recruitment program will be a very promising starting point to study the molecular genetic basis of affective disorders in Bosnia and Herzegovina. This project is important, as it will allow to discussing the variability of genetic basis of complex diseases across population.

A population specific view on complex genetic disorder is also required because of gene and environmental interaction; environment is strongly dependant on cultural factors and the society as Marušič from Ljubljana demonstrated by reference to suicidal attempts and completed suicide. It is now evident that suicidal behavior is genetically influenced. Although specific susceptibility genes have not yet been identified with certainty, environmental factors are known. Particularly alcohol availability and consumption increases suicidal risks, which is also reflected by differential incidence rates across countries. But environment is obviously not the only source of variation in national suicide frequencies; similar genetic background (Fino-Ugrian) produces a comparable excess in suicide risk in spite of different cultural backgrounds.

The beneficial effects of the progress in genetic research for our patients will come from the understanding of genomic effects of available efficacious treatments. The currently growing knowledge will, as Jakovljević from Zagreb

demonstrated – help o better predict treatment outcome and to identify the crucial molecular targets of treatment which are essential for successful outcome. Thus it can be expected within the upcoming years to use genetic markers to select the most appropriate drug for an individual patient and to develop new, more causal and therefore more effective treatments.

Genetic markers may also help to predict the conversion to psychosis in subjects presently with prodromal symptoms as Džubur-Kulenović from Sarajevo demonstrated. Thus early treatment will become possible targeted to prodromal subjects with high, genetically mediated risk for schizophrenia. Neurodegeneration which might occur early in the course of schizophrenia might be prevented by these means and the clinical long - term course of psychosis can be modified too the benefit of the patient.

Spahić-Mihajlović from Chicago discussed the relationship between schizophrenia and diabetes and the metabolic syndrome. Both conditions are genetically influenced as well as this might be the case for their excess co-morbidity. Atypical antipsychotics have specific impact on this relationship, but this is even more the case for conventional antipsychotics. However, the variety of risk factors is also operating.

The Symposium also discussed humanistic and conceptual views that are challenged by the molecular genetic revolution. Kulenović from Sarajevo reminded the audience to the origins of molecular understanding of life by reference to the great biologist and most innovative scientist and thinker in the filed: Jacques Monod. Life and evolution of nature and humans is not only under the control of causal mechanisms, the necessity principle. There is also space for Randomness and Freedom. Thus, our future is not predetermined.

However, there are serious concerns and fears that the human nature might be manipulated by gene technological interventions. With unpredictable – but in any case dangerous outcome as Novaković from Bijeljina argued. Thus it is evident that the ethics of molecular genetic research in disorders and of the dissemination of research results to affected and the public require substantially more attention than it currently receives.

The participants of this high-quality exciting conference hope that genetic research in psychiatry is stimulated particularly in South East Europe. It becomes evident that the success chances in this field are high.

PROGRESS ON THE GENETICS OF SCHIYOPHRENIA AND BIPOLAR DISORDER

Wolfgang Maier and Marcella Rietschel

Introduction

The distinction between schizophrenia and bipolar disorder was historically based on distinct phenomenologies and long-term courses. A differential nosology and etiology was postulated, however never convincingly proven. The dichotomic postulate was maintained despite of striking similarities in prevalence rates and risk factors between both "lifetime" disorders: Lifetime prevalence rates are similar (- 1%) and stable across countries and cultures, male-to-female ratios of affected subjects are balanced, age at onset reveals a broad overlap in the age period of 18-30 years. However, there is also evidence for differences in risk factors (Mortensen et al., 2003): e.g., premorbid IQ score presents a risk factor for schizophrenia but not so for bipolar disorder (Zammit et al., 2004).

Both disorders are under genetic control with very similar recurrence rates of 5-10% for siblings and parents, similar concordance rates (- 10% for DZ and > 50% for MZ twins), and about 60-80% of the variance being influenced by genetic variants in both disorders, and the remainder being due to individuum-specific environmental factors which are not shared among the twins; a monogenic, Mendelian transmission cannot be observed for any of both disorders; both also reveal genetic-familial bonds to unipolar depression; variation of prevalence/incidence rates by season of birth seems also to be a characteristic of both disorders.

The similarity in risk factors between both disorders is now extended by neurobiological communalities: There is rapidly growing evidence that the psychopathology or/and the nosologically based disease entities are not based on distinct pathogenic processes. For example, both disorders share morphometric features as enlarged ventricles and reduced hippocampal volumes, neurophysiological patterns as reduced amplitudes of evoked potentials (as P300), and various memory dysfunctions. Recently, common cellular and molecular patterns were observed: e.g., glutamate-mediated excito-toxicity in the cingulum (woo et al., 2004). Similar patterns of gene expression in post-mortem hipoocampal tissues (e.g., reduced GABA-ergic markers or neurotrophic factos as BDNF; Knable et al., 2004) pointing at similar oligodendrocyte dysfunction (Tkacher et al., 2003), or similar alterations in MRNA of receptors in crucial

prefrontal and hippocampal areas pointing at similar dysfunctions in signal transduction (Lopez-Figueroa et al., 2004) are common between schizophrenia and bipolar affective disorders. Furthermore, in both disorders similar abnormalities of intracellular molecules linking different neurotransmitter systems with intracellular enzymes (like PSD95) that mediate signaling and provide links between different neurotransmitter systems were found, e.g. in key areas of the thalamus (Clinton and Meador-Woodruff, 2004). These neurobiological communalities are supplemented by the recent progress in genetic research.

We review the current evidence of a genetic relationship between schizophrenia and affective disorders on an epidemiological and molecular level.

Family and twin studies

For many decades familial aggregation of schizophrenia and bipolar disorder without cosegregation of both disorders was cited as a strong argument for the dichotomic position. The empirical proof of this argument requires family and twin studies covering simultaneously bipolar disorder and schizophrenia in comparison to controls. Only a very few family studies fit these criteria. The empirical evidence in favor of a nosological dichotomy of schizophrenia and bipolar disorder emerging from these studies is not in agreement with a dichotomic position. A hallmark stdy, the lowa-500 study by Tsuang et al. (1980), revealed an excess of bipolar disorder in families of probands with schizophrenia. Other controlled family studies observed a link of Schizophrenia as well as of bipolar disorder to unipolar depression (Gershon et al., 1988; Maier et al., 1993), or an aggregation of psychotic affective disorders in families of probands with schizophrenia and bipolar disorder (Kendler et al., 1993). More recent family reports producet an even more distinct overlap of bipolar disorders in families of schizophrenic patients and vice versa (Valles et al., 2000; Maier et al., 2002). The most recent extended study in siblings was conducted trough case registers in New Zealand (Osby et al., 2003, verbal communication) reporting a relative risk (odds ratio) of 3.6 (2.9-4.4) for bipolar disorder among first-degree relatives of probands with schizophrenia, and of 4.4 (3.5-5.4) for schizophrenia among first-degree relatives of probands with bipolar disorder (comparator: unaffected controls). In comparison, the recurrence risks for schizophrenia in relatives was increased by a factor of 7.4 (6.8-8.1), and for bipolar disorder in relative by a factor of 12.8 (10.6-15.3). given that diagnoses were derived from registers, misclassifications cannot be excluded and possibly influenced the magnitude of reported results However, other studies with extremely carefully

validated diagnostic rocedures are pointing in a qualitatively similar direction. High-risk studies in offspring of probands with schizophrenia in comparison to probands with affective disorders demonstrated excess cosegregation: more cases with psychotic disorders in kids of affective disorder probands, more cases with affective disorders in kids of schizophrenia parents compared to the general population (Erlenmeyer-Kimling et al., 1997). The reanalysis of the Maudsley twin series demonstrated that the overlap of familial vulnerabilities is due to genetic factors shared between schizophrenia and mania (Cardno et al., 2002): diagnosis-specific additive genetic variance was reported as 33% for schizophrenia, and as 19% for mania, compared to 49% and 68% respectively of common additive genetic variance.

There is also symptomatic overlap between bipolar disorder and schizophrenia: psychotic affective disorders share both symptom clusters. It might be suggested that this symptomatic subgroup combines the two disorders and carries the genetic vulnerabilities to each of them. Thus, clustering of psychotic symptoms in families of patients with psychotic affective symptoms have to be explored. This possibility might at least partly explain excess risk for schizophrenia in relatives of bipolar probands; the empirical evidence for this constellation, however, is controversial (Tsuang et al., 2004).

Furthermore, aggregation of bipolar disorder in families of schizophrenic patients could be restricted to psychotic bipolar disorder which might put psychotic bipolar disorder in a distinct nosological position. Empirical evidence for these possibilities is available (e.g., Kendler et al., 1993; Maier et al., 2002) but not consistently so.

Despite of this inconsistency, there is now growing evidence of a cosegregation between affective disorders (including bipolar disorder) and schizophrenia.

Search strategies for disposition genes

Both, schizophrenia and bipolar disorder are genetically complex diseases driven by genetic as well as environmental factors. The search of disposition genes can be accomplished by linkage and association studies: Linkage studies identify candidate regions which are likely to host disposition genes. Candidate regions might cover broad areas on the genome (10-30 cMorgans corresponding to 10-30 milion base pairs).

Association studies identify smaller regions with markers in linkage disequilibrium including those for disposition genes Linkage disequilibrium describes dependency between the distribution of two genetic markers on the same chromosome; it is dependent on the sampled population and ranges up to 100.000 base pairs in the mean in Caucasian populations, however, meaningful genetis analyses assume linkage disequilibrium of 15.000 (Schulze et al., 2004). Thus, the associated marker is not necessarily the directly influential variant; the marker is, however, in linkage disequilibrium with the pathogenic mutation. Association studies can be parformed only with preselected specific candidate genes or can be performed across limited regions on a chromosome; genome-wide association studies require high through-put technologies with a very high number of markers (> 1 milion) and are currently not yet feasible. Given the high risk for false positives, replication of postulated linkage and association findings are obligatory for their validity.

Currently, there are confirmed linkages between schizophrenia and markers in specific candidate regions and confirmed association findings in some candidate regions; some of them were also found to be linked to bipolar disorder. We are exploring the possibility of genetic variants whicht exert pleitropis effects by influencing both schizophrenia and bipolar disorder. How can linkage and association studies contribute to the clarification of this possibility? Is the overlap between candidate regions for bipolar disorder and for schizophrenia informative to this question? Is the association of a marker to schizophrenia as well as to bipolar disorder able to provide evidence for common genetic determinants?

Linkage analyses

Genome-wide linkage analysis is a most efficient tool in detecting causal disease genes in monogenic disorders. This technique is less efficient in genetically influenced complex diseases with multiple contributing genes. Initially, a high degree of inconsistency was noted among implicated candidate regions in about 20 schizophrenia and about 18 bipolar disorder scans, and the appropriateness of this strategy for genetically complex disorders was critically discussed (DeLisi et al., 2002). Most investigated samples, however, were underpowered to be able to replicate postulated linkages to candidate regions. Metaanalyses combining all published genome-wide scans provide a feasible opportunity to circumvent this limitation.

Two meta-analyses were performed for schizophrenia as well as for bipolar disorder; fortunately, the data analysis was perfomed in parallel for

schizophrenia and bipolar disorder allowing conclusions on the overlap of candidate regions. Overall, both metaanalyses conclude that there is substantially more consistency than expected on the basis of a comparison between suggestive linkage results of specific linkage studies. However, the analytic technique for a metaanalysis of linkage studies is not straightforward: Given the variation of linkage disequilibrium between nearby markers across populations the positional information of a linkage signal is not accurate. We have two sources of evidence for linkage on the basis of multiple studies: (a) the actual linkage scores combined across various studies at a specific marker locus, and (b) the aggregation of linkage signals in a small region with multiple markers in linkage disequilibrium. The various techniques of metaanalyses give differential weight to these alternative rationales resulting in different candidate regions. Yet, as both modes of reasoning are appropriate, all metaanalytic conclusions based on different analytic techniques might be true. Yet, two different analytic methods were applied resulting in different confirmed linkage findings:

Badner and Gershon (2002) used only a subset of published genome scans and found strong evidence for linkage

(a) for schizophrenia on 13q31 and 22q11-13, with the 13q-linkage showing strongest evidence ($p < 6x10 -6$),

(b) for bipolar disorder on 8p22, 13q31 and 22q 11-13, with the 13q-linkage showing the maximal evidence ($p < 7x10-5$).

Thus, most of the identified candidate regions (22q and 13q) with strongest evidence (i.e., genome-wide statistical significance) reveal an overlap between both disorders.

The more comprehensive meta-analyses by Lewis et al. (2003) for schizophrenia and by Segurado et al. (2003) for bipolar disorder found strong evidence for linkage only for schizophrenia; evidences for linkage to bipolar disorder were only moderate. Ignoring this difference the strong and/0r moderate evidence for linkage was found in the following chromosomal regions:

(a) for schizophrenia primarily on 2q, but also on 5q, 3p, 11q, 6p, 1q, 22q, 8p, 20q and 14p,
(b) for bipolar disorder on 10q 11.21-22, 9p22-21, 14q24-32.

Thus, just considering the strongest p-values for candidate regions no overlap of candidate regions between schizophrenia and bipolar disorder can be concluded

according to this more comprehensive analyses. Yet, there is still aconsidrable number of overlapping candidate regions if nominal p-values (P avgRnk > 0.05) are also considered:

- 2q22. 1-q23.3 for both disorders,
- 8p22 (8pter-p22 for bipolar disorder, and 8p22-p21-p21. 1 for schizophrenia),
- 14q13.1 (14q13.1-q24. 1 for bipolar disorder, and 14pter-14q31.1 for schizophrenia).

These are three overlapping candidate regions among 12 candidate regions for schizophrenia and 21 candidate regions for bipolar disorder with nominally significant pvalues.

Previously, a systematic review of the published genome scans exploring regions of overlap between replicated diagnosis-specific candidate regions prposed five common linked regions (Berrettini, 2003): 18p 11.2, 13q32, 22q 11-13, 8p22, 10p14.

Thus , although the combined analysis of available genome-wide linkage analyses provided different results depending on the applied method, a considerable overlap of validated candidate regions between schizophrenia and bipolar disorder can be observed – for all modes of biometric analyses.

Pleiotropis genetic effects with a specific DNA-sequence variation influencing the manifestation of tvo different disorders can only be proven if the pathogenic mutation is known. Linkage to the same candidate region and association to the same marker can only propose the possibility of common genetic determinants.

Cytogenetic abnormalities

Several defined cytogenetic alterations of the DNA string reveal major psychiatric syndromes. Most extensively explored are a translocation on chromosome 1q, and chromosome 22q microdeletions. It is most noteworthy that both abnormalities are associated with the occurrence of schizophrenia as well as of affective disorders in the same families.

A balanced reciprocal translocation (1;11) (q42; q14.3) was found to cosegregate with psychotic syndromes in a large Scottish family (Blackwood et al., 2001). Strong linkage at this region q42; q14.3) was found to cosegregate with psychotic syndromes in a large Scottish family (Blackwood et al., 2001).

Strong linkage at this region q42 was calculated for schizophrenia (LOD score = 3.6), and even higher for affective disorders (LOD score = 4.5); linkage can be maximized by considering all kinds of psychotic and affective disorders as affected (LOD score = 7.1). A refined clinical analysis reveals that the DISC phenotype includes schizophrenia, schizoaffective disorder, bibolar disorder and recurrent major depression but also neurophysiological abnormalities in absence of clinical diagnoses; particularly, the most common abnormality among carriers of the balanced t (1; 11) translocation shows a reduced P300 amplitude in response to an oddball discrimination task which occurred in carriers with the various mentioned diagnoses as well as in unaffected carriers (Blackwood and Muir, 2004). This neurophysiological abnormality is also consistently observed in schizophrenia but also in affective disorders (friedman and Squires-Wheeler, 1994; Pierson et al., 2000).

The translocation disrupts two genes which were called DISC1 and DISC2; they might be involved in the cytoskeletal regulation which is relevant for neuronal development, neuronal architecture and intracellular transport. In particular DISC1 interacts with a variety of cytoskeletal proteins, some of them are associated with cortical development (Ozeki et al., 2003). The translocation is up to now only observed in a single family. Could the family-specific linkage be of more general relevance? The answer is yes, as the translocated 1q region is closely located to markers showing linkage to schizophrenia in two Finnish samples (Hovatta et al., 1999; Ekelund et al., 2001). Thus, the DISC 1 and DISC2 genes are hot candidates for susceptibility genes of major disorders. Yet, the pathogenic mutations still have to be identified.

The Velo-Cardio-Facial syndrome (VCFS) – also called DiGeorge syndrome – is a monogenic disorder caused by interstitial deletions in a specific region of chromosome 22:q11. This syndrome is characterized by facial malformations and congenital heart disease. It reveals a sharp excess of prevalence in major psychiatric syndromes with more than 25% (Bassett et al., 2001) – mainly with severe psychotic and affective disorders which are similar to both schizophrenia and bipolar disorder (Carlson et al., 1997). These
disorders are also segregating and cosegregating in the VCFS families.

Given the very small prevalence (> 1%) of VSFS in the general population, the impact of this syndrome-specific genetic association on schizophrenia as vell as on bipolar affective disorders might be negligible. Yet, the 22q 11 microdeletions are also slightly more common in unselected samples of patients With schizophrenia (2% compared to 1% among 4000 in the general population) (Scambler, 2000); similar figures for bipolar disorder are not

available. Although linkage of schizophrenia to 22q11 did not show up in a recent large-scale multicenter study (Mowry et al., 2004), it revealed to be one of the most consistent and strongest findings emerging in another meta-analysis covering all published genome-wide scans (Badner and Gershon, 2002). Thus, it is possible that the same gene in 22q22-q22 is impacting on schizophrenia and bipolar disorder in the VCFS families as well as in larger samples of multiplex families. There is considerable dispute which of the genes in the 22q 11 region presents as disposition gene; e.g., there is some but still insufficient evidence for COMT (Shifman et al., 2002) and PRODH2 (Liu et al., 2002) in schizophrenia; the COMT gene is also discussed as disposition gene for bipolar disorder (Shifman et al., 2004) (s.below).

Susceptibility genes

There is rapidly growing evidence for DNA-sequence variations in specific genes to be implicated in the manifestation of schizophrenia. A substantial proportion of these genes are apparently also involved in the etiology of bipolar disorder. Currently, it is quite evident that the neuregulin-1 gene, dysbindin gene, G72/G30 gene and possibly also the COMT gene are involved in schizophrenia (Chumakov et al., 2002; Shifman et al., 2002; Straub et al., 2002; Schwab et al., 2003; Stefansson et al., 2002, 2003). Subsequently, it was recognized that the G72/G30 gene and possibly also the COMT gene are also involved in the etiology of bipolar disorder evidenced by identical markers for both disorders (Hattori et al., 2003; Schumacher et al., 2004).

Complex behaviours as psychotic and affective disorders are influenced by multiple genes, and an influencing gene generally affects multiple behavioural components at various physiological functions (Kas and van Ree, 2004). In this context it is of interest that each of the idenfitied genes is involved in multiple physiological pathways; simultaneously, the physiological targets are, however, very similar between the identified susceptibility genes (with the exception of the COMT gene): They are involved in the glutamatergic transmission (Collier and Li, 2003), in the development and the survival of neurons and glia cells. However, the functional consequences of each of these genes are currently only poorly understood.

G30/G72 gene:

The strongest support for specific susceptibility genes common to schizophrenia and bipolar disorder comes from the G30/G72 gene in the 13q candidate region (Hattori et al., 2003; Addington et al., 2004; Chen et al., 2004; Korostishevsky et al., 2004; Wang et al., 2004). There is a curiosity with this gene locus: G30 and G72 are two overlapping genes with G30 including G72; the over-transmitted marker variants and naplotypes differ between populations (Koorostishevsky et al., 2004). A haplotype in this gene was found to be associated with schizophrenia in a Russian and a Canadian sample, and was subsequently replicated in several other samples including a German one (Chumakov et al., 2002; Schumacher et al., 2004). Associations of other G30/G72 haplotypes were also reported for schizophrenia in the Ashkenazi population (Korostishevsky et al., 2004) and for childhoodonset schizophrenia (Addington et al., 2004). The pathogenic mutations are not yet identified but might be located in the vicinity of this gene complex or in the regulatory region (Korostishevsky et al., 2004).

The overlap of the candidate regions in this chromosomal section between schizophrenia and bipolar disorder motivated the genetic association studies in bipolar disorder with the identical G30 haplotype being found to be associated with bipolar disorder and schizophrenia. However, in some populations the at-risk haplotypes are shared between schizophrenia and bipolar disorder (Schumacher et al., 2004). The function of both genes is not yet known. However, G30 is in vitro interacting with the DAOA gene (D-amino-acid oxidase activator); gnetic variants of this gene were also found to be associated with bipolar disorder and schizophrenia (Schumacher et al., 2004) but not consistently so (Hattori et al., 2003). DAOA is of particular interest as it degrades serine which is modulating the glutamaergic NMDAR1 receptor which is differently expressed in both disorders, schizophrenia and bipolar disorder (Law and Deakin, 2001).

Leboyer et al. (1998) discussed the possibility that the more basic symptoms might be in a stronger relationship to susceptibility genes than those symptom patterns which are defining disorders which are validated through clinical conventions. They proposed the concept of candidate symptoms; a refined phenotype analysis by schulze et al. (in preparation) applied this idea and searched for symptoms in strong relationship to the G30 at-risk haplotype among subjects with bipolar disorder. Persecutory delusions were the only symptom with this property; this finding is unlikely to be a false positive as it could be replicated in an independent sample. As a consequence a distinct

etiological status of bipolar disorder with this specific psychotic symptom can be concluded.

BDNF gene:

The brain-derived neurotrophic factor (BDNF), the gene being located on chromosome 11p13 outside of confirmed candidate regions, is belongin to the family of neurotrophic factors and is involved in neuronal development, migration, growth and survival of neurons, but also in active-dependent neuronal activity and learning processes as long-term potentiation (Green and Craddock, 2003). BDNF transcripts are reduced in the hippocampus of both bipolar disorder and schizophrenia (Knable et al., 2004). The gene is expressed in hippocampus and neocortex, and reveals several polymorphisms; one of them results in an amino-acid substitution with functional impact in cell models on activity-dependent secretion (Val66Met); the more common variant of this polymorphism is also associated with enhanced episodic memory achievement (Egan et al., 2003). Two other polymorphisms in nonexpressed sections of the gene are also known. Thus, the BDNF gene presents as an apriori ideal candidate gene for foth bipolar disorder and schizophrenia. And indeed, two convincing family-based association studies proposed an association of the common Val-variant with bipolar disorder in Caucasian populations (Neves-Pereira et al., 2002; Sklar et al., 2002) which could, however, not be replicated in Japanese populations (Nakata et al., 2003).

An unexpressed dinucleotide repeat (GT)n polymorphism located close to the promoter region of the BDNF gene was also reported to be associated with schizophrenia in one but not in several other samples (Muglia et al., 2003). Furthermore, in a single study another polymorphism using a novel nucleotide substitution (C270T) was investigated in a recent case-control sample with cases with schizophrenia, and provided a positive result (Szekeres et al., 2003).

Taken together, the positive association results with multiple polymorphisms are difficult
to interpret in the context of negative reports both for schizophrenia and bipolar disorder. The BDNF gene, thus, remains a very promising candidate gene which might have a modest effect on each of both disorders.

COMT gene:

The catechol-O-methyltransferase (COMT) gene is located in the 22q 11 candidate region for schizophrenia and bipolar disorder which is confirmet in

meta-analysis. The gene product is a dopamine-degrading enzyme; dopamine is involved in the pathophysiology of schizophrenia and bipolar disorder. The COMT gene carries multiple polymorphisms with at least one of them is of functional relevance: Val158Met. The Met-variant causes a substantially reduced enzyme activity inducing an increased level of dopamine in the prefrontal area which is involved in working memory (which is impaired in schizophrenia as well as in bipolar disorder); consequently, carriers of this more common Val-variant are less efficient in working memory tasks independent of their affection status (Egan et al., 2001).
The Val-variant was also proposed to be over-transmitted in subjects with schizophrenia, but up to now the cumulative evidence is only spurious with maximally a very small effect size (Glatt et al., 2003). However, haplotypes tapping three other polymorphisms turned out to be more strongly associated with schizophrenia (Shifman et al., 20029. The same haplotype turned out to be also associated with bipolar disorder (Shifman et al., both disorders, but the functional Val 158Met variant is probably not of pathogenic impact.

PIPK2A gene:

A most interesting candidate regionin this context is 10p12. Linkage to schizophrenia has been confirmed in several family studies (Faraone et al., 1998; Schwab et al., 1998: Foroud et al., 2000). Linkage to bipolar disorder was also reported (Rice et al., 1997). These linkage results are complemented by associations to a variant of the PIP5K2A gene, both of schizophrenia and bipolar disorder (Stopkova et al., 2003). Schwab et al. (1998) also detected variants of the closely nearby placed PIPK2 gene for schizophrenia. Remarkably, both genes are involved in the phosphate inositol metabolism. Phosphoinositol pathways are involved in intracellular signal transmission, particularly in the context of long-term potentiation, a mechanism relevant for episodic memory which is impaired in schizophrenia; furthermore, phosphoinositol is modulated by lithium. It is speculated that an inositol deficit contributes to bipolar disorder. The phosphoinositol-related findings might introduce a hypothetical common neurobiological basis for schizophrenic and manic syndromes with therapeutic implications. Yet, the implication of phophoinositol-related genes still have to be confirmed. In this context it is particularly relevant to noticethat PIP5K2A is a critical component of the phosphoinositide and inositol phosphate pathways are modulated by lithium, an effective drug for bipolar and schizoaffective disorders.

Other candidate genes can also be discussed to be related to schizophrenia as well as to bipolar disorder, particularly DNA-sequence variants in genes coding for proinflammatory factors: the interlleukin-1ß in the interleukin-1 cluster on chromosome 2q13 (outside of the candidate region 2q22) (Papiol et al., 2004), and the tumor necrosis factor alpha gene located in the schizophrenia candidate region 6p close to the HLA region (Schwab et al., 2003; Pae et al., 2004); the empirical basis, however, is limited and far from being convincing, further replications are required.

Conclusion

There is emerging evidence that schizophrenia and bipolar disorder define no etiologically distinct disorders. A Series of family and twin studies as well as molecular-genetic studies propose some etiological overlap which is at least partially due to genetic factors. In conclusion there is overlapping inheritance which might be due to shared polygenic mutations in the same disposition genes in terms of cosegregation in families and twins..

A first common disposition gene (G30/G72) was identified for both disorders, and it can be expected that other disposition genes of this kind will follow given the overlap of candidate regions detected by linkage analysis and common functional and molecular neurobiological features. Although some association studies observed the same at-risk haplotype associated with both disorders. As the pathogenic variants are not yet identified it still remains unclear if both disorders are driven by the identical genetic variants and mechanisms (e.g. , in the gene G30/G72). Given the strong DNA-sequence variability observable in many genes two nearby located but different variants within the same gene coding specifically for each of both disorders remain a possibility.

Diagnostic entities are based on clinical conventions which might lack etiological and pathophysiological validity. Therefore, the observed genetic overlap between schizophrenia and bipolar disorder might have different meanings. The most plausible alternatives are:

(a) Both or one of both disorders are etiologically and pathophysiologically heterogeneous, e.g., there is a distinct subtype (e.g., psychotic bipolar disorder) explaining the overlap but not being considered as a separate diagnostic entity.

(b) Both disorders share common symptomatic and or neurophysiological features which have their own genetic underpinning which is consequently shared between both disorders

(c) Complex behaviour is driven by multiple genes, and each variant in these genes is predisposing to different aspects of behaviour depending on spatial distribution of geneexpression patterns which might be triggered by environment (pleiotropy); thus, the same genetic factor might induce schizophrenia as well as bipolar symptoms depending on the context.

Although some arguments were discussed in favor of possibility (b), the current status of knowledge does not allow a conclusive decision.

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A GENOME 4-WIDE LINKAGE SCAN OF BIPOLAR DISORDER IN BULGARIAN AND ROMA FAMILIES

Radka Kaneva, Bulgaria

INTERPLAY OF GENES AND ENVIRONMENT AS CONTRIBUTORY FACTORS IN SUICIDAL BEHAVIOUR

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Family studies show that the risk of suicide is increased when there is a suicide in the family, particularly when a violent method is used (Linkowski et al. 1985). One of the limitations of family studies is that they do not differentiate between genetic and other factors, as family members share both genetic as well as environmental factors. Classic research methods, which differentiate between that which is genetic and that which is acquired within the family, are the "natural experiments" of twin and adoption studies. In the case of identical twins the concordance of suicide is much greater than is the case with fraternal twins (Roy et al. 1991). Genetic model fitting on one of the largest studies of twins showed that 43 percent of the variability of suicidal behaviour can be explained by genetics (McGuffin et al. 2001).

If so, how can genetic factors increase the probability of suicidal behaviour in an individual? Suicide does not of course show a simple Mendelian pattern of transmission; there is not a 'suicide gene'. As in the case of other complex behaviour patterns, it is likely that the predisposition towards suicide consists of numerous genetic and environmental factors, which manifest themselves as suicidal behaviour only when a certain threshold of liability is crossed. In other words, we can talk about a polygenetic multifactorial aetiological model of suicidal behaviour.

How do we locate and identify genes that are involved in suicidal behaviour? The most practicable approach currently is to focus on so-called candidate genes, that is genes involved in metabolic pathways in the brain that could plausibly have something to do with suicidal behaviour. As it is thought that the variability of serotonergic neurotransmitters plays a pivotal role in individual differences in mood, impulsiveness and aggression, it is no surprise that molecular genetic studies of suicide and suicidal behaviour focus on serotonergic genes. Candidate genes can be classified into three subgroups:

- gene involved in synthesis of serotonin (*tryptophan hydroxylase – TPH* is the rate -limiting enzyme in serotonin synthesis);
- genes involved in serotonergic neurotransmission (*serotonin transporter - 5-HTT* regulates re-uptake of serotonin into pre-synaptic neuron and different *serotonin receptors* that also regulate neurotransmission), and
- genes involved in serotonin catabolism (*monoaminoxidase – MAO*).

One of the problems that has pervaded association studies in psychiatry, and indeed studies of many other common forms of disease, has been that attempted replications have been on so small a scale as to have little power to confirm original positive findings (Owen et al. 1997). In an attempt to overcome such shortcomings and problems of genetic studies of suicide, recently first meta-analyses of association studies of suicide behaviour were reported. Lalovic and Turecki (2002) reported negative results for intron 7 based on publications with an overall number of 1290 cases and 2295 controls. On the other hand, an analysis by Rujescu et al (2003) including only those studies that were performed on samples of similar ethnic origins and geographic closeness provides strong evidence for an association of suicide-related behaviour with an A218 nucleotide polymorphism in the TPH gene in Caucasians.

So far little attention has been paid to the possible interplay of genes with environmental factors. As such, a simultaneously performed candidate gene analysis and a study of life events and social support might be a way forward. As a nice example of such simultaneously performed research, Caspi et al (2003) tested why stressful experiences lead to depression in some people but not in others, by using a prospective-longitudinal study of a representative birth cohort. The functional polymorphism in the promoter region of the serotonin transporter (5-HTT) gene was found to moderate the influence of stressful life events on depression. This epidemiological study provides evidence of a gene-by-environment interaction, in which an individual's response to environmental insults is moderated by his or her genetic makeup.

Until we know more about the genetic factors significant in the development of suicidal behaviour, most of the work related to suicide prevention will be directed at improving environmental conditions, particularly with respect to those individuals where the tendency towards suicidal behaviour is most pronounced. One should not however forget that identifying people at risk already has some societal implications and, therefore, raises a number of etical issues regarding public policy (Meltzer 2000). First of all, it will soon become very important to protect the confidentiality of data on individuals, from whom

material for molecular genetic research on suicide will have been taken. Secondly, we must make sure that potential subjects can provide informed consent. Thirdly, the question of to whom, when and how to present information once genetic testing becomes more commonplace will have to be addressed.

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POPULATION GENETICS AND EPIDEMIOLOGY – SCHIZOPHRENIA AS A THRESHOLD CHARACTER

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Most behavioural traits fit into the category of quantitative characters and are believed to be subject to a complex system of genetic determination, and simultaneously highly influenced by environmental factors (polygenic model of multifactorial inheritance). Accordingly, there is a set of several or many genes (a polygene), each having a relatively small contribution to the hereditary basis of a quantitative trait. The most important form of interaction between the genes of a polygene is considered to be addition. However, notwithstanding their equal mendelian nature, genes constituting a polygene do not have equal effect in the determination of a trait. The polygenic model has long been the fundamental model of quantitative (biometrical) genetics, although a "single locus with two alleles" model can in some instances account for a continuous phenotypic variation.

Heritability defined as genetic/nongenetic variance ratio can be estimated depending on the nature of the character and its variation in an array of different ways. Many methods have been developed for this purpose. But it is still fairly exceptional to apply population genetics techniques in the procedures of estimating heritability of psychiatric nosologic entities.

A case of heritability/liability estimation, using the specific data from several schizophrenia studies is presented. The aim is to explore the possibility of applying population genetics and its models in the epidemiological investigation of schizophrenia and related diseases which can be regarded as "threshold characters".

GENETIC POLYMORPHISMS INFLUENCING RESPONSE OF ANTIDEPRESSANTS

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Pharmacogenetic studies in mood disorders were performed only during recent years involving short term antidepressant response. Antidepressant drugs are the first line treatment for major depression but the therapeutic response in clinical practice is expected in about two thirds of patients. The large inter-individual variability in the pharmacological response pattern has been partially ascribed to heritable factors. We investigated the possible influence of a set of candidate genes as possible genetic predictors of antidepressant response efficacy. The functional polymorphism in the upstream regulatory region of the serotonin transporter gene (5-HTTLPR), the A218C gene variant on the tryptophan hydroxylase gene (TPH), the G-protein beta3-subunit (Gbeta3) C825T gene variant and the Circadian Locomotor Output Cycles Kaput (CLOCK) gene variants were independently associated with short term SSRIs antidepressant efficacy. The effects of 5-HTTLPR and TPH polymorphisms were more pronounced in subjects not taking pindolol, while this effect was not observed for Gbeta3 and CLOCK. CLOCK variants were associated with insomnia time course during treatment. We observed a significantly higher presence of insomnia throughout the trial in homozygotes for the C variant. A Neural network approach was developed for analyzing multiple gene polymorphisms simultaneously. The inclusion in the model of baseline depressive scores, polarity, presence of psychotic features and fluvoxamine plasma levels did not influence the observed association. DRD2, DRD4, Mao-A and 5-HT2A gene variants were not associated with outcome. These results shed further light on the genetically determined component of the response to pharmacological treatments.

GENETICS AND ETHICS

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"Do not wish death to the enemy, but long life to yourself"

Arabian adage

Today the most difficult thing to do is to study genetics and regain ethics. One does both things in order to provide better to the others. It is difficult to reach the aim.

Genetics, as a science, studies inherited factors, which affects the human development. Lesser degree of scientific knowledge emphasis higher degree of "no ones fields" in which many diseases are attributed to hereditary. Modern science tries to get inside all factors of human continuum and behaviour, and genetics gets more and more its exact position, which naturally belongs to it.

Multidisciplinary is deteological position of approach to all modern scientific studies, even those studying genetics. Some sciences are radically involved into human beginning among which are; psychology, sociology, economics, ethics. Other sciences are also in multidiscipline touch with beginning: biology, biochemistry, eugenic, medicine. Great number of sciences observes moral development and human beginning: andrology, politics, economics, law, philosophy to Freud's tautology.

Introduction

Genetics is a science about inherited. In philosophic sense, genetic refers to the beginning-genesis, concerning the history of beginning and development of plants and animals, heredity or science of heredity. Human genetics is a scientific discipline studying the influence of inherited factors on human development.
Moral and deteological in science are replaced with standards and regulations. In this way, one shows his/hers creativity, but within the limits which exist in modern medicine and psychiatry. In this normative part, the truth is ultimate, and

in the junction of two cannons there is multidiscipline. Today it is exactly scientific and legal need.

Ethical (moral) end of human action remains in all aspects, but the question is: Is the influence real or more frequently archaic and scholastic?

Researches Genetics

Biological factors in etiology of many diseases in psychiatry understand the influence of: anatomical-morphological, biological, physiological and biochemical factors.

Genetic factors are roughly investigated in the beginning of mental diseases, in investigation of epidemiological genetics and molecular biology.

Epidemiological genetics accepts the knowledge that dominant disease in psychiatry "schizophrenia occurs more frequently in certain families. Multimorbidity risk for members of patient's family (close relatives) is between 4% and 9%, not so close relatives have less chance to develop the disease" (S.Loga).

There are several assumptions how the disease can be inherited Monogenetic - certain gene is responsible for the development of the disease Poligenetic- many genes are responsible for the development of the disease Heterogenetic- the disease is not simple; it is heterogeneous, caused by the influence of many different factors.
Lowering of number of mutual genes lessens the risk of developing the disease
This disorder occurs on both, mother and father, sides

Mental disorders are inherited poligenetically and multifactorally. Voulnerability to mental disease is normally distributed in the family, but it is manifested only in those in which it passes the threshold of reaction.

Molecular genetics is performed at the level of chromosomal analysis with biological markers. In this way, the search for known genetic markers in families in which mental disease occurs is performed. Also, in this way we find the field of lingag analysis, which tells us that sensitive places are differently disseminated in the genome. There are positive findings on chromosomes 13q, 22q, 6p, 8p.

There are :1.linkage analysis researches, 2. gene cloning researches and 3. mRNA brain expressiveness researches.

Behaviour genetics studies the influence of inherited factors on psychological characteristics and behaviour. This genetics is based on the studies of families with psychological characteristics (mental backwardness) or illness. During past three centuries, heredity has been observed in "exotic mental illnesses", according to MKB-10.

Genetic psychology is genetic structuralism or Geneva school with great French influence. The school is an interesting collection of expansive psychoanalysis, Soviet objectivistic school and Anglo-Saxon behaviourism. In these scientific places results have emerged and become visible during 1950-s and they influenced psychological idea (thought) of that period.

Genetic psychology emphasizes the connection between genetic model and development of psychological functions. Genetic code is showed according to the development sequence during the lifetime, and with that process, complex psychological functions are developed. From the psychological point of view, the school prefers cognitive functions in mental development of the human being and gives it the name of cognitive orientation. (20).

Intellect is developed by senso-motoric period, which lasts 18 months and ends by controlling of the first symbols and development of abstract schemes. It is continued by the "preparation period" (until 6 to 7 years) in which symbolically development of speech happens together with process of abstraction and understanding of classification. Then a child than begins to make concrete conclusions, operates with negations, identity, reciprocity and it is able to close the value.

In period from 10 to 12 years formal-operational period starts and full thinking normalization, and ability of "superoperational" performing are achieved. This period lasts after the puberty. This is the characteristic of abstract thinking of an adult human being, which is very important for the development of moral reasoning. Logical reasoning is a base for moral reasoning, and ethics is the logic of human acts. In that way fundaments for moral reasoning and acting are formed.

In later phases of development under the behavioural influence, one cedes a lot of things to the moral judgment. On the bases of this one compromises basic moral principles (compromise compromitans). One actually lives between two

basic wombs, mother's and ground's, keeping the part of what he/she inherited and with(out) obligation to accept what the environment imposes.

Two latest great awards in the world in the field of the most finest researches of the brain structures tell us about the biggest expansion of genetic researches of the brain tissue. Daily information about human cloning (without knowledge about its legality) brings the human race into the reality: *Where is the human genetics going?*

Etics

Eugenic is one of the most important branches of social politics. It is also called race hygiene, the science about the conditions, which lead to production of physically and mentally healthy posterity. It prevents production of unhealthy and incapable posterity. It is a tendency of creating certain benevolent conditions.

Data suggest that F. Galton founded eugenic in 1870. He divided it into: positive, which improves human race by stimulating reproduction. Negative prevents the situation in which human being dies early. Blood relation helps appearance of physical and mental deficiencies. Applied eugenic could provide avoidance of reproduction if both parents are at risk. There can be found some legal problems of regulation of eugenic issues.

French law in January 1975 says: "a pregnant woman can bring her condition in unwanted situation; she can ask her doctor to perform an abortion. This abortion can be performed only before the completion of 10 weeks of pregnancy. Law allows the abortion in case when it is known that a fetus is abnormal."(12).

Today there are possibilities of interventions during the pregnancy with embiopathy caused by immunological postinfective dysfunction. Multiple pregnancies in which a mother and fetus are in danger represent indication for eugenic abortion. In past three decades an intentional abortion can be performed if two doctors, after the physical examination, reach unique attitude about it. This approach depends on the country legislation.

So, human and scientific factors determine the lasting of the human life after the conception in ordered societies. The World Health Organization finds that birth rate is reciprocally proportional to conditions of life and it brings a man closer to nature. In psychiatric sense according to MKB-10, probable psychiatric

consultations will remain in "exotic mental disorders." *Is this a new conflict between exotic and nature?*

New-old ethical dilemas

"Moral principles are easy to understand, but very difficult to apply in practice"

J. Maric

Does the man know to respect patient's personality?
Does the man know to respect personality of other man before the conception?
Does the man know to respect human and medical secret?
Does the man know to respect the person of a doctor inside him/herself?
Does the man know when, how and where to create a new human being?

Attitude towards the patient is the basic obligation in doctor's work. Psychiatric attitude can be more specific with more understanding for suffering of the body and soul of the patient. But, there is a Freud's thesis which says: " Three things cannot be learnt: to be a good parent, psychotherapist and nations leader." Empathy is small obligation of each doctor; above this are obligations of psychiatrist and psychotherapist. Who knows what are other obligations-one will remain human or human will disappear.

Conception is a real beginning of a human life, but modern man thinks that it is some kind of intrigue in which something bad will happen to him (more frequently) or something good (less frequently). Rarely one thinks that it is planned conception of a new life. Conception cannot be destroyed. If there is real contraception monsters will not be born. (20).

Abortion is an act of ceasing the pregnancy, death of homo novus. Abortionist kills the fetus. Legal, social and ethical factors will follow human being before, during and till the end of her/his life.

Euthanasia is a way of ceasing of human life, which is performed by the doctor as an act of mercy. A dilemma as old as medicine is to help or not the dying man. Today this dilemma is in different legislative procedures (from approval to strong disapproval). All cultures in the world have their own principles and customs about helping man in dying. Only doctor can understand is he needed to the patient any more.

Codex of medical work exists in form of Hippocratic Chapter and that is the entrance into the modern medicine. Apparently it will be simple and sufficient if it is respected. It is difficult to maintain the idea that my colleagues are my brothers (9) where man distances from the other man.

General practitioner becomes what he/she becomes by studying and finishing modern medical studies. Codex prescribes practitioner's behaviour, a family establishes a part of family superego and the society establishes a part of social superego. It is simple how one possesses something remedial inside him/herself and resists all temptations. It is difficult when passions affect your mind.

Moral principles are easy to understand and learn, but very difficult to apply in practice. (12). By analyzing ethics of youth makes it more difficult. Their universe is full of passion of the youth; it is less permeable, more passionately protected than some distant gens when a respectable thinker from modern civilization studies it. It seems that there is an important difference between the old and young-first ones cannot spend what they have, second ones will be able to spend everything if they have it.

Today in ethical understanding of fighting and existence, there are a lot of ruined ethical characteristics of this area. It must be honestly admitted that we possesses a part of our own guilt and insufficient understanding of change in social relations and situations.

However, essential maintenance of ethic in atheism, benevolent dictatorship and other social situations show collective spirit of survival. Principles and customs left a part of culturology, other part we sank by our own.

When adults play a war game like children and then get angry, the young must show them immaturity. Immaturity calls for maturity. Maturity belongs to the immature regardless the period of life. If one wants to grow, there is no point in waiting. Politics should be left to those who, apart of knowledge, have some other qualities. This shows that nobody needs politics. It would be better to cure wounds of bad politics and then create pawn for new investments in world heritage, but without avoiding ethical, spiritual and cultural in ones broad sense of understanding. This cannot be just politics (20).

Discussion

Genetics predetermines inherited characteristics of a human being. Modern science, by studying genetics, tries to change human characteristics. Genetic

researches have some regulations, but they are separately presented in certain legislations. In this way genetic researches include those, which are completely approved and those, which are at the edge of common sense and ethics.

Researches preventing diseases or changing the course of the disease and improving quality of human life are within the borders of all human benevolences. They are privilege of the rich and other parts of mankind wait for these results.

Other group of researches is a "cosmological scope with a great number of marketing studies." Huge part of these researches is not just at the edge of ethics, but at the edge of necessity as well. They are expensive genetic researches whose aim is to improve human biological environment. These improvements are done not to make people love more but to help a type of modern communication on a subculturological level. A lot of people come in Socrates position "to suffer not just from own defects but from virtues as well." This happens before one becomes perfect, because the place for a perfect man is in the museum.

Third part of researches moves towards cloning. This new creation means being the same as before with a little bit improvement than before. Here, a lot of things are unclear (Does everyone has right to be cloned? What will destiny of clones be? Does clone reach ideality without having any possibility to change human characteristics by nature? Will clones overcome those who are not clones? Where will people live when clones take over the planet?) *Homo meiducus waits for the product of homo technocraticus as homo novus.*

Conclusion

By wishing to humanize nature and to naturalize human being, one reaches the perfection and gets even further. Problems start when one moves away from human nature and loses extent in his wishes. This lost of extent creates virtual picture of superman and subnature. To show real situation we can paraphrase one literary mother's advice: "My son, don't go at the edge of the road. A tile can fell and kill you. Don' t go in the middle of the road either, something can run over you. Just go somehow like this." Today man can stay "somehow like this" not only in Bosnia, but all over the world.

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MODERN PSYCHOPHARMACOTHERAPY: BETWEEN GENETICS DETERMINISM AND FREEDOM OF CHOICE

Miro Jakovljević

RETROSPECTIVE OF HUMAN POPULATION GENETICS STUDIES IN B&H

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Abstract

The earliest medical records on antroposcopic and antropometric traits in B&H population date from the end of 19th century. However, first exact results of human population-genetic research were obtained in 1930's using analysis of genotype frequencies of blood group systems. Further research was significantly intensified in the period 1960 – 1980 trough comprehensive analysis of complex phenotype systems of qualitative and quantitative traits as described in numerous reports by Berberovic and his collaborators, as well as adoption of improved sampling methodology and data collection and interpretation (Hadziselimovic 1981a, 1981b; Hadziselimovic *et* Berberovic 1981; Hadziselimovic *et al.* 1981; Hadziselimovic *et al.* 1990). After the end of war aggression on Bosnia and Herzegovina (1992-1995) the survey on local human communities in respect to

their demographic structure and ethnic and cultural characteristics have been continued using methodology of molecular genetic markers (Hadziselimovic 2002; Pojskic *et al.* 2003; Marjanovic *et al.* 2003, Kapur *et al.* 2003; Marjanovic *et al.* 2004a, Pojskic *et* Hadziselimovic 2004a, Kapur *et al.* 2004, Pojskic *et al.* 2004b, Marjanovic *et al.* 2004b - *in press* and Marjanovic *et al.* 2004c - also *in press*). The main objectives of population genetic research in Bosnia and Herzegovina are determination of diversity of local human populations especially isolated populations, as well as three main ethnic groups (Bosnian Bosniacs, Bosnian Serbs and Bosnian Croats).

Obtained results had particular significance in the process of identification of human remains after war in Bosnia and Herzegovina. During the history of population-genetic study of human population in B&H various genetic markers have been employed for complex analysis: qualitative hereditary biochemical and physiological, static-morphological and dynamic-morphological traits, microsatellite markers (15 autosomal STR loci), hipervariable regions of mtDNA (HV1 and HV2), Y-STR (12 loci) and 28 Y-biallelic markers.

All mentioned results consistently showed following population structure characteristics:

- higher intra-ethnic than inter-ethnic variability;
- rather small genetic differentiation and genetic distance among different ethnic groups in Bosnia and Herzegovina;
- observed genetic diversity of human (sub)populations in Bosnia and Herzegovina is correlated with their geographic rather than ethnic parameters;
- genetic specificity of isolated human (sub) groups no matter their ethnic origin;
- the significant effects of violent (war-caused) migrations on genetic structure of local and isolated human populations in Bosnia and Herzegovina.

FAMILY BASED ASSOCIATION STUDY OF BIPOLAR DISORDER: CURRENT STATE IN BOSNIA AND HERZEGOVINA

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Abstract

Bipolar disorder, Type 1 (BP1) is a severe psychiatric disorder characterized with episodes of mania and depression, with complex genetic background. The mode of inheritance is still unknown, but most probably polygenic. Theoretically, single gene effect in complex genetic phenomena is rather small and could not be easily revealed in samples with limited size. Therefore, the large sample size is needed for primary identification of candidate genes and further confirmation testing in geographically distinct populations. Since BP1 disorder is a broad and heterogeneous phenotypic category, diagnostic methodology and sample collection need to be harmonized within the collaborative groups that will also enable genetic association analysis with specific symptom clusters. In the framework of the collaborative study with the Department of Psychiatry, University of Bonn (2001-2004) almost 80 BP1 triads have been collected in Bosnia and Herzegovina using the same standardized phenotypic instruments.

EARLY INTERVENTION IN PSYCHOSIS

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Abstract

Over the last few decades, there has been increasing interest and research in the issues surrounding the onset of psychotic illness. From an epidemiological point of view, although schizophrenia is said to afflict up to one per cent of the population at some time in their lives, the incidence seems to be affected by

various social factors, which vary in different populations; migration is an example. It has been observed that there is often major change in the psychosocial functioning of many people with psychotic illnesses within the first three years of the onset, thereafter, the deterioration tends to plateau out, so that the first three years of the illness could be described as a 'critical period' in which the future course of the illness was set. It is suggested that intervention in a psychotic illness at the earliest possible time, particularly in this 'critical period' may offer the best chance of improving the prognosis of patients.[1]

Recently the 'prodrome' or 'at risk mental state' phase of the illness has achieved significant attention and this phase is seen, arguably as one potential target for improving the outcome of psychosis [2].

With regards to specific interventions and individual case management, it is felt that the adherence of the individual to a treatment plan is facilitated if his/hers initial contact with mental health services is a positive one, thus minimizing unnecessary delay in the initiation of adequate treatment and possibly avoiding admission to a hospital[4].

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UPS AND DOWNS OF ATYPICAL ANTIPSYCHOTICS

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Abstract:
The metabolic effects of treatment with antipsychotic medications have been under considerable debate. The knowledge base about metabolic effects is evolving. The two questions are: first, whether individuals with schizophrenia or bipolar disorder are at greater risk for obesity and type 2 diabetes than the general population even without taking antipsychotic medications, and secondly, to what extent antipsychotic medications increase rates of obesity and type 2 diabetes. The most important risk factor for development of diabetes in patients taking antipsychotics are still unclear. Possible risk factors include age, gender, race, BMI prior to treatment, diet, physical activity and comorbid conditions. Unfortunately, there have been few prospective studies to determine importance of these factors, and most of the retrospective studies attempt to control for these factors. Atypical antipsychotic drugs should be chosen to minimize the risk of weight gain, diabetes, and hyperlipidemia. Weight gain and metabolic disturbances associated with atypical antipsychotics should be controlled aggressively due to associated risk of coronary artery disease. Coronary artery disease is significant cause of mortality in these patients. However, because these conditions can be treated with lifestyle modifications such as diet, exercise, and medications, these adverse events should not discourage use of the atypical antipsychotics. Some patients who experience significant weight gain may benefit from switching to an agent with less potential weight gain. It is important that psychiatrists who prescribe atypical antipsychotics educate their patients about risks and warning signs of metabolic dysfunction associated with these agents and ensure follow up.
Key words: Schizophrenia, bipolar disorder, atypical antipsychotics, metabolic syndrome, obesity, diabetes, dyslipidemia.

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JACQUES MONOD: FROM GENETICS TO PHILOSOPHY

Tvrtko Kulenović

Abstract:

Jacques Monod, together with two other French biologists received a Nobel Prize in Physiology and Medicine in 1965, for his research in the field of molecular biology. His name is perhaps more familiar to the wider audience as the author of *L' hazard et la necessite*, that is considered to be the most important work on the philosophy of nature written in the Twentieth century. Monod's horizon in this book is wide, his thought deep and its literary quality is extraordinary. Leftist in his youth, perhaps even a communist, later a member of French Resistance, he defines socialism as a 'painful dream of the nineteenth century'.

This book was accepted with harsh criticism both from the left and from the right. The Right, naturally connected with creational interpretation of the origins of the world, attacked Monod for his denial of the Divine principles, for his statement that 'our numbers have come from the gambling table in Monte Carlo'. The Left, on the other hand has projected historical causality to the world of nature, creating and defending its God – Marx. Monod's teaching ('Destiny exists only if happens, otherwise not.') is close to the philosophies of the Far East, particularly the philosophy of ancient China in the 'Book of Prophecies' (*I King*) that states that the individual should base his preparations for the future based on his knowledge of the present, and not on prophecies.

The hazard, of course does not assume disorder because …'once encoded in the structure of DNA, singular, and therefore unpredictable event, will be mechanically precisely replicated and transferred, multiplied in millions and billions of examples. Uprooted form the realm of sheer hazard, it enters the realm of necessity, the realm of undisputable safety. Because, at the macroscopic level, on the level of living organisms, natural selection is taking place. Even the most brilliant minds of today still cannot accept and understand that it is possible to derive the *son* of the whole music of the biosphere form one source of noise', says Monod. Darwin's philosophy of nature is still applicable today, only, it is based on new assumptions about the function of hazard in the evolution.

REPORTS
The First Symposium of Zoonoses
with international participation

Sarajevo, Aopril 22-23, 2005

Conference report

Mirsada Hukić, president of the Organizers Committee

On the 22nd and the 23rd of April 2005, the Microbiology - and the Society of Infectiology of Bosnia and Herzegovina and the Clinical Centre of the University of Sarajevo, Institute of Science and Development, organised under the auspices of the Academy of Sciences and Arts of Bosnia and Herzegovina, the First International Symposium on Zoonoses with emphasis on Hemorrhagic Fever with Renal syndrome (HFRS), Q-Fever and Brucellosis.

Participants from Slovenia, Croatia, Belgium, Finland, United States of America, Macedonia, Serbia and Montenegro, Kosovo and Bosnia-Herzegovina enjoyed two days of high-level scientific presentations and discussions. There were 77 Institutions as participants of the Symposium.

In the field of HFRS, participants presented data concerning the epizoonotical and epidemiological status of their respective regions and provided a unique total picture of the past and current situation.

Q-Fever and Brucellosis, both re-emerging infections of concern to the entire Balkans region and the respective Public Health systems, were discussed in detail and valuable suggestions and recommendations were brought forward for management, standardisation of sampling and detection and prevention.

The close collaboration between medical doctors of all relevant specialities (microbiology, nephrology, pathology, infectology, epidemiology, etc), veterinarians and scientists thus provided an excellent platform for establishing collaborations and exchange of knowledge. The new ideas concerning treatment, patient management, surveillance for zoonoses and early warning systems that "emerged" from this conference will, no doubt, have a positive impact on future public health efforts in the region.

Sponsored by Bosnalijek, Pharma Swiss, Diamedica and Seđan, this initiative to look over the borders, detect mutual problems and suggest common solutions, was more successful than could be expected and will hopefully become a tradition. In two years time, the Second International Symposium on Zoonoses will be held in Banja Luka.

HTV

The main epidemiological characteristics of Hemorrhagic Fever with the renal syndrome in Republic of Serbia

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Haemorrhagical Fever in Mačva District from 1997. to 2003.

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The management of surveillance of haemorrhagic fever with renal failure syndrome

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Ophthalmic disorders three years after the acute glaucomatous attack in patient with hemorrhagic fever with renal syndrome

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HFRS outbreak and Pumala virus in Croatia in 2002

Lidija Cvetko [1], Alemka Markotić [1], Angelina Plyusnina [2], Josip Margaletić [3], Marica Miletić-Medved [4], Nenad Turk [5], Zoran Milas [5], Tatjana Avšič-Županc [6], Alexander Plyusnin [2]
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Influent of climatic factors on the outbreak of epidemic of hemorrhagic fever with renal syndrome

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Hemorrhagic fever with renal syndrome in Požega Area (review on clinical and laboratory indicators of epidemic HFRS in 2002. at County Hospital Požega)

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Genetic Analysis of Wild-Type and Human Strains of Hantaviruses Causing Hfrs in Slovenia

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Fatal Chronic Kidney Disease – Balkan Endemic Nephropthy and Hantaviruses

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Early Activation Mechanisms in Lung Fibroblasts Infected with Hantaviruses

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Renal Pathohistological Findings in Hfrs Patiens in Biopsy and Autopsy Speciments - Experience From Balkan

Dimitrijevic J.(1), Zaki S.(2), Micic S.(1), Radosavljevic R.(1), Brajuskovic G.(6), Cerovic S.(6), Bogdanovic R.(3), Kovacevic Z.(4), Skataric V.(4), Aleksic A.(4) and Gligic A.(5)

Hemorrhagfic Fever with Renal Syndrome in Croatia - what are the news and what have we learned?

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Hemorrhagic Fever with Renal Syndrome (Hfrs) in Kosova

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Puumala and Dobrava Viruses Inducement Hemorrhagic Fever with Renal Syndrome - Glomerular Filtration Rate Convalescents Nine to Ten Years after Acute Phase

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Crimean-Congo Hemorrhagic Fever (CCHF) in Kosova

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Emerging and Reemerging Viral Zoonoses

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Hantaviruses in Western Europe

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Diagnostic of Hemorrhagic Fever with Renal Syndrome

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Serological Evidence on Presence of Puumala Like and Belgrade/Dobrava Hantaviruses at the Haemorrhagic Fever With Renal Syndrome Suffering Patients on North- Western Bosnia

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Clinical and Epidemiologic Characteristics of Hemorrhagic Fever with Renal Syndrome

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Epizootology and Epidemiolgy Characteristics of Hfrs in Former Yugoslavia

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Mitochondrial Factors And Extracellular Matrix Molecules Are Responsible For Apoptosis Caused By Hantaviruses

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Seroprevalence Of Antibodies To Hantaviruses In Patients From The Health Centre Tuzla

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HTV posters

Outbreak Of Hemorrhagic Fever With Renal Syndrome In Central Bosnia In 2002.

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Chronic Renal Failure As A Consequence Of Hemorrhagic Fever with Renal Syndrome (Hfrs)

Veselin Skataric, Z. Kovacevic, V. Rabrenovic, D. Jovanovic, K. Obrencevic, A. Gligic.
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Hemorrhagic Fever With Renal Syndrome During Pregnancy Resenatation Of Two Patients

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Haemorragic fever with nephrotic syndrome (Nephroso Nephritis) in the municipality of Konjic form 1984 to 2004 - *the extract*

Armina Lazarević, Amira Alajbegović-Dzumhur
Opća Bolnica Konjic

Importance Of Ultrasonography In Diagnostic Of Hemorrhagic Fever With Renal Syndrome

Jasna Topalović, Snježana Mehanić, Vesna Hadžiosmanović, Nermina Bajramović, Meliha Hadžović
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The Clinical Characteristics Hemorrhage Fever With The Kidney Sindrom In Epidemic 2002 Year.

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3 Q fever

Epidemiologic Characteristics Of Q Fever In Croatia

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Q- Fever In Serbia

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Q Fever In Animals In The Regions Around The River Drina

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Laboratory diagnosis of Q fever

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Q Fever At Cattle-Breeding Farm Prača

Vesna Hadžiosmanović, Snježena Mehanić, Jasna Toplović
Merdina Ferhatović, Meliha Hadžović
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Q-fever (Coxiella burnetii) investigations in dairy cows

Branka Vidic, Ružica Ašanin, S. Bobos,D. Bugarski
Scientific Veterinary Institute "Novi Sad"

Nomadic Grazing Of Sheep And Widespread Epidemic Of Q Fever In Republic Of Srpska

Jelena Maric, Violeta Santrac, M. Saric, B. Bjelajac, S. Golubovic, Z. Djeric, D. Kubelka
Veterinary Institute of RS "Dr Vaso Butozan" Banjaluka: Faculty of Medicine, Banjaluka; Ministry of Agriculture, Forestry and Waterpower Engineering of RS, Bijeljina

Q Fever In Human And Veterinary Pathology In Montenegro

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Q fever poster

Q-fever in Croatia

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Cross Reactivity During The Serotesting Of Blood Samples Upon Brucellosis And Q-Fever

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Q-fever: case report

Emir Halilbasic, Edita Sijercic
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Seroprevalence Specific Antibody To Rickettsia In Humans Sera From Different Locality In Serbia

Samardzic S.1, Dasch G.2, Djuricic B.3, Lako B.4, Ristanovic E4, Bozovic B.5, Vukov B.6, Simovic T.7, Milic A.1,Eminovic M.8, Stajkovic N.4,ekanac R.34and Gligic A.5.
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Chronic Fatigue Syndrome after Q Fever a Case Report

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Q- Fever In Belgrade

Ljubic Bozidar, Radmila Dmitrovic, Snezana Radivojevic, Slobodan Stanojevic
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Q – fever – clinical course of disease Case review

Horozić M., Ahmetagić S., Mešanović J., Delalić L.
UKC TUZLA Clinic of Infectious deseases

Brucelosis

Monitoring Possibilities Of Brucellosis By Elisa Testing Of Milk

Bradarić Abida[1], Kapetanović Osman.[1], Zahirović Lejla[2]
Health ministry of Canton Sarajevo – sanitary inspection[1]
Cantonal veterinary station Sarajevo[2]

Efficiency Of Veterinary –Sanitary Measures For Ruminant Brucelosis Control

Mitrovic N., Nedic D., Kubelka D., Marković T.
Veterinary Institute of Bijeljina

Seroprevalence of Brucellosis In Ruminants In Republic Of Srpska From 2001 To 2004

Jelena Maric, Novalina Mitrovic, Violeta Santrac, M. Saric, D. *Despotovic, D. Nedic, D. Kubelka; Veterinary Institute of Republic Srpska "Dr. Vaso Butozan" Banjaluka; Ministry of Agriculture, Forestry and Waterpower Engineering, Bijeljina; Veterinary Institute of Bijeljina*

Dynamic of Specific Antibodies Titers in Patients with Brucellosis

Nurkić M[1], Delibegović Z[1], Kikanović H[1], Numanović F[1], Tihić N[1], Iamamović A[1], Nurkić J[1], Džafić F[1], Sabitović D[1], Hadžihafizbegović S[1], Mešanović J[2],
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2.Clinic for Infectious diseases of University clinical center Tuzla.

Current Zoonoses Brucellosis and Q-fever – Epidemiological aspects of hospitalized patients at the Clinic for Infectious Diseases

Drnda A, Pinjo F, Osmić A, Ferhatović M, Jusufi I, Gazibera B.
Clinic for Infectious Diseases, KCU Sarajevo

Investigation Of Sensitivity And Specificity Of Some Serological Methods In Brucellosis Diagnostics

Asanin Ruzica, Vidic Branka, Grgic Z., Matovic K. And Nisavic J.

Distribution Of Brucellosis In Serbia

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Institut of Epidemilogy, Medical Military Academy, Belgrade

Clinical and lab characteristics of brucellosis in Tuzla Canton

Poljakovic A1, Zahirovic M1,Ahmetagić S1, Horozic M1 , Piljic D1 , Stojic V1 , Petrovic J1 , Sinanovic A1 .
1 Clinic for infection Disease UKC Tuzla

Human brucellosis in Croatia and at the University Hospital for Infectious Diseases «Dr. Fran Mihaljević» in Zagreb

Balen Topić M, Beus A, Cvetnić Ž, Skuhala T, Desnica B.

Bottom of Form Human brucellosis: an overview

I.Curic, I.Kuzman, M.Ostojic, S.Puvacic, S.Curic, B.Vidovic
Clinical Hospital Mostar Department for infectious diseases

Microbiological diagnosis of brucellosis: modern approaches and Serbian experiences

Ristanovic Elizabeta, Lako Branislav,
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Zoonosis In Infectious Practica

Dautović-Krkić S. Lukovac E. Mostarac N. Hadžović M. Gazibera B. and Muratović P.
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Presentation, Diagnosis And Treatment Of Acute Brucella Meningitis (a case report)

*Dragan Mikić, *Milomir Đokić, °Branislav Lako, *Milomir Milanović
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Clinical And Epidemiological Characteristics Of Brucellosis In Patients Treated During The 2002-2004 Period

Snježana Mehanić, Fikret Pinjo, Vesna Hadžiosmanović, Jasna Topalović, Merdina Ferhatović, Meliha Hadžović
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Brucellosis Foci Never Before Detected In The Area Of Belgrade

Ljubic Bozidar, Radmila Dmitrovic, Vera Babic- Dunjic, Tijana Relic, Snezana Radivojevic, Slobodan Stanojevic, Dragica Vojinovic, Jadranka Zutic
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Brucellosis In The Republic Of Macedonia-Clinical Experiences

Bosilkovski M, Krteva Lj, Caparoska S, Dimzova m, Sopova Z.
Klinika za infektivni bolesti i febrilni sostojbi Skopje, R. Makedonija

Brucella Melitensis Biotip 3 – Description Of An Epidemic

Violeta Santrač, Jelena Marić, Sonja Nikolić, Sonja Radojičić, D. Sando, D.Kubelka
Veterinary Institute of Republic of Srpska «Dr. Vaso Butozan» Banjaluka

Brucellosis in the municipality of Konjic - *the extract*

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Brucellosis in Herzegovina

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2. Department of infectious diseases of RMC "Dr.Safet Mujić" in Mostar

Brucellosis – posters

Spondylodiscitis as a rare manifestation of brucellosis

Vesna Turkulov, Nadežda Madle-Samardžija,Grozdana Čanak, Radoslava Doder, Jovan Vukadinov, Siniša Sević
Clinic for Infectious Diseases, Clinical centre Novi Sad

Brucellosis- case report

Zahirovic M1,Poljakovic A1 Horozic M1 ,Hadzic E2, Piljic D1 , Stojic V1 , Petrovic J1 , Sinanovic A1 .
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Brucellosis as a protracted febrile illness- case report

T. Skuhala, A. Beus, B. Desnica, M. Balen Topić
Clinic for Infectious diseases «Dr. Fran Mihaljević» Zagreb, Croatia

Zoonose – posters

Zoonoses: The vet - profession of a high risk

R.Velić
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Farmyard manure as transmitter of zoonoses

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Zoonoses In The Postwar B&H Important Common-Health Problem

E. Bektaš , Z. Smajlagić
Javna ustanova «Dom zdravlja sa poliklinikom» VISOKO

Zoonotic agents as biological weapons

Ristanovic Elizabeta, Lako Branislav
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Soluble Fas And Transforming Growth Factor Beta-2 In Acute Viral And Bacterial Zoonoses Infections

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Zoonosis In Tuzla Canton – Always A Current Problem

Ahmetagić S[1], Topalović B[2] , Mešanović J[1], Šabović S[1],
Pavić G[3].
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Viral And Bacterial Zoonosis In Forestry Workers

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Smalll Rodents And The Forest Ecosystem In Croatia

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Conclusions

1. Zoonosis are still actual problem in Bosnia and Herzegovina, as well in South-east countries in Europe, important for human and veterinary medicine, as well for the edonomy and biosafety of the countries.
2. There were noticed different aproach to the prevention, diagnostics and therapy of zoonoses.
3. For the resolution of these problems it is recommended close cooperation of scientists and workers – investigators from human as well veterinary medicine and the govermental institutions (including all levels protection).
4. It is recommended creation of a Permanent body for zoonoses in B&H, with the representatives of human and veterinary medicine, organized by the sectors of different diseases (hemorrhagic fever, brucellosis, Q-faver, rabies, trichinellosis and so far (under the experts principe)

5. The main goal of this body for zoonoses in B&H is to bring the protocols and the procedures for diagnostics, therapy and prevention of zoonoses and to create the laboratories which will work on all the tetitory on B&H.
6. It is a need to organize the referent centres for zoonoses and equipe the laboratories with biosafety levels 3.
7. To carry out a registration of the farms and to mark all animals due to control the moving of animals.
8. To promote a work of veterinary, sanitary and public health inspection.
9. The institutions and individuals from veterinary and human medicine schould be included in continuing education of population.
10. It is necessary toinclud in the Meditteranean program of zoonoses.
11. To apply the international standards in the control of zoonoses in a people as well in animals (monitoring)
12. To create a regular and extraordinary system of reporting about zoonoses between the competent servicas in human and veterinary medicine, as well with the new dewerb on zoonosis in neighboring countries.
13. To make the maps with the regions in wich zoonoses are existing.
14. It is necessary to advance the scientific-research work in area of zoonoses. A specially attention schould be direct to the isolation and identification the news or the old changed of pahtogens which presumably circulating on the region of B&H. To study a existance – persistance of sequelle after the past diseases of hemorrhagic fever with the kidny syndrome. To promote and develop diagnostic procedures of zoonoses to be efficient in practice.
15. To organize symposiums every second year and congress of zoonoses every fourth year.

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