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Editorial

We inform our readers and collaborators that the interest for publication in Bosnian Journal of Basic Medical Sciences is growing continuously. Numerous letters and messages (e-mail) of support, in particular from our scientists working abroad, are sufficient confirmation. This exchange resulted in the idea (E. Kanlic, MD PhD, Associate Professor, Dept. of Orthopedic, Texas) that our scientists working in the USA edit one issue (preliminary deadline in April 2005) that would be dedicated to the latest understandings and achievements in a specific area that would be of interest for our experts. We are looking forward to successful cooperation with other colleagues engaged in fundamental science.

It is with great pleasure that we inform you that, after anticipating a reply from the Department of Health and Human Services for some time, on the 6th July 2004 we received an information from Mr. Sheldon Kotzin, Executive Editor MEDLINE/Index Medicus stating that Bosnian Journal of Basic Medical Sciences has received a positive score (very good) upon reviewing and has been accepted for indexation in MEDLINE/Index Medicus.

We are proud that our issues are mainly filled with papers by young researchers which agrees with our primary intention that the Journal grows into a vehicle for the exchange of knowledge among young researchers in the world. With this issue we complete the cycle for this year. We are convinced that, despite significant difficulties, we will find adequate support and ensure regular publication of research results from the entire Bosnia and Herzegovina.

With sincere greetings and gratitude,

Sarajevo, October 2004

Editorial and Advisory Board
M.Mujic

This Journal is Indexed in:
CAB Abstract / Global Health databases and
Index Medicus/MEDLINE.
APOTEKE SARAJ EVO oglas 1/1
CMYK
THE BCL-2 PROTEIN: A PROGNOSTIC INDICATOR STRONGLY RELATED TO ER AND PR IN BREAST CANCER

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3 Department of Oncology, University Clinical Center Sarajevo, Bolnička 25, Sarajevo, Bosnia and Herzegovina

*Corresponding author

Abstract

Bcl-2, the protein product of the Bcl-2 gene, is a member of the Bcl-2 family of proteins that play a crucial role in a complex mechanism of apoptosis. It was recently proposed that bcl-2 could inhibit cancer progression. In this study, we evaluated the expression patterns of Bcl-2, estrogen receptors (ER), progesterone receptors (PR) in 71 primary invasive breast carcinomas and their association with other clinicopathological parameters.

Samples from 71 patients with invasive breast cancer with follow-up ranging from 4-103 months (median 57 months) were included in the study. Forty-six patients (66%) obtained a complete response, while 5 (9%) were considered non-responders during the follow up period of 103 months. Eighteen (25%) patients died, 15 (21%) from primary disease and 3 (4%) from other disease. In univariate analysis, tumor size (<2 cm), lymph node (<4 lymph nodes), hormonal status and Bcl-2 expression are correlated with longer overall (OS) and relapse-free survival (RFS). Patients with 4 or more positive axillary lymph nodes had significantly shorter OS (p=0.01) and RFS (p=0.009). Higher expression of Bcl-2 was associated with longer OS (p=0.02) and RFS (p=0.03), and this result were independent of axillary lymph nodes and tumor size in Cox multivariate analysis.

Introduction

Programmed cell death, apoptosis, is a physiological mechanism of cell death that plays an important role during development, metamorphosis and organ involution in many diseases, including cancer. (1) Regulation of apoptosis is a complex process and involves a number of genes, including Bcl-2 and related family members. (1-3) Abnormalities of apoptosis may lead to uncontrolled cell proliferation and ultimately carcinogenesis. BCL2, first identified by its involvement in the t(14;18)(q32;q21) characteristic of follicular lymphomas (4) is a major negative regulator of apoptosis. The mechanism of the anti-apoptotic function of BCL-2 is only partially understood, involving decreased mitochondrial release of cytochrome c, which is, in turn, required for procaspase-9 activation and initiation of the apoptotic cascade. (5) The association of bcl-2 protein with a prognostically favorable phenotype is interesting and difficult to understand given its antiapoptotic function. One reason may be the expression of other members of the bcl-2 family that are proapoptotic such as Bax and can counteract the effect of bcl-2. High levels or aberrant patterns of bcl-2 expression occur in various tumors including breast cancer. (6,7). However, besides the experimental evidence showing the role of the bcl-2 protein as an inhibitor of apoptosis, some studies have suggested a growth-suppressing effect of bcl-2 associated with a retardation of mammalian cell proliferation. (8)

In this study, we wanted to evaluate the prognostic value of Bcl-2 protein in breast cancer and its correlation with OS and RFS. Also we wanted to evaluate the association between Bcl-2 protein and other prognostic clinical parameters.

Materials and Methods

Patient selection

The biopsy specimens from 71 patients with invasive breast cancer diagnosed at the Department of Pathology, University Hospital Sarajevo, Bosnia and Herzegovina, from January to December 1998 were randomly selected for this study. Clinical data were collected from the Department of Oncology, University Hospital Sarajevo, Bosnia and Herzegovina. Follow up ranged from 4 to 103 months (mean: 57 months). The last follow-up data were obtained in June 2004. Three patients were excluded due to inadequate sample and one due to lost follow up. Breast cancer specimens were reviewed using morphologic and immunohistochemical criteria according to the WHO classification of breast cancer. (9) The degree of malignancy was assessed according to the Elston and Ellis grading system, which classifies tumors into grade I (well differentiated), grade II (moderately differentiated), and grade III (poorly differentiated). (10) All clinicopathological data together with clinical outcome are summarized according to treatment arm in Tables 1.
Immunohistochemical staining

Formalin-fixed, paraffin-embedded tissue was cut at 5 μm, dried overnight at 60°C and deparaffinized in xylene. Subsequently, sections were rehydrated through graded alcohols into water. Heat-induced epitope retrieval was achieved by boiling sections in the EDTA buffer at pH 8.9 in the Electrolux microwave oven at 1000W for 20 minutes (4x5min). After boiling, sections were allowed to cool at room temperature for 20 minutes, rinsed thoroughly with water and placed in Tris-buffered saline (TBS) for 5 minutes. Endogenous peroxidase was blocked with Peroxidase Block solution provided in the EnVison+® kit (DakoCytomation, Glostrup, Denmark) for 5 minutes and slides rinsed/washed with TBS. Primary antibodies used in the study are listed in Table 2. The visualization was performed using EnVision+® (DakoCytomation, Glostrup, Denmark) method according to the manufacturer’s instructions. Appropriate positive and negative controls were used. Staining for ER and PR was evaluated semi-quantitatively using the H score system according to the method described by McCarty et al. (11) which considers the intensity and percentage of cells. The score was calculated as follows: \( H_{\text{score}} = (\%3 + \text{cell} \times 3) + (\%2 + \text{cell} \times 2) + (\%1 + \text{cell} \times 1) \). The intensity of ER and PR immunostaining was visually estimated and stratified into 4 groups. Bcl-2 expression was scored semi-quantitatively: score 0 (0-10%), score 1 (10-20%), score 2 (20-50%), and score 3 (>50%) cells were positive.

Statistical Methods

Descriptive statistics comparing Bcl-2, ER and PR expression with conventional markers of tumor aggressiveness were analyzed by standard Chi-square tests and Pearson test. Estimates of relapse-free survival (RFS) and overall survival (OS) were calculated by the Kaplan-Meier product-limit method and the differences assessed by the log-rank test. Multivariate survival analysis using Cox’s proportional hazard regression model was carried out to assess the independent contribution of each variable to survival. Probabilities of RFS and OS were calculated from the date of breast carcinoma diagnosis to either the date at which relapse from breast carcinoma was clinically identified or the date of last contact. All p-values were two-tailed and the 0.05 level was considered statistically significant. A computer program package (SPSS 11.5) was used for all statistical testing.

Table 1. Characteristic of 71 patients with breast cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>71</td>
</tr>
<tr>
<td>female</td>
<td>71 (100)</td>
</tr>
<tr>
<td>male</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>6 (8)</td>
</tr>
<tr>
<td>41-60</td>
<td>43 (61)</td>
</tr>
<tr>
<td>&gt;61</td>
<td>22 (31)</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
</tr>
<tr>
<td>ductal</td>
<td>47 (66)</td>
</tr>
<tr>
<td>lobular</td>
<td>9 (13)</td>
</tr>
<tr>
<td>other</td>
<td>15 (21)</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>19 (27)</td>
</tr>
<tr>
<td>2-5</td>
<td>38 (53)</td>
</tr>
<tr>
<td>&lt;5</td>
<td>14 (20)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>21 (30)</td>
</tr>
<tr>
<td>2</td>
<td>22 (31)</td>
</tr>
<tr>
<td>3</td>
<td>28 (39)</td>
</tr>
<tr>
<td>Lymph node</td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>30 (42)</td>
</tr>
<tr>
<td>1-3</td>
<td>24 (34)</td>
</tr>
<tr>
<td>4-9</td>
<td>12 (17)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Overall survival</td>
<td></td>
</tr>
<tr>
<td>No evidence of disease</td>
<td>47 (66)</td>
</tr>
<tr>
<td>alive with disease</td>
<td>6 (9)</td>
</tr>
<tr>
<td>dead of disease</td>
<td>15 (21)</td>
</tr>
<tr>
<td>dead of other disease</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Therapy</td>
<td></td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>29 (41)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>33 (47)</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>9 (13)</td>
</tr>
</tbody>
</table>

Table 2 Primary antibodies used for immunohistochemical staining

<table>
<thead>
<tr>
<th>Antibody (Clone)</th>
<th>Dilution</th>
<th>Incubation time (Temp)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER (1D5)</td>
<td>1:20</td>
<td>30 min (RT)</td>
<td>DakoCytomation, Glostrup, Denmark</td>
</tr>
<tr>
<td>PR (PgR 636)</td>
<td>1:20</td>
<td>30 min (RT)</td>
<td>DakoCytomation, Glostrup, Denmark</td>
</tr>
<tr>
<td>Bcl-2 (124-BCL-2)</td>
<td>1:20</td>
<td>30 min (RT)</td>
<td>DakoCytomation, Glostrup, Denmark</td>
</tr>
</tbody>
</table>

RT room temperature
Results

Characteristic of seventy-one patients with breast cancer are shown in Table 1.
Forty-seven (66%) tumors were of ductal type, nine (13%) lobular and fifteen (21%) other types of breast cancer. Twenty-one (30%) tumors were grade I, 22 (31%) were grade II and 28 (39%) were grade III. The histologic distribution according to the WHO classification was as follows: grade 1, 21 cases (30%); grade 2, 22 cases (31%); and grade 3, 28 cases (39%).

Association between clinical and histological parameters with expression of bcl-2, ER and PR

Grade 1 of breast cancer was associated with better OS (p = 0.001) and RFS (p = 0.01). (Figure 1) Tumor size <2 cm was associated with better OS (p=0.003) and RFS (p=0.007). (Figure 2) Also, patient with smaller number of positive lymph node than 3 had longer OS (p=0.003) and RFS (p=0.002). (Figure 3) Distribution of H-scores for ER and PR, as well as percent of Bcl-2 positive cells with varying degrees of intensity is given in Table 3. The results of ER, PR, and bcl-2 expression are illustrated in Image. 1-9 Bcl-2 positive immunoreactivity was detected in fifty one (72%) tumor samples. Six (9%) of this showed immunoreactivity in 10-20% of the tumor cells, scored as 1+, 10 (14%) samples showed staining in 21-50% of the tumor cells, scored as 2+, and 35 (49%) samples revealed immunoreactivity in more than 51% of the tumor cells, scored as 3+. Twenty (28%) samples showed immunoreactivity in <10% of the tumor cells, and were scored as 0. (Table 3) Survival analysis revealed a favorable OS (p=0.02) and relapse free survival (p= 0.03) for patients with strong expression of bcl-2. (Figure 4) In general, more lower-grade of breast cancer expressed Bcl-2, ER and PR (Table 4), than the high-grade breast cancer did. Grade of breast cancer (p>0.000, linear-by-linear Association) and tumor size (p=0.01, linear-by-linear Association) were negatively associated with bcl-2 expression. (Table 4-6) Also, Bcl-2 protein expression was positively associated with ER (r=0.408, p<0.001) and PR expression. (r=0.413, p<0.001, Pearson test). (Table 7 and 8)

Table 3. Immunohistochemical results of, ER, PR and Bcl-2

<table>
<thead>
<tr>
<th>Percent bcl-2 (%)</th>
<th>H-score ER (%)</th>
<th>H-score PR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20 (28)</td>
<td>25(35)</td>
</tr>
<tr>
<td>1</td>
<td>6(9)</td>
<td>12(17)</td>
</tr>
<tr>
<td>2</td>
<td>10(14)</td>
<td>6(9)</td>
</tr>
<tr>
<td>3</td>
<td>35(49)</td>
<td>28(39)</td>
</tr>
<tr>
<td>Total</td>
<td>71 (100)</td>
<td>71(100)</td>
</tr>
</tbody>
</table>

Table 4. Correlation between clinical parameters and Bcl-2, ER and PR expression

<table>
<thead>
<tr>
<th>Age</th>
<th>Diagnosis</th>
<th>Grade</th>
<th>Size</th>
<th>LNS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>P=0.023</td>
<td>P=0.517</td>
<td>P&lt;0.001</td>
<td>P=0.285</td>
</tr>
<tr>
<td>PR</td>
<td>P=0.389</td>
<td>P=0.167</td>
<td>P&lt;0.001</td>
<td>P=0.04</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>P=0.315</td>
<td>P=0.915</td>
<td>P&lt;0.001</td>
<td>P=0.01</td>
</tr>
</tbody>
</table>

*LNS-Lymph node status

Table 5. Negative association between bcl-2 expression and Grades of Breast cancer*

<table>
<thead>
<tr>
<th>GRADE</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>bcl-2</td>
<td>0</td>
<td>2 (10)</td>
<td>4 (20)</td>
<td>14 (70)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1(17)</td>
<td>2 (33)</td>
<td>3 (50)</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<td>4 (40)</td>
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<td></td>
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<td>14(40)</td>
<td>7 (20)</td>
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<tr>
<td>Total</td>
<td>21 (30)</td>
<td>22 (31)</td>
<td>28 (39)</td>
<td>71(100)</td>
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</table>

* Data are given as number (percentage of row total). P<0.001, linear-by-linear Association.
Table 6. Negative association between bcl-2 expression and size of breast cancer*

<table>
<thead>
<tr>
<th>SIZE</th>
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<th>2-5</th>
<th>&gt;5</th>
<th>Total</th>
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<tbody>
<tr>
<td>bcl-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2(10)</td>
<td>11(55)</td>
<td>7(35)</td>
<td>20(100)</td>
</tr>
<tr>
<td>1</td>
<td>2(33)</td>
<td>2(33)</td>
<td>2(33)</td>
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<tr>
<td>2</td>
<td>3(30)</td>
<td>6(60)</td>
<td>1(10)</td>
<td>10(100)</td>
</tr>
<tr>
<td>3</td>
<td>12(34)</td>
<td>19(54)</td>
<td>4(12)</td>
<td>35(100)</td>
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<tr>
<td>Total</td>
<td>19(27)</td>
<td>38(54)</td>
<td>14(20)</td>
<td>71(100)</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage of row total). P=0.01, linear-by-linear Association.

Table 7. Positive association between bcl-2 and ER expression*

<table>
<thead>
<tr>
<th>ER</th>
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<th>3</th>
<th>Total</th>
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<td>0</td>
<td>13(65)</td>
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<td>1(5)</td>
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<td>20(100)</td>
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<tr>
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<td>2(33)</td>
<td>1(17)</td>
<td>3(50)</td>
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</tr>
<tr>
<td>3</td>
<td>4(11)</td>
<td>2(6)</td>
<td>11(32)</td>
<td>18(51)</td>
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<td>20(28)</td>
<td>6(9)</td>
<td>20(28)</td>
<td>25(35)</td>
<td>71(100)</td>
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* Data are given as number (percentage of row total). P<0.001, linear-by-linear Association.

Table 8. Positive association between bcl-2 and PR expression*

<table>
<thead>
<tr>
<th>PR</th>
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<th>Total</th>
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<tr>
<td>bcl-2</td>
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<td></td>
<td></td>
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</tr>
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</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>12</td>
<td>6</td>
<td>28</td>
<td>71</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage of row total). P<0.001, linear-by-linear Association.

Table 9. Cox regression test results

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>Wald</th>
<th>df</th>
<th>Sig.</th>
<th>Exp(B)</th>
</tr>
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<tbody>
<tr>
<td>GRADE</td>
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<td>.628</td>
<td>.039</td>
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<td>.844</td>
<td>.884</td>
</tr>
<tr>
<td>ER</td>
<td>.004</td>
<td>.005</td>
<td>.624</td>
<td>1</td>
<td>.430</td>
<td>1.004</td>
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<td>PR</td>
<td>-.006</td>
<td>.006</td>
<td>1.144</td>
<td>1</td>
<td>.285</td>
<td>.994</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>-.029</td>
<td>.014</td>
<td>4.181</td>
<td>1</td>
<td>.041</td>
<td>.972</td>
</tr>
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<td>THERAPY</td>
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<td>3.529</td>
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<td>.060</td>
<td>.808</td>
</tr>
<tr>
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<td>1</td>
<td>.041</td>
<td>5.341</td>
</tr>
</tbody>
</table>

LNS-Lymph node status
Bcl-2 expression in breast cancer was independent of tumor size and lymph node status in multivariate analysis. However, strong ER and PR expression was not independent of size and lymph node status as a favorable prognostic factor for OS and RFS in multivariate analysis. (Table 9)

Discussion

Bcl-2 is an oncogene that contributes to malignancy by inhibiting apoptosis and thereby extending cell survival and is one of the most studied apoptotic genes in breast cancer.

Overexpression of Bcl-2 occurs in 40% to 80% of human breast tumors (12, 7). In primary human breast cancer specimens, high Bcl-2 expression is associated with good prognosis as well as the expression of estrogen and progesterone receptor and low tumor grade. (12-18). In our study bcl-2 expression correlated positively with other predictors of favourable clinical course of the patient and less aggressive behaviour of tumour such as ER and PR positivity, smaller tumour size, lymph node negativity and lower tumour grade. Also, Bcl-2 expression in breast cancer was independent of tumor size and lymph node status in multivariate analysis. However, strong ER and PR expression was not independent of size and lymph node status as a favorable prognostic factor for OS and RFS in multivariate analysis.

According to Sjöström et al. (19) low bcl-2 expression was associated with shorter time to progression and shorter overall survival. Yang et al. (20) demonstrated that bcl-2-positivity was associated with favorable prognosis and their Cox proportional hazard model demonstrated that bcl-2 protein is an independent prognostic factor in invasive breast cancer, which is similar to our results.

One possible explanation for the phenomenon that bcl-2 is associated with better outcome (in clinical studies of prognostic factors in breast cancer) is that bcl-2 positive tumours often have ERs and, therefore more favourable prognosis. This could partially explain a favourable prognostic value of bcl-2, due to endocrine treatment that followed operative procedure.

We must also stress the possible inhibitory effects of bcl-2 on the progression of tumour which might explain why in our study bcl-2 overexpression is inversely associated with tumour size and grade. This seems paradoxical be-
cause, in experimental models, bcl-2 overexpression protects cells from apoptotic death and decreases cell-cell adhesion leading to the lost of the contact inhibition. (6, 21, 22) In this way, bcl-2 is supposed to enhance cancer cells survival and promote tumorogenesis. (6,22,23) But, some other studies found inverse correlation between Bcl-2 expression and proliferation index in invasive tumours. (e.g. malignant melanoma). This suggests the possible role of bcl-2 in the regulation of cell proliferation. (24-26)

Also, we found that absence of Bcl-2 expression is strongly associated with high proliferation rates and high tumor grade. A number of mechanisms by which Bcl-2 exerts its positive growth-supporting effects and its anti-apoptotic function have been proposed. It has been shown that Bcl-2 plays a role in the regulation of cell division. As in our study, Bcl-2 overexpression (27) has previously been shown to be associated with negative axillary lymph node status. We found that 50% patients with negative lymph node as well as 58% patients had bcl-2, 3+ expressions, without significant correlation. However, besides the experimental evidence showing the role of the bcl-2 protein as an inhibitor of apoptosis, some studies have suggested a growth-suppressing effect of bcl-2 associated with a retardation of mammalian cell proliferation. (8)

A study by Hellemans et al. (13) showed no prognostic significance for bcl-2 expression in node-negative patients, but bcl-2 negativity correlated with reduced survival among node-positive patients. The evaluation of bcl-2 expression and extent of apoptosis may provide useful prognostic information on breast cancer patients; however while increased apoptosis is strongly associated with the progression from primary carcinomas to lymph node metastases; bcl-2 does not seem to play a significant role in this process. (28) However, lymph node metastases are not only the result of altered apoptosis, but are caused by several other genetic alterations, for example alterations in adhesion proteins, (29) cell motility and angiogenesis, among others. The way in which these proteins affect the other parameters involved in lymph node metastasis is not known.

Briasoulis E et al. (30) were recently found that quantita-
tive assessment of bcl-2 expression constitutes a new approach in early breast cancer with potential clinical implications. We consider that molecular sub-staging of patients with stage II breast cancer by level of bcl-2 expression provides additional important prognostic information and prompts for investigation of its clinical significance on the issue of adjuvant systemic therapy.

Recently it was proposed that bcl-2 could inhibit cancer progression (8). The level of bcl-2 protein in T cells has been connected with the retardation of the G1/S transition through the sustained level of p27 (8) or through dephosphorylation of RB (31). Therefore, the protective effect of bcl-2 against cell death may be accomplished by slowing down the cell cycle progression (by increasing the length of the G1 phase) what in the end has positive effect on the overall survival of the patients with breast cancer.

We confirm with studies Coradini D et al (32) that no single biomarker was able to identify patients with the best (or worst) prognosis or those which would be responsive to a given therapy. Novel findings derived from gene-expression analysis indicate that the simultaneous consideration of molecular alterations contributing to the hallmarks of cancer might provide clinically useful prognostic, and perhaps therapeutic, information.

References


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HANTAVIRUS HOST/VIRUS INTERACTIONS WITHIN SOUTHEAST EUROPE

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Abstract

Viral studies have historically approached their phylogenetic analysis without consideration of the impact of the role the host plays in evolution. Our study examines host/viral interactions through analysis of the phylogenetic relationship between hantavirus genetic sequences and host cytochrome B sequences. Phylogenetic analysis of known Hantavirus genetic sequences were performed using PAUP 3.1.1 (vers. 4.0.0d64). Only sequences available through GENBANK were analyzed.

Phylogenetic analysis of hantavirus sequences revealed distinct patterns based upon geographic area. These patterns coincided with the known ranges of reservoir hosts. Multiple hosts for individual viruses and multiple viruses in a single host species for hantaviruses have been described. This may be due to accidental exposure, host-switching, co-speciation, or broad co-accommodation. Since the host is the actual environment that the virus survives in, changes in the host over time could potentially directly influence changes in the virus. Multiple viruses and hosts collide in Southeastern Europe increasing the prospect of finding distinct viral/host relationships. Rodent Cytochrome B is very well conserved and can be used to track host lineage. By tracking the relationship of infected hosts, we theorize that patterns in host DNA will emerge that will mirror patterns in viral sequences. This analysis of the host DNA could provide an understanding into the causes of variation in hantavirus sequences, pathogenicity, transmissibility, infectivity, viral range and expand our knowledge of viral/host interactions. Surveillance for viruses in the field should include analysis of the host DNA in combination with the viral analysis.

Introduction

Hantaviruses are enveloped viruses with a diameter of approximately 90-120 nm and a negative sense RNA genome. The genome is tripartite and consists of a small (S), a medium (M) and a large (L) segment, which are separately packed in helical nucleocapsids. The S genomic segment encodes the nucleocapsid while the M genomic segment encodes (1) two glycosylated envelope proteins (2). The L genomic segment is presumed to encode the L protein, which is assumed to be the RNA-dependent RNA polymerase based on its transcriptase as well as replicase functions (3). Viruses replicate in the cell cytoplasm and virus particles mature by budding into vesicles near the Golgi apparatus. Hantaviruses are serologically related rodent viruses representing a genus within the family Bunyaviridae. Unlike other bunyaviruses, hantaviruses are excreted in the saliva, urine, and feces of infected rodents. Humans may become infected through inhalation of aerosols of dried excreta, or by bite of infectious rodents (4). The distribution of hantaviruses is worldwide and different viral species circulate among natural populations of rodents (5). Cases of hemorrhagic fever with renal syndrome were first documented within Croatia in 1954 (6). Periodic outbreaks have continued to arise sporadically, with the largest epidemic occurring in 1995 (7). Types of hantavirus identified from Croatia include Dobrava, Pu- umala (8) and Tula (9). Although accidental infections by hantavirus of other mammalian hosts have been found, each viral species within the genus Hantavirus is believed to be primarily associated with a single rodent species (10). Recent evidence brings into question the validity of this one virus/one host concept. Since the 1993 outbreak of Sin Nombre virus in the western United States, great emphasis has been placed upon analysis of viruses found in rodents of the North and South American subfamily Sigmodontinae. The majority of this research has concurred with the occurrence of a single viral species or lineage within each rodent host species or race. Exceptions to this include Oran and Andes viruses, which have both been isolated from Oligoryzomys longicaudatus (11) and Black Creek Canal (12) and Muleshoe (13) isolated from Sigmodon hispidus. The presence of 2 distinct hantaviruses in this single South American rodent species suggests that the co-occurrence of multiple viruses in a single host species is possible. Considerably less is known about hantavirus within other murid rodent subfamilies. One rodent subfamily, Arvicolinae has a distribution in both the old...
and new world and hantavirus strains have been isolated from several of its genera, including Apodemus and Microtus.

**Dobrava Analysis**

*Apodemus* is restricted to the Old World and members of this genus are known to be infected with both Hantaan (HTN) and Dobrava (DOB) viruses. *Apodemus flavicolis* is thought to be the primary host of Dobrava virus in Europe. Recently however, DOB was isolated from *A. agrarius*, the primary host of HTN in Estonia (14), Russia (15) and Hungary (16). The presence of DOB in a second host over such a wide geographic range within Europe brings into question the generality of the one virus per single host species concept.

The nucleotide sequence identities between Hungarian (Tazar) DOB and other related viral lineages shown in Figure 1 included: Russian DOB from *A. agrarius* 88%; Estonian DOB from *A. agrarius* 86%-87%; Bosnia DOB from *A. flavicolis* 88%; Greek DOB from *A. flavicolis* 88%-85%; Sapporo Rat virus 70%; HTN 68%; Khabarusk 57%; PUU 56%; TUL 53%; Sin Nombre 48%. Although the sequence data obtained are limited, phylogenetic analyses linked DOB isolated from *A. agrarius* into a group with DOB previously isolated from *A. flavicolis* (16). The *A. agrarius* DOB obtained from Russia (15) did not support monophyly (common ancestry) for DOB isolated from *A. agrarius* populations in Hungary and in Russia. Other representative hantaviruses, including PUU, TUL, HTN and Sapporo rat virus, were more basal in the phylogeny (see Figure 1).

Hantaan virus is known to infect *A. agrarius* populations in Asia, whereas the virus has not been isolated in Europe. Direct enzyme-linked immunosorbent assay has demonstrated the presence of Hantaan-like antigens in *A. agrarius* in the former republic of Czechoslovakia [5.5%, (17)], the European regions of the former Soviet Union [5.3%, (18)] [28.5%, (19)], and Serbia [2.2%, (20)]. As there are no reports of HTN sequences from Europe, and given the similarities in immunological response between HTN and DOB, one might assume that earlier findings of DOB lineages, implying an older age than DOB in *A. flavicolis*.

*Apodemus flavicolis* ranges throughout much of Western Europe eastward to the Ural Mountains and *Apodemus agrarius* ranges from Eastern Europe eastward to the Pacific Ocean, covering the majority of the Asian continent (22). Given the extensive ranges of both rodent species, it would be interesting to examine other populations within each species as well as other species of *Apodemus*. This would allow one to relate the viral phylogeny to the rodent host phylogeny. It could well be that the pattern of divergence seen for hantaviruses in the New World peromyscine rodents is mirrored in Old World arvicoline rodents. Therefore, more than one hantavirus in *Apodemus agrarius* may reflect either geographic variation within the species or host-switching in regions where two host species are potentially sympatric.

**Tula Analysis**

Tula Virus (TUL) within Southeastern Europe also provides an interesting opportunity to examine virus/host relationships. When TUL was initially described by Plyusnin et al. (23), the virus was found to infect both *Microtus arvalis* and *M. rossiaemeridionalis*, and Song et al. (Unpublished Genbank sequence) found TUL in *Pitymys subterraneus*. While most European systematists classify Pytmys as a separate genus, Nowak (22) classifies Pytmys as a subgenus of *Microtus*. Scharninghausen et al., (9) also found *M. agrestis* in Croatia infected with TUL. Using Nowak’s classification, *M. agrestis* is the fourth species of Microtus found to be infected with TUL.

Sampling within Croatia has revealed TUL in two separate rodent species. The high percentages of the same TUL sequences in *M. agrestis* and *M. arvalis* from Croatia suggests that the co-occurrence of this virus in two *Microtus* species may not be the result of accidental infection. If *M. agrestis* were accidentally infected with TUL, the percentage of PCR positive animals should be lower than that of *M. arvalis*. Because the infection rate in *M. arvalis* (11.8%) was less than half of that found in *M. agrestis* (27.6%), it is unreasonable to assume that the predominance of positive *M. agrestis* could be due to accidental exposure. It is much more likely that the Croatian virus is circulating between both rodent species (9). While
it has been noted in numerous studies that several rodent species can be infected in with a single hantavirus (14; 15; 16; 21) circulation of viruses between closely related hosts has not been demonstrated. Scharninghausen et al. (16) found that although Apodemus agrarius was infected with DOB in Hungary, phylogenetic analysis of viral genetic sequences indicate that this was an ancient relationship and not evidence of host switching.

The presence of TUL in 2 different Croatian rodent species may be due to broad co-accommodation (24), where the same parasite establishes itself in an existing host without cospeciation between the host and the parasite occurring. Since no geographic isolation has occurred between M. arvalis and M. agrestis populations occupying the study area and viral exchange between the two species appears to be occurring, speciation of the virus in M. agrestis has not happened yet occurred. Clustering of TUL sequences within the phylogenetic trees (Figure 2) roughly corresponds with the known historic range of Microtus arvalis subspecies. Subspecific ranges of M. arvalis are as follows: (1) M. a. arvalis ranges through the plains of southwest central Europe from northeast France to the western portion of the Czech Republic; (2) M. a. gregarius ranges through Germany and the Czech Republic, north through the Baltic states; (3) M. a. levis ranges through the mountains of central and eastern Europe, south to Bulgaria through northern Greece; (4) M. a. duplicatus ranges from the Oder River and northern Slovakia east to the Ural Mountains (25-27). Although the subspecies of the host can be estimated based upon the historic range, no host DNA was available from previously reported viral sequences. Therefore, it was not possible to determine weather or not rodents parasitized by each viral sequence were genetically and phylogenetically distinct as would be expected if viral distinction coincided with host subspecific distinction. Based on distributions, the predicted subspecies of M. arvalis analyzed in this and other studies are indicated in Figure 2. Samples from the Czech Republic are annotated with more than 1 subspecies, as the ranges of 4 subspecies come together in this area. It may be that viral differences observed in M. arvalis differentiate along subspecific lines of the host, but until the host has been properly surveyed, this remains an untested hypothesis.

**Legend to Fig. 1:**
Cladogram derived from nucleotide sequences of DOB and various other hantaviruses. Numbers denote Genbank accession numbers. The cladogram was derived from the neighbor-joining estimated phylogeny and bootstrap analysis using p-distance estimates. The phylogenetic analysis was performed using PAUP 3.1.1 (vers. 4.0.0d64) (30). Numbers at each internode or bifurcation represent bootstrap support based on 1000 replicates. A Sin Nombre virus sequence (L37904) was used as the outgroup to root the tree. The sequences labeled Tazar were collected from the Military Base at Tazar, Hungary, (16).
Discussion

Southeast Europe offers a unique opportunity to examine the relationship between hantaviruses and their hosts. Multiple viruses and hosts collide in this region, increasing the prospect of finding distinct viral/host relationships. Since the host is the actual environment that the virus survives in, changes in the host over time or across its range could potentially directly influence changes in the virus.

To test the theories on the relationship of DOB and TUL with their hosts, direct comparisons between the phylogeny of the virus strains and the phylogeny of the infected rodents is necessary. Rodent DNA can be identified to species level by using standardized primers to examine Mitochondrial DNA (28). Using mitochondrial DNA it is possible to trace the maternal lineage of the host and determine relationships between separate populations.

PCR may also be used to determine the sex of the host (29). Host tissue can be preserved in 95% ethanol for ease of storage. Mince a small piece of tissue approximately the size of a pea for suspension in the alcohol or mix 0.5 ml of blood in 1 ml of 95% ethanol. This will preserve the DNA indefinitely at room temperature. Analysis of the genetic sequence should follow standard phylogenetic methods. By tracking the relationship between infected hosts, we theorize that patterns in host DNA will emerge that will mirror patterns in viral sequences. The analysis of the host DNA could provide an understanding into the cause of hantaviral sequences, pathogenicity, transmissibility, infectivity, viral range and expand our knowledge of viral/host interactions. Surveillance for viruses in the field should include analysis of the host DNA in combination with the viral analysis.
References


Abstract

Classical works dealing with the possibility of mother-child incompatibility with regard to basic ABO blood groups give contradictory conclusions (e.g. -(1,2)). Bioreproductive and population-genetic indicators have been studied in a sample of live births and in two pregnancy samples with different "a priori" and "a posteriori" risk assessment. The analysis points out that ABO blood groups can influence fertility of different parental pairs, and consequently - assessment of the individual pregnancy risk.

Key words: ABO blood groups, bioreproductive, population-genetics indicators.

Introduction

There are various views, which are in conflict with each other, about clinical importance of immunity incompatibility mother-child with regard to belonging to basic blood groups of ABO system. Some authors think that this type of incompatibility has a reflection on fertility of different parental pairs (1,3), while the others have different conclusions (with certain reserve; (4,5). The manifestations of hemolytic anemia of newborns due to ABO incompatibility are generally mild and don't have big clinical importance (6). However, it is not possible to completely disregard the influence of belonging to ABO blood groups on complex immunity interaction between a mother and a child (2). It is very probable that these interactions could have influence on the level of risk of individual pregnancies, having in mind especially the occurrences in the early stages of pregnancy. In other words, the chances for successful pregnancy could depend on specific arrangement of parental pairs by blood groups of ABO system (7).

Materials and methods

We want to show one attempt of usage of population-genetics analysis in order to contribute to the solution of this medical problem. Furthermore, in this paper the theoretical assumptions for possible detection of this controversial factor of fertility are being studied using population-genetics indicators. In the last few years the bioreproductive and population-genetics indicators have been analyzed in the samples of pregnant women, with a goal to determine the indicators of high risk for unsuccessful pregnancy. The basic population-genetics parameters of certain systems of group variations have been found earlier in observed groups (8, 9).

Results and discussion

In accordance with the existing medical (immunity) theory, the parental pairs could be divided into two basic groups, and those are: risky parental pairs (by which can appear different symptoms of incompatibility) and not risky parental pairs. According to the same theory, the problems due to ABO incompatibility are threatening only to the mothers with the O blood group. This is followed by the conclusion that the fertility of corresponding pairs in population should be lower. On the other hand, the frequencies of reciprocal types of pairing in balanced population should be equal, by population-genetics theory (model of genetic balance). In other words, the appearance of ABO incompatibility should reflect in significant differences in frequency of critical and reciprocal types of pairing, and those differences should depend on the level of risk of different studied groups (samples of pregnancies). The well known theoretical basis of population-genetics analysis of basic groups of ABO system (10) are shown in Table 1.

In Table 2 the theoretical frequencies of critical types of pairing are shown, and those are the types of pairing which can result in ABO mother-child incompatibility. From the shown data (Tables 7, 8, 9) which is of descriptive character it is noticeable that this parameter is decreasing with the decrease of the prior estimate of the level of risk.
Table 1. The phenotype and genotype composition of balanced population with regard to the basic blood groups of ABO system.

<table>
<thead>
<tr>
<th>BLOOD GROUP</th>
<th>A</th>
<th>B</th>
<th>AB</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>p^A</td>
<td>p^O</td>
<td>p^B</td>
<td>p^O</td>
</tr>
<tr>
<td>Theoretical frequency</td>
<td>p^2</td>
<td>2pr</td>
<td>q^2</td>
<td>2qr</td>
</tr>
</tbody>
</table>

Table 2. Critical types of pairing in population.

<table>
<thead>
<tr>
<th>Type of pairing</th>
<th>Total theoretical frequency</th>
<th>Theoretical frequency of risky pairs</th>
<th>Relative risk of individual pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>AxO</td>
<td>pr^2(p+2r)</td>
<td>pr^2(p+r)</td>
<td>p + r</td>
</tr>
<tr>
<td>BxO</td>
<td>qr^2(q+2r)</td>
<td>qr^2(q+r)</td>
<td>q + r</td>
</tr>
<tr>
<td>ABxO</td>
<td>2pqr^2</td>
<td>2pqr^2</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3. The size and the estimate of risk of studied samples.

<table>
<thead>
<tr>
<th>Studied samples</th>
<th>Symbol</th>
<th>N</th>
<th>General prior estimate of the risk of studied pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsuccessful pregnancies (spontaneous miscarriages)</td>
<td>E</td>
<td>480</td>
<td>++ +</td>
</tr>
<tr>
<td>Risky pregnancies (genetic advising)</td>
<td>Amniocentesis executed</td>
<td>A1</td>
<td>409</td>
</tr>
<tr>
<td></td>
<td>Amniocentesis not executed</td>
<td>A2</td>
<td>346</td>
</tr>
<tr>
<td>Live births (Bui 1966)</td>
<td>B</td>
<td>265</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. Proportion of genes p^A, p^B and p^O in studied samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>p^A</th>
<th>p^B</th>
<th>p^O</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>0.24</td>
<td>0.12</td>
<td>0.64</td>
</tr>
<tr>
<td>A1</td>
<td>0.29</td>
<td>0.14</td>
<td>0.57</td>
</tr>
<tr>
<td>A2</td>
<td>0.37</td>
<td>0.17</td>
<td>0.46</td>
</tr>
<tr>
<td>B</td>
<td>0.28</td>
<td>0.13</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Table 10. shows that the main part of the critical types of pairing are the pairings of the type AxO. The expected frequencies were calculated from the corresponding population-genetics parameters (p,q,r), which are characteristic for each sample. In this Table the data for the samples A1 and A2 are merged into one record. It could be said that for this condensed record the general prior estimate of risk has conditional value of 1.5. As it could have been expected, the test shows that the samples are heterogeneous. This is understandable taking into consideration the fact that the samples are formed by strictly differential criteria of biological characteristics of pregnancy (unsuccessful pregnancies, risky pregnancies, successful pregnancies). The obvious excess of critical and reciprocal types of pairing (with the exemption of normal pregnancies) has a very unequal statistical importance. As the Table shows, statistically the most important excess of reciprocal pairings is in sample E and sample A2. Among the types of pairings (AxO, BxO, ABxO), whose fertility is endangered by ABO incompatibility mother-child, exists inequality in the level of risk. Furthermore, ABO incompatibility with the mother O exists among all the children of the pairing ABxO, while the level of risk
### Table 5. Absolute frequencies of types of pairing.

<table>
<thead>
<tr>
<th>Type of pairing</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
</tr>
<tr>
<td>AxA</td>
<td>26</td>
</tr>
<tr>
<td>AxB</td>
<td>18</td>
</tr>
<tr>
<td>AxAB</td>
<td>16</td>
</tr>
<tr>
<td>AxO</td>
<td>82</td>
</tr>
<tr>
<td>BxA</td>
<td>39</td>
</tr>
<tr>
<td>BxB</td>
<td>1</td>
</tr>
<tr>
<td>BxAB</td>
<td>7</td>
</tr>
<tr>
<td>BxO</td>
<td>41</td>
</tr>
<tr>
<td>ABxA</td>
<td>18</td>
</tr>
<tr>
<td>ABxB</td>
<td>8</td>
</tr>
<tr>
<td>ABxAB</td>
<td>0</td>
</tr>
<tr>
<td>ABxO</td>
<td>12</td>
</tr>
<tr>
<td>OxA</td>
<td>108</td>
</tr>
<tr>
<td>OxB</td>
<td>48</td>
</tr>
<tr>
<td>OxAB</td>
<td>25</td>
</tr>
<tr>
<td>OxO</td>
<td>31</td>
</tr>
</tbody>
</table>

### Table 6. Absolute frequencies of critical and reciprocal types of pairing.

<table>
<thead>
<tr>
<th>Type of pairing</th>
<th>Critical</th>
<th>Reciprocal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>A1</td>
</tr>
<tr>
<td>AxO</td>
<td>82</td>
<td>54</td>
</tr>
<tr>
<td>BxO</td>
<td>41</td>
<td>26</td>
</tr>
<tr>
<td>ABxO</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Σ</td>
<td>135</td>
<td>92</td>
</tr>
</tbody>
</table>

### Table 7. Theoretical proportions of critical types of pairing.

<table>
<thead>
<tr>
<th>Type of pairing</th>
<th>Theoretical proportion</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>A1</td>
</tr>
<tr>
<td>AxO</td>
<td>( pr^2(p+2r) )</td>
<td>0.149</td>
</tr>
<tr>
<td>BxO</td>
<td>( qr^2(q+2r) )</td>
<td>0.069</td>
</tr>
<tr>
<td>AbxO</td>
<td>2pqr^2</td>
<td>0.024</td>
</tr>
<tr>
<td>Σ</td>
<td>0.242</td>
<td>0.219</td>
</tr>
</tbody>
</table>
of incompatibility for other "critical" pairs differs (Table 13) and it depends from population-genetics parameters (incompatibility can appear only in half of heterozygote fathers of A and B blood group, and participation of heterozygote in population and all its parts has a theoretical value of 2pr or 2qr). The risk is equal to the possibility of heterozygosis of the father. These differences are not taken into consideration in showed analysis.

**Conclusion**

It should be mentioned that these findings do not give completely unified conclusions in terms of presence or absence of ABO incompatibility mother-child, or conclusions about the value of population-genetics analysis for detection of the consequences of such incompatibilities. The analysis of frequency of critical and reciprocal parental pairs confirms the thesis that the belonging to ABO blood groups plays a certain role in complex immunity interactions between mother and the child. In other words, it can have an effect on the estimate of the risk of pregnancy.
Table 11. Test of homogeneity of samples (11) with the regard to the frequency of studied types of pairing. (* Table is in the text with the original data).

| Estimate of the risk | Sample |  T8*  | T6*  | T6*  | e      | o_re  | o_crit | (o_re-e)^2 | (o_re-e)^2/e | (o_re-e)^2/e | Σ [(o-e)^2/e] | df |
|---------------------|--------|-------|------|------|--------|-------|--------|-------------|---------------|---------------|---------------|---------------|----|
| 2                   | E      | 116.1 | 135  | 181  | 357.21 | 4212.01 | 3.08   | 36.28       | 39.36         | 1             |
| 1                   | A      | 147.2 | 149  | 210  | 3.24   | 62.80  | 0.02   | 0.43        | 0.45          | 1             |
| 0                   | B      | 59.8  | 47   | 59   | 163.84 | 0.64   | 2.74   | 0.01        | 2.75          | 1             |
| Pool.               |        |       |      |      |        |        |        |             |               | 42.56         | 3             |
| tot.                |        | 323.1 | 331  | 450  | 62.41  | 16103.61| 0.19   | 49.84       | 50.03         | 1             |
| diff.               |        |       |      |      |        |        |        |             |               | 7.47          | 2             |

Table 12. Statistical importance of deviation of the frequency of critical and reciprocal types of pairing from their expected frequency calculated from population - genetics parameters.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Crit</th>
<th>Rec</th>
<th>theor</th>
<th>χ²crit-theor</th>
<th>p</th>
<th>χ²rec-theor</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>135</td>
<td>181</td>
<td>116.1</td>
<td>3.08</td>
<td>&gt;.25</td>
<td>36.28</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>A1</td>
<td>92</td>
<td>107</td>
<td>89.5</td>
<td>0.07</td>
<td>&gt;.25</td>
<td>3.42</td>
<td>&gt;.25</td>
</tr>
<tr>
<td>A2</td>
<td>57</td>
<td>93</td>
<td>57.7</td>
<td>0.01</td>
<td>&gt;.25</td>
<td>21.60</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>B</td>
<td>47</td>
<td>59</td>
<td>59.8</td>
<td>2.74</td>
<td>&gt;.25</td>
<td>0.01</td>
<td>&gt;.25</td>
</tr>
</tbody>
</table>

Table 13. Relative risk of appearance ABO incompatibility mother-child in critical types of pairings.

<table>
<thead>
<tr>
<th>The possibility of heterozygosis of the father</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
</tr>
<tr>
<td>AxO</td>
</tr>
<tr>
<td>BxO</td>
</tr>
<tr>
<td>AbxO</td>
</tr>
</tbody>
</table>
References


Abstract

We retrospectively analyzed patients with larynx cancer that were treated at ENT Clinics in Sarajevo in the last 5 years. According to the tumor site, TNM classification, operative procedure and postoperative treatment we found the following:

Over the last 5 years 156 patients underwent surgical treatment - 143 (91,6%) male and 23 (8,4%) female. According to the age 1 patient belonged in the age group 30-40 years (0,64%), 25 in the age group 40-50 years (16%), 51 in the age group 50-60 (32,7%), and 79 were over 60 years of age (50,6%). Of those 145 were smokers (93%), and 11 non-smokers (7%). Histological findings showed 100% cases with squamous cell cancer.

Most of the patients were surgically treated with total laryngectomy with unilateral or bilateral dissection and thyroidectomy or lobectomy (29%) or total laryngectomy with thyroidectomy or lobectomy (23%), total laryngectomy (22%). The rest of the patients underwent total laryngectomy with unilateral or bilateral dissection (16%) chordectomy (4%), supraglottic laryngectomy (3%), hemilaryngectomy (2%) and hemilaryngectomy with dissection (1%).

All patients were postoperatively irradiated and chemotherapy was combined with irradiation only in younger patients.

Key words: incidence of larynx cancer, surgical treatment

Introduction

Tumors located in the interior of larynx are considered as larynx tumors. They have different symptoms, metastases and prognosis than hypopharynx tumors. According to pathohistological findings most frequent larynx cancer is squamous-cell cancer while other types like adenocancers, cylindromas and fibrous tissue malignancies are rather rare in larynx interior.

In 2001 incidence of larynx cancer in FB&H, treated in Clinics Center Sarajevo, was 110 new cases, 93 male and 17 female(1). In the same period in Croatia the incidence was 491 new cases, 397 male and 34 female, with ratio 12:1(2).

In other countries incidence is similar or slightly increased and male-female ratio ranges from 5:1 to 20:1. The highest incidence of larynx cancer is found in Sao Paulo, Bombay and Thailand. Industrial regions have higher incidence in comparison to other regions. In USA incidence is higher in African Americans than in Caucasians, and male are more frequently affected in comparison to female.

When tumor is revealed, during the first examination, 65% of patients have local disease, 30 % neck metastases and 5% distant metastases (3). According to the age, male individuals in 60-75 age class are the most frequently admitted patients. In our material 50% of all patients are male individuals over 60 years of age.

In ethiology of larynx cancer the abuse of tobacco and alcohol play an important role. They act synergistically and increase the risk of larynx cancer, along with chronic irritation with cement dust, asbestos, in workers in nickel and oil industry. According to the statistics 88-98% of patients with larynx cancer are smokers (4). Predisposing factors could be some forms of chronic laryngitis, kerathosis, papillomas and leukoplakia. Laryngopharyngeal reflux is also associated with this disease, but it has not been proven yet (5).

Goals

- Determination of larynx cancer incidence in the last 5 years in relation to sex, age and habits.

- Determination of tumor stadium according to TNM classification.

- Analysis of surgical treatment of patients with larynx cancer.

Material and methods

We retrospectively analyzed patients with larynx cancer that were admitted at ENT Clinics in Sarajevo over the last 5 years. All research described in the submitted publication involving human subjects and material derived from human subjects complies with ethical principles in World Medical Association Declaration of Helsinki- Ethical Principles for Medical Research Involving Human Subjects (initiated in June 1964, amended in October 1975; October 1983; September 1989; October 1996; October 2000). According to the tumor site, TNM classification, operative procedure and postoperative treatment we divided patients in groups.
Results

Graph 1. INCIDENCE RELATED TO AGE GROUPS

Graph 2. TNM CLASSIFICATION IN %

Graph 3. PATHOHISTOLOGY

Ca planocellular laryngis 156

Graph 4. SURGICAL TREATMENT
Discussion

Larynx cancer is an important health and social problem. In most number of cases it is diagnosed late and with grave prognosis. Thus, it requires special attention from the point of prevention, diagnostics and therapy. Increase in number of patients with larynx cancer is obvious from year to year, and since 1998 the rate is 5-17%. We proved this tumor prevalence in male in comparison to female patients which fits the data from Western Europe and USA where 95% of all patients are male (6).

According to the age group larynx cancer incidence is highest in the group of over 60 years (50%), than 50-60 years (33%), 40-50 years (16%), and 30-40 years (1%). According to pathohystological findings, in our material, only squamous-cell cancer was found (100%). We did not encounter other types of cancer unlike other authors data who noticed anaplastic cancer in few patients, while other types of cancers were not encountered.

Incidence of squamous-cell cancer in the group of larynx cancers is by far the highest and according to European and American data amounts to 90-93%, while in our material it was 100% (7).

According to TNM classification our data showed highest incidence of advanced-stage tumors, 39 cases T3N0M0, T4N2M0-17; T2N0M0-14; T2N1M0-13; T1N0M0-10 cases. The results reveal the fact that number of advanced stage tumors is high what leads to the conclusion that diagnosis is established late and the amount of " time lost " is significant. Thus, the prognosis is questionable.

According to surgical treatment there is a prevalence of total laryngectomies with unilateral or bilateral dissection and lobectomy or thyreoidectomy (29%), followed by total laryngectomies with lobectomy or thyreoidectomy (23%), total laryngectomies (22%), total laryngectomies with unilateral or bilateral dissection (16%), chordectomies (4%), supraglottic laryngectomies (3%), hemilaryngectomies (2%) and hemilaryngectomies with dissection (1%).

Conclusion

Malignant tumors of larynx are very complex group of diseases in sense of diagnosis, treatment and rehabilitation. In total amount of ENT tumors, malignant tumors of larynx are present in more than 50% patients and their number is increasing every year.

From our results it is obvious that the disease is diagnosed in an advanced stage which diminishes therapeutic possibilities. Modern diagnostic procedures enable quick diagnosis, thus time from first examination to definitive diagnosis is short, but the period from the onset of disease until first examination in most cases is rather long.

Stage analysis proved that most of our patients were in T3N0M0 stage, and according to that, the majority of surgical treatment consisted of total laryngectomies with dissection - 41.9%.

Larynx cancer is second most frequent cancer in University in Sarajevo Clinics Center, while in European and American data it is not found amongst ten most frequent malignancies. That is the most impressive aberration from the quoted statistics which points to the necessity of further investigation. This could be partially explained by extensive smoking habit and low living standard.

References

INFLUENCE OF LONG TERM STRESS EXPOSURE ON SOMATISATION SYMPTOMS OUTCOME

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*Corresponding author

Abstract

Long term stress exposure results in somatisation symptoms appearance. Cardiovascular, respiratory, gastrointestinal and muscle-bone symptoms arise because of intensified activity of autonomic nervous system caused by chronic stress. The aim of the study was to examine the relationship between long term war stress exposure and appearance of somatisation. 40 students of health-care faculties in Sarajevo, of both sexes, were included in investigation and divided in two groups-somatisation and control. Somatisation group subjects (N=20) lived in B&H under war conditions, from 1992-1995. Control subjects (N=20) spent the same period outside B&H. For evaluation of somatisation symptoms we used SCL-90-R test. The obtained data were statistically evaluated using Student's t-test and \( \chi^2 \) test. Confidence level was set at \( p < 0.05 \). Our results showed statistically significant difference in somatisation level between somatisation and control subjects group. Different intensity of appearance of certain symptoms in male and female was established. The score of somatisation dimension between somatisation and control group showed statistically significant level (\( p < 0.0001 \)). Study results confirmed correlation of chronic stress exposure (living in war environment) and somatisation symptom appearance. Individual organic systems had various level of symptom expression. The influence of sex on intensity of individual symptoms of somatisation is possible.

Key words: somatisation, stress

Introduction

Long-term exposure to an extreme traumatic event causes psychological abnormalities that persist long after the removal of stress-precipitating agent. Somatisation has been described as the psychological distress in the form of physical symptoms. It has also been suggested that somatisation is a way of defense against psychological distress expression (1). Long-term stress exposure is cause of changes in autonomic nervous system functioning. It happens at two levels: the basal tone and stress-related reaction (2). Stress, by activating the sympathetic nervous system, hypothalamic-pituitary axis, renin angiotensin system causes the release of stress hormones such as: cateholamines, corticosteroids, glucagon, growth hor-
Results and discussion

SCL-90-R completed by subjects in both groups was analyzed. Total level of somatisation higher than 50 was considered abnormal. Our results showed that individual members of somatisation group had higher level of somatisation than individual members of control group (Graph 1.). Graph 1. shows relation between somatisation level of individual members of somatisation and control group. The obtained differences in total level of somatisation between the two groups is displayed on Graph 2. In the somatisation subjects group total level of somatisation was higher compared to the control subject group.

In Graph 2. we displayed the difference of total level of somatisation between somatisation and control group. The obtained experimental data were statistically evaluated by using Student’s t test. Data were expressed as mean ± SD. Statistically significant difference was established between two groups p<0.0001. Intensity of single somatisation symptom appearance in two observed groups was analyzed. Then we compared intensity of symptom expression of male subjects between somatisation and control group. Intensity of symptom expression of female subjects in two observed groups was analyzed separately (Table 1.). Intensity of symptoms was expressed on 0-4 scale. Table 1. shows the differences in intensity of individual symptoms of somatisation between male and female subject groups. Data were evaluated by χ² test.

The obtained experimental data show statistically significant difference (p 0.0001) in symptoms intensity between somatisation and control group. Statistically significant difference in intensity of all somatisation symptoms between the two groups was confirmed. Symptom such as "pains in heart and chest" showed statistically significant difference (p 0.01) that is lower than the difference for other somatisation symptoms (p 0.001). For female subjects, a higher level of significant differences between somatisation and control groups was obtained for symptoms such as "headaches", "faintness or dizziness", "nausea or upset stomach", "hot or cold spells", "heaviness in limbs". We found higher level of difference between male subjects of somatisation and control group for symptoms "pains in heart or chest" and "pains in lower back" compared to other symptoms.

Conclusion

Long-term stress exposure leads to psychological abnormality and increased activity of autonomic nervous system. This type of alteration causes a variety of pathophysiological changes. Biological changes in the functioning of noradrenergic and serotoninergic system, hypothalamo-hypophyseal-adrenaline axis and endogenic opiatice system gradually leads to the expression of organic disorders (5). Chronic stress exposure influences the appearance of symptoms in cardiovascular, respiratory, gastrointestinal and muscle-bone systems. We confirmed the relationship
between chronic stress exposure (living in the war environment) and somatisation symptoms occurrence in post war period. We established variation in the expression of symptoms within individual organic systems. Our investigation shows possible influence of sex on the intensity of individual symptom expression. Long-term stress exposure is a risk factor in the development of many diseases. On the basis of our investigation, we can expect appearance of clinical symptoms and changes in biochemical parameters (glucose intolerance, increased blood lipids, changes in serum enzymes activities and other abnormalities) in somatisation subjects group (6). Changes of biochemical parameters are caused by long-term intensified activities of vegetative, endocrine, cardiovascular and immunological system (7). Intensity of the expression of individual somatisation symptoms in male differs from that in female subjects. This indicates possible influence of sex on clinical symptom appearance (8,9).

Table 1. Expression level of individual somatisation symptoms in somatisation and control subjects group

<table>
<thead>
<tr>
<th>SOMATISATION SYMPTOMS</th>
<th>SG₂/C₃</th>
<th>SG₂/C₄</th>
<th>SG/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>headache</td>
<td>*</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>faintness or dizziness</td>
<td>*</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>pains in heart or chest</td>
<td>**</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>pains in lower back</td>
<td>**</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>nausea or upset stomach</td>
<td>ns</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>soreness of muscles</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>feeling of loosing breath</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>hot or cold spells</td>
<td>ns</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>numbness or tingling in part of the body</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>feeling of having lump in the throat</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>weakness in body parts</td>
<td>ns</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>heaviness in limbs</td>
<td>ns</td>
<td>**</td>
<td>***</td>
</tr>
</tbody>
</table>

SG₂/C₃ - symptom intensity difference between male subjects of somatisation and control group;
SG₂/C₄ - symptom intensity difference between female subjects of somatisation and control group;
ns not statistically significant p>0.05;
* statistically significant p<0.05;
** statistically significant p<0.01;
*** statistically significant p<0.001;
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Abstract

Angiotensin converting enzyme (ACE) plays an important role in blood pressure regulation not only in the state of rest, but also during physical exercise. The aim of this study was to estimate the serum ACE activity in response to acute dynamic exercise.

The study involved a group of young, healthy, male volunteers (average 22 years of age). Exercise testing was carried out on ergometer bicycle according to the protocol of individually adjusted continuous, constant workload (3W/kg). The activity of ACE in serum was measured in venous blood, in the period of rest, in 4th, 8th and 12th minute of exercise and 1st, 3rd and 6th minute of recovery by spectrophotometric method.

Marked inter-individual differences in basal serum ACE activity were determined (range 8.31 - 63.72 U/L). Serum ACE activity did not significantly vary during exercise and in the period of recovery. Systolic blood pressure changed during exercise compared to values during rest period in accordance with the applied type of dynamical exercise. Diastolic blood pressure did not vary considerably during exercise. Statistically significant correlation between mean arterial blood pressure and ACE activity in the serum was not found.

The lack of increase of ACE activity in the serum, in spite of changes in blood pressure values, most likely shows the presence of alternative ACE independent pathway involved in the production of vasoactive substances that have important role in the regulation of cardiovascular system response to acute dynamic exercise.

Key words: angiotensin converting enzyme, acute exercise, healthy volunteers, male

Introduction

Angiotensin I converting enzyme, dipeptidyl carboxypeptidase (ACE, kininase II: EC 3.4.15.1, peptidase P) is a part of the renin angiotensin system that plays important role in electrolyte balance and blood pressure regulation not only in the state of rest, but also during physical exercise. ACE is also involved in the calicrein-kinin system. Besides being present in plasma, ACE could be located at luminal surface of vascular endothelial cells in various places in body. The activity of ACE was determined not only in blood serum but also in most of vascularized tissues. (1)

Circulating ACE levels or ACE activities in plasma are highly genetically determined (2.3). There are two forms of ACE gene in humans: D (deletion), or I (insertion), and there are three possible configurations: DD, DI and II. It was demonstrated that I/D dimorphism related to ACE genes concurs with serum and tissue activity of ACE. The ID polymorphism is physiologically important because it was observed that the I allele is accompanied with lower (3, 4), and D allele with relatively higher activity of circulating and tissue ACE (4). This was confirmed in studies conducted in populations of various ethnic groups. The variations in levels of ACE activities related to ID polymorphism of ACE genes probably do not influence the systemic level of angiotensine II and blood pressure (5).

In healthy persons the levels of ACE in plasma can show as much as 5-fold inter-individual variations (6), but intra-individual variations are small, and there are no marked variations in serum activity of ACE during 24 hours (7).

Physiological role of ACE is still not completely revealed. ACE cleaves dipeptide (histidyl-leucine, His-Leu) from the C terminal end of decapeptide chain of angiotensin I and produces angiotensin II, potent vasopressor and aldosterone stimulating peptide. At the same time, ACE deactivates bradykinin in systemic circulation that is potent vasodilator and stimulator of prostaglandin production. Physical activity stimulates the renin angiotensin system (8, 9). The inhibition of ACE does not inhibit this stimulation completely (10).

There is disagreement in reports about changes of serum ACE activity in response to physical effort. Some of them showed the lack of changes in serum ACE activity (11) while the others reported an increase (6).

Because of the above-mentioned controversial findings, we aimed this study to estimate the serum ACE activity in healthy, male subjects in response to acute dynamic exercise.
Subjects and methods

Subjects

The study involved 14 healthy, young, male volunteers (average 22 years of age). All of them signed written informed consent. The School of Medicine Ethic Committee, Sarajevo approved the study protocol.

Procedure

Before exercise testing each subject underwent the same procedure consisting of clinical history, physical examination, routine laboratory tests, electrocardiogram (ECG) and blood pressure measuring in the period of rest. The results of this procedure showed that these subjects had no evidence of present or past health problems. No subject was taking any medication in the study period.

Exercise test

The exercise testing was conducted in quiet environment with temperature ranging from 22° to 24° C. Each subject, immediately after period of warming up that lasted 3 minutes (workload 1,5W/kg) carried out one exercise test of 12 minutes, in upright, sitting position on bicycle ergometer (LODE-Corival 400) under the protocol of continuous, constant, individually adjusted workload (3 W/kg) and cycling rate of 60 RPM.

Blood pressure was measured using standard cuff method in the period of rest, in the 4th, 8th and 12th minute of exercise and in the 1st, 3rd and 6th minute of recovery.

ECG was continuously monitored during exercise and in the recovery period using Quinton ECG monitoring system Q-5000.

The indications for aborting the test were: unusual changes in ECG or blood pressure reactions, pain in legs, dyspnoea and dizziness.

Serum ACE activity

Blood samples were drawn through a canilla placed into the left cubital vein in the period of rest, in the 4th, 8th and 12th minute of exercise and in the 1st, 3rd and 6th minute of recovery. The ACE activity in serum was determined by spectrophotometric method according to Filipoviæ et al. modification (12) using hippuryl-l-histidyl-l-leucine as a substrate ("Sigma", St. Luis, USA). Spectrophotometer used was "Perkin Elmer" 550 S model. The values of serum ACE activity were expressed in U/L.

Statistical analysis

Values were expressed as mean +/− SEM. In the analysis of changes of serum ACE activity and blood pressure values, we used t test for small dependent samples. Differences were considered statistically significant at the p < 0.05. The coefficient of correlation was determined by Pearson’s method.

Results

The average values of serum ACE activity in period of rest, during exercise and in recovery period is shown in Table 1.

There were marked inter-individual variations in basal values of serum ACE activity amongst the subjects (range 8,31 - 63,72 U/L). No statistically significant changes in serum ACE activities were found either in the exercise period or in the recovery period. The dynamics of blood pressure changes is shown in Figure 1.

Systolic blood pressure (SBP) increased significantly in comparison to the value measured in the period of rest. The dynamics of changes is in accordance with the type of applied dynamic, rhythmic exercise on bicycle ergometer. In the recovery period systolic blood pressure decreased successively, and at the end of this period there were no statistically significant differences in comparison to the value in the period of rest.

During exercise there were no significant changes of diastolic blood pressure (DBP). In the early period of recovery (in the 1st minute) DBP decreased significantly compared not only to the values in the period of rest but also at the end of exercise. At the end of recovery period the value of DBP was statistically significantly lower than in the period of rest. The dynamics of mean arterial pressure changes and serum ACE activity is shown on logarithmic scale in Figure 2.

Our results did not show significant correlation between SBP, DBP or MAP and serum ACE activity either in the period of rest, during exercise or in the recovery period.

Discussion

Acute physical exercise demands fast adaptation of entire cardiovascular system. The most important hemodynamic changes are due to the need for matching the blood flow with increased metabolic needs of active tissue (13). The increase in cardiac output and changes in peripheral resistance caused by local vasodilatation in active tissues influence arterial blood pressure values.

Both humoral and nerve mechanisms are involved in the regulation of blood pressure (14). They maintain the balance between the needs for increase in arterial blood pressure that is important for obtaining appropriate blood flow through active parts and decrease of arterial blood pressure caused by peripheral vasodilatation (for delivering nutrients and oxygen, and elimination of vaste products and carbon dioxide as well as thermoregulation).

Angiotensin converting enzyme is one of the key molecules in the production of potent vasopressor, angiotensin II, which effectivly influences blood pressure values. The values of basal serum ACE activity in subjects involved in our study showed marked inter-individual differences, which is in accordance with the results by Woods et al. (6). They related their results with polymorphism of ACE genes. It was observed that the subjects with DD genotype...
had higher average values of serum ACE activities in basal conditions, while the subjects with II genotype had lower values.

Reports on serum ACE activity in response to acute physical exercise are not consistent. There were problems in comparison of the obtained results due to the use of numerous different protocols of exercise testing (different in the intensity of workload and duration of workload) and different equipment used in exercise (bicycle ergometer or treadmill). The results may also be influenced by the personal characteristics of the subjects (age, gender, ethnicity, level and type of usual physical activity). The important differences in findings of different studies are due to frequency of blood sampling (6, 11) not only in the period of exercise but also in the recovery period.

Our results show that there were no significant changes in serum ACE activity either during exercise or in the recovery period in comparison to the basal values in the period of rest. Miura et al. (11) also found no changes in serum ACE activity during acute dynamic exercise lasting 30 minutes. Nevertheless, they found that concentration of angiotensin II in serum increased significantly. They presumed that this is due to the presence of "kinine-tensine system" that is involved in the production of angiotensin II independently of serum ACE. They also found that there were no statistically significant changes in concentrations of ACE (U/L) at the end of exercise in comparison to basal values. Their results suggested the presence of alternative pathway of angiotensin II production during acute physical exercise where one or more enzymes from the serine protease group are involved.

As opposed to that, Woods et al. (6) found significant increase in serum ACE activity compared to basal values not only immediately after 20 minutes of exercise at the

### Table 1. The serum angiotensin converting enzyme (ACE) activity in period of rest, during exercise and in recovery period

<table>
<thead>
<tr>
<th>Type of activity</th>
<th>Serum ACE activity (U/L)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period of rest</td>
<td>40.04</td>
<td>4.76</td>
</tr>
<tr>
<td>Exercise (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>38.51</td>
<td>2.58</td>
</tr>
<tr>
<td>8</td>
<td>37.08</td>
<td>2.51</td>
</tr>
<tr>
<td>12</td>
<td>36.14</td>
<td>1.90</td>
</tr>
<tr>
<td>Recovery (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>40.87</td>
<td>3.47</td>
</tr>
<tr>
<td>3</td>
<td>37.44</td>
<td>3.05</td>
</tr>
<tr>
<td>6</td>
<td>38.15</td>
<td>3.84</td>
</tr>
</tbody>
</table>

* Values are expressed as mean value ± SEM (number of subjects) = 14
level of 70% of VO2 max, but 40 minutes later as well. In this study, the basal values of serum ACE activity were significantly higher in subjects with DD genotype (45.5 ± 2 nmol His/Leu/ml/min) than in subjects with II genotype (24.9 ± 1.8 nmol His/Leu/ml/min) although the whole group showed an increase in serum ACE activity. Our results are difficult to compare with the results of the above-mentioned studies because of differences in the design of the studies. The differences are, primarily, in the duration of acute exercise, type of workload, dynamics of blood sampling not only in the period of exercise but also in the recovery period. We found marked differences in basal values of serum ACE activities amongst the subjects included in our study which concurs with Woods et al (6). However, the Miura et al. (11) results are more in accordance with ours and we can accept their presumption that the lack of increase in serum ACE activity during acute exercise is due to the presence of ACE independent pathway in the production of vasoactive agents during acute dynamic exercise.

**Conclusion**

Acute dynamic exercise does not cause an increase in serum ACE activity in healthy, young male subjects. Statistically significant correlation between serum ACE level and systolic, diastolic or mean arterial blood pressure either in the period of rest, during exercise or in the recovery period was not found. The lack of increase in ACE activity in the serum, in spite of changes in blood pressure values, most likely indicates presence of alternative ACE independent pathway involved in the production of vasoactive substances that have important role in the regulation of cardiovascular system response to acute dynamic exercise.
References


Abstract

Brunkow exercises starting with dynamic contraction of hands and feet with fixed point on the wrist or/and heel. Dynamic contraction from the beginning, transferring through kinetic chain, leads to isometric contraction of the group of muscles, which has to be included in the exercise. Starting positions determinates the group of muscles to be trained.

The purpose of this study is to investigate influence of Brunkow exercises on spinal motion improvement and pain relief and to evaluate use of Brunkow exercises, as a routine method for lower back pain in Physical Medicine and Rehabilitation Centres.

Thirty-four patients with symptoms of low back pain were included in study. Patients received a mean of 14.9 treatments with standard deviation of 8.96.

All patients were assessed before and after the treatment for spinal mobility and flexibility as well as pain intensity. All parameters for spinal movements showed statistically significant improvement in patients with low back pain who practiced Brunkow exercise program at the end of treatment in relations to pre-treatment values, with significant difference of p<0.01 for all motions.

Pain was reduced on VAS for X=1.7 with S.D. 1.97. Difference Test was t=6.020 with significant difference p<0.01.

Flexibility of spine increased, so average difference in values before and after treatment for Shober test was 0.5 cm with SD 0.65. Difference test was t=3,794 with significant difference p<0.01.

Brunkow exercises for low back pain are beneficial treatment for increasing flexibility and mobility of spine and improving the pain.

Key words: Brunkow exercises, low back pain, spinal mobility

Introduction

Therapeutic approaches in treating low back pain are different and very often controversial. In general, physiotherapy treatments for low back pain, can be sorted in "passive" treatments, such as thermo and cryo procedures, manipulation, massage, orthosys, traction and electrotherapy, and "active" treatments like kinesitherapy procedures. There are different types of exercises for back pain, such as flexion exercises, extension exercises, or some specific exercises that are combination of these two types. Decision which type of exercises can be applied is very individual, depends of physician's approach and there is no prescription which one is the most appropriate for each patient. (1-10)

Exercise is typically aimed at strengthening back extensors or flexors and increasing back flexibility to reduce injury risk, improving mood and pain perception to reduce the impact of injury. (11-15)

Brunkow exercises (16) can be called "pushing exercises" and they can be done in all starting positions. They are starting with dynamic contraction of hands and feet with fixed point on the wrist or/and heel. Dynamic contraction from the beginning, transferring through kinetic chain, leads to isometric contraction of the group of muscles, which has to be included in the exercise. Starting positions determinates the group of muscles to be trained.

The purpose of this study is to investigate influence of Brunkow exercises on spinal motion improvement and pain relief in patients with symptoms of low back pain. Although Brunkow exercises are isometric exercises, and their main goal is strengthening paravertebral muscles we were interested in their influence on improvement of spinal mobility, flexibility and pain relief. The aim was also, to evaluate use of Brunkow exercises, as a routine method for lower back pain in Physical Medicine and Rehabilitation Centres.

Methods

Participants

Thirty-four patients with symptoms of low back pain were included in study, which was approved by the Ethics Committee of the Sarajevo University Faculty of Medicine. Patients were recruited from Physical Medicine and Rehabilitation outpatient Clinic in Community Based Rehabilitation Centers in Sarajevo and from University Clinical Center Sarajevo - Institute for Physical Medicine and Rehabilitation. Subjects were referred for physiotherapy treatment from Primary Health Care physicians or from specialist Clinic (orthopedic, traumatology, neurology etc). Patients with lower back pain, but without motor or
sphincter deficit were included in the study. Professionals who had minimal training in Brunow method did instruction for exercises and supervision of patients.

Measures
All patients were assessed before and after the treatment. Spinal range of motions were measured using centimeter and measuring a distance between top of the third hand finger and floor while patient were asked to move forward, backward and on right and left lateral side.

Visual Analog Scales (VAS) were measured and recorded as numeric rating scales (0-10). VAS was given to each subject to show his pain intensity on scale graded from 0 to 10, where grade 0 means that patient doesn't have a pain and grade 10 is sign for the worst possible pain. The patient marks a certain length of this line that was equivalent to the intensity of pain experienced. The distance of this mark from "no pain" end of the scale was measured. Shober test was used for measuring flexibility of spine. While patient was in standing position, a horizontal line on the level of edge of iliac bones was marked, and second line 10 cm proximal and parallel with first one. In normal spinal condition, this space increasing for 5 centimeters more, so difference between lines will be 15 centimeters. If patient has pain in lower back, his spinal movements are limited by pain, and Shober test has decreased values. Shober test values before and after the treatment can show results of treatment.

Exercise Therapy
Brunow exercises were performed individually according to need and possibility of each patient. Patients attended exercise program daily, under supervision of physiatrist and physiotherapist in the Clinic for Physiotherapy and Rehabilitation, and they were asked to do the same exercise program at home - five times a day in series of 5 to 10 repetition each time, depending of stage of disease and pain intensity. Type of exercises and number of repetitions in each session were created individually for each patient. Starting positions for Brunow exercise program gradually increase pressure on vertebra (prone position, standing, supine position and sitting). The training contraction was performed as a maximal contraction held for 5 seconds, followed by a rest for 1 minute.

Statistics
Results were expressed as mean +/- SD. Differences in group means were examined by Student t-test.

Results
A total of 13 men (38%) and 21 women (62%) participated in this study. Mean age was 42 years (+/- 13,8 years) - error of 2,36. (Figure 1.) 41% of patients experienced first symptoms of lower back pain in the year of assessment, 15% has 4 years experience of pain, 20% 10 years and 26% more than 10 years. (Figure 2.) According to Quebec Task Force of Spinal Disorders (24) all patients were grouped in 3 groups: acute stage of disease - pain less than 7 days, subacute stage - pain from 8 days to 7 weeks and chronic stage - pain more than 7 weeks. There were 9% of participants in acute pain, 50% in subacute and 41% in chronic pain in Brunow group. (Table 1.) Patients received a mean of 14,9 treatments with standard deviation of 8,96. Three patients didn't complete the treatment. No adverse effect occurred. Thus, 31 patients were enrolled into the statistical analysis.
Data analysis

Measurements of spinal movements and flexibility of spine showed significant improvement in all patients with lower back pain after exercising Brunkow program. Average measurements (X) for Shober test before treatment were 13.0 with standard deviation (SD) 2.33 were increased after treatment to X = 13.5 and SD 2.12.

Average difference in values before and after treatment was 0.5 cm with SD 0.65. Difference test was t=3.794 with significant difference p<0.01. (Figure 3.) All parameters for spinal movement showed improvement after exercising Brunkow program for lower back pain. (Table 2.)

Difference in measurements before and after treatment showed that flexion increased for 3.4 cm in average, extension for 1.7 cm; right side flexion for 2.0 cm and left side flexion for 2.2 cm in average. All of these parameters showed statistically significant improvement at the end of treatment in relations to pre-treatment values, with significant difference of p<0.01.

Mean pain intensity was reduced significantly as a result of treatment.

Pain intensity pre-treatment, on VAS was in average X=5.8, with S.D. 2.01 and at the end of treatment was decreased to X=4.1 with S.D. 2.25.

Pain was reduced on VAS for X=1.7 with S.D. 1.97. Difference Test was t=6.020 with significant difference p<0.01. (Figure 4.)

Intensity of pain (VAS) showed that among 31 patients who exercises Brunkow program for low back, 7 patients pain didn't have pain relief (23%). Comparing pre-treatment and post-treatment data in all participants, it was found that among 31 patients who performed Brunkow exercise program for low back pain 9 patients didn't have any improvement in spinal flexibility, so values of Shober test were the same before treatment and after the treatment for 30% of participants in this study. Spinal mobility score in Brunkow group showed that 13% of patients didn't improve in flexion score, 9% in extension, 9% in right side flexion and 23% in left side flexion. (Figure 5)

Results in this study showed that Brunkow exercises for low back pain was beneficial for participants in our study but there was certain number of patients who didn't achieve any improvement.

Table 2. Spinal mobility before and after Brunkow exercise program in patients with the low back pain.

<table>
<thead>
<tr>
<th>Motion</th>
<th>Statistics</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>Difference</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexion</td>
<td>X - average</td>
<td>24.2</td>
<td>20.8</td>
<td>3.4</td>
<td>t=4.593</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>SD - standard deviation</td>
<td>20.8</td>
<td>19.3</td>
<td>4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>X</td>
<td>62.5</td>
<td>60.8</td>
<td>1.7</td>
<td>t=5.118</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>5.7</td>
<td>6.0</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateroflexion right</td>
<td>X</td>
<td>49.9</td>
<td>47.9</td>
<td>2.0</td>
<td>t=6.909</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>6.2</td>
<td>6.6</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateroflexion left</td>
<td>X</td>
<td>50.2</td>
<td>48.0</td>
<td>2.2</td>
<td>t=5.519</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>5.8</td>
<td>6.2</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

This study investigated use of Brunkow exercises in the treatment of patients suffering from lower back pain and its influence on pain relief, spinal movements and flexibility of spine. Kinesitherapy is an "active" physiotherapy treatment that can be applied to the patients with lower back pain. Decision which type of exercises can be used is very individual, depends on physician's approach and there is no define prescription to specify type of exercises for different patients and to indicate when they should be applicable. (1-10).

Although Brunkow exercises, as isometric exercises for paravertebral muscles have mainly goal to tone the muscles, we were interesting to their influence on spinal movements, flexibility of spine and pain relief. Our treatment consisted of one session of exercises daily under supervision of professionals and recommendation for few more sessions at home, depending of pain intensity and general medical conditions of patient. As was recommended, treatment also focused on correcting of body posture. All patients were assessed before and after the treatment and their spinal movements, spinal flexibility and intensity of pain are measured.

Pain was the most impairing symptom in this patients' sample and each patient experienced pain before treatment, average rate of VAS was 5,8 before treatment (on 10 rate VAS). After treatment a significant pain reduction occurred. Comparing pre-treatment and post-treatment results, all participants in this study have pain relief with a difference in VAS of 1,7.

Elasticity of spine, measured by Shober test, also showed significant improvement at the end of treatment comparing with pre-treatment measurements, with average difference of 0,5 cm. All patients showed some evidence of restricted ROM before the treatment, mostly because of pain limitation. Spinal mobility measurements were used as a predictor for functional evaluation and pain reduction (more pain relief, better functional results), so this functional test functioned as a pain provocation test. An improvement in all parameters of spinal motion was seen.

Low back pain is frequently associated with persistent joints stiffness from capsular, ligamentous, or para-articular muscles and tendon contractures, and that is another reason for limited spinal mobility in our participants.(17) Brunkow exercises started as dynamic contraction of distal parts of upper or lower limbs, but they are finishing with isometric (static) contraction of paravertebral muscles. Strong muscle contractions activate muscles’ ergoreceptors (stretch receptors). (18) The afferents from the receptors cause endogenous opioids to be released and also cause the release of beta-endorphin from pituitary. These secretions may cause both - peripheral and central pain to be blocked. (19) Reduction of the pain immediately after training has also been reported in some researches done with patients with the low back or cervical pain (20) Increased level of endorphins after training can also decrease activities related pain. Results of this study confirming that Brunkow exercises can decrease the pain and increase spinal flexibility and mobility.

Conclusion

Brunkow exercises for low back pain are beneficial treatment for increasing flexibility and mobility of spine and reducing the pain.

All participants in this study have pain relief with a difference in VAS of 1,7.

Elasticity of spine, measured by Shober test, also showed significant improvement at the end of treatment comparing with pre-treatment measurements, with average difference of 0,5 cm.

Spinal mobility measurements before and after treatment, showed that flexion increased for 3,4 cm in average, extension for 1,7 cm; right side flexion for 2,0 cm and left side flexion for 2,2 cm in average.

Spinal mobility, flexibility and pain relief in patients with lower back pain, can improve by performing Brunkow program of exercises for lower back pain.
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SIGNIFICANCE OF THE INTERFERON (IFN) IN THE THERAPY

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Abstract

Interferons belong to the group of the regulatory glicoproteins, of low molecular mass. They are the products of infected cell - genome, but not virus, as a consequence of the case answer by different inductors. Human IFN are divided on the sequence of amino - acids into three groups: Alpha, Beta and Gamma interferons. Recently are discovered new types of IFNs: Omega and Tau, but bigger than alpha molecules. Also, has been performed the division into two types: I and II. Besides the antiviral and antiproliferative effects, they have also the effect in the treatment of malignant diseases, and act protectively against the radiation.

Key words: Interferon, IFN, Alpha, Beta, and Gamma.

Introduction

The discovery of the interferon (IFN) is closely related to the names of the investigators Issacs and Lindemann in the period of the 1957. The cells with virus infections or other ethiologic agents excrete IFN. It's product of lymphocytes (lymphokines), macrophages, fibroblasts, and other cells. They are included into physiological barrier of the mechanism of the non-specific immunity of the host. The cells could produce and excrete IFNs a few hours after sensibilization. Except viruses there are a few different intracellular micro-organisms as well as inducers: (chlamydia, brucella, ricketcia), listeria, mycoplasma, coliforms, protozoa, bacterial products (endotoxin, nucleic acids), different cells including neoplastic, and chemical compounds (synthetic double stranded RNA, tiloron, acridin stains, specific antigens for sensibilization of the lymphocytes), immune stimulators or mitogenes, double stranded polynucleotides (poly I: C, poly dA: dB), synthetic polymers (polysulphates, polyphosphates, piran), and some antibiotics (kanamycin, cyclohexamid).

The basis of the IFNs activity is directed to inhibition of the synthesis of the RNA (Ribonucleic acid) and DNA (Deoxyribonucleic acid) in the cells of the host. Nowadays, it's clearly known except antiviral activity (inhibition of viral replication, synthesis of proteins, and as a terminal stage excretion from the infected cells) IFN posses antiproliferative and immunoregulation effects (with enlarge activity of the macrophages, cytotoxic T lymphocytes, as well as natural killer cells - NK) too.

The United States Food and Drug Administration (FDA) for clinical use until 1986 did not approve interferon, nearly 30 years after its discovery (1,2).

Chemical and biological features of IFN

IFN belongs to the group of regulatory glicoproteins of low molecular mass from 16, 000 to 70, 000 Dalton. It has carbohydrate components except proteins, which is very important for their antigenic difference. They show the resistance related to the nuclease action, stability within the wide diameter pH from 2 to 10, and are not antigenic. From other side they show the extreme sensitivity according to the proteolitic enzymes especially trypsin. For IFN we can say that the virus is specific, which means that between the viruses that provoke formation IFN (interference virus) and virus which discover the interferon action (interfered virus) there is no antigen relation. However, according to the type of the cells in which is produced it discovers the expressive specificity ("species specificity") which means by the relation of the cell that selectively adsorbs and releases in itself only the homologue IFN molecules.

Division and types of IFN

Depending on the origin of IFN can be produce within human and animal organism, and in that case we speak about the endogenous or natural IFN (serum, liquor, various secrets, etc.). The exogenous IFN is the outer product of viruses or different inductors senzibilated cell in the cell culture, of human or animal origin. It is significant to emphasise also the production of the recombinant IFN which posses the minimum undesirable effects and it's significant place in the application. The second division of the IFN is according to the cell type which they produce as well as on basis on sequence of amino - acids to: Alpha (leukocytes), Beta (fibroepithelialis), Gamma (immune) (3,4).

IFN α (alpha) make the greatest (more than 20 members) and mainly applicable group. They produce them by virus infected leukocytes (macrophages, B-lymphocytes, dendrite cells) and they are resistance to the low pH. They are antiviral and antiproliferative effects (induction of enzyme oligo (A) synthetaze, protein kinaze P1 and MHC (Major Histocompatibility Complex) antigens I. and II., and they belong to the cells NK activators. IFN α amplifies the cellular immune response stimulating T helper cells and increasing the diameter of T helper cells according to T suppressers.

IFN β (beta) posses only one protein molecule (I subtype) the product of fibroblast as well as the epithelial cells.
resistant to the low pH. As well as α is antiviral and antiproliferative effect.
IFN γ (gamma) also has only one protein (1 subtype) immune interferon the product of T lymphocytes as well as NK cells. Carbohydrate component is not necessary for biological function. Predominantly they have immunoregulation effect. It acts first of all as non-specific actor of activation (MAF) amplifies the activity NK cells and expressiveness of the molecule MHC on the target cells. Also, gamma IFN inhibits replication of viruses in the infected cells and acts synergistic with TNF α (cytotoxic) in killing the target cells. Besides the mentioned, we differ recently also IFN omega and tau which are similar to IFN only for their molecules bigger than alpha molecule. Except mentioned divisions IFNs are divided according to a type as well. IFN alpha, beta omega and tau mutually are similar according to the function and structure (consist of one molecule of amino - acids) and they belong to IFN type I (5). Contrary to this IFN gamma is built in the form of dimmer (two mutually stranded copies of protein) and make IFN type II. The basic differences type I and II are that the type I most successfully leads the cell into antiviral condition while the type II more significant influence on the immune mechanism of immune system of the host.

Biosynthesis of IFN

According up to now examinations of IFNs are the product of eukariotic cell but not virus and the information about the biosynthesis of the same is found in the nucleus DNA that is in genome of the cell of the host. Normally IFNs are not present in the cell the production to them is "inhibited" and their production is the consequence of the response to the different inductors. As an inductor of the production of IFN type I can be the interproduct of viral double stranded RNA occurred during the viral replication. It is considered that only one molecule of the viral RNA is sufficient for the induction of the production of interferon in the cell. A few hours after infection appear IFN type I while gamma IFN appear a little bit later with activation of lymphocytes T. In the cells where there are produced IFN inhibit viral replication leave the infected cell and connect with interferon receptors of the cytoplasmatic membrane of the closest cells and in it express the same effect. It is emphasised "species specificity" which means that the activity according to the definitive type of the cell. Biosynthesis of IFN begins by the synthesis of the essential enzymes: oligo (A) synthesis and protein kinaz. The activity of oligo A synthesis is expressed in the activation of endoribonucleasis who's the main task is degradation of the viral RNA while enzyme protein kinase degrades the initial factor polypeptide εIF-2, which is necessary for the synthesis of the viral protein. Nowadays is known that besides the antiviral action IFN acts antiproliferative and immunoregulatory. Antiviral and antiproliferative effect of IFNs (α And β) is confirmed on the different cells, viruses and tumors in vivo and in vitro (3,4). Immunoregulatory the effect of IFN gamma appears on macrophages, lymphocytes T and B as well as NK cells. Also, IFN inhibit the growth of same malignant cells.

Treatment

IFN is the subject of clinical examinations and all in the purpose of their application in the treatment of the various virus infection as well as different malignant diseases. It's know that the cells of some type of leukaemia osteosarcoma as well as the kidney cancer expressively sensitive to the action of IFN. Application of exogenous IFN has the significant effect in initiative phase's virus infection by the stoppage of viral replication. The use of IFN in therapeutic purposes show some an undesired effect of side phenomena in the major measure of IFN α and β. As the most significant side phenomena appeared the symptoms in the form of influence syndrome the expressive malaise, myalgia, high temperature followed by fever, inclination to bacterial infections as well as the different haematology disorders first of all thrombocytopenia and granulocytopenia. We shall mentioned some examinations and application IFN in the treatment of some diseases:

Alpha IFN

Treatment: HBV (Hepatitis B Virus), HCV (Hepatitis C Virus), papillomas, laryngeal papillomas, HIV infection, leukaemia, Kaposi sarcoma, and tumors of kidney and colon.
Patients with diagnosis of fulminate (progressive) form of HBV tried with application of alpha IFN.
IFN alpha is got by recombinant DNA technique on the bacteria or yeast's and acts inducing the cell enzymes, which interfere with the synthesis of virus protein. In the treatment of chronic form HBV - (HbeAg positive patients) the efficacious is the long -term application of high doses of recombinant alpha IFN (5 -10 i.m. 3x weekly s.c. during six months).
Besides the type we have also lymphoblast type which is got by the stimulation lymphoblast cell lines by virus. The younger patients, the shorter duration of the disease (before integration HBV in the cell genome of hepatocytes), the grater activity of aminotranspherases in sera, female sex, achieving HBV infection in the adult age, the lower values HBV DNA in sera - favour the factors for good response on the application of alpha IFN (6,7). IFN alpha 2b is efficient in the treatment of chronic form HBV in adolescents. Such individuals have present “surface antigen” (HbsAg) for the period of six months. All the individuals with the decomposition of the liver (encephalopathia, ascites, high values of serum bilirubin, the prolonged protrombic time) generally should not be
treated by IFN 2b. The recommended dosage for IFN alpha 2b in the treatment of the chronic form HBV is 5,000,000 units daily, whether i.v. or i.m. for the period of 16 weeks. For the time of the therapy is necessary the monitoring of patient’s eventually present side phenomena reduced on minimum. There is as parental form (Roferon A, ROCHE; Intron A, Schering Plough) for i.m. or s.c. application. The half-life of the drug is from 2 to 3 hours. The most common adverse effects are the symptoms like to the flu like syndrome, gastrointestinal disturbances, and depression. By many adult with chronic HBV infection there is insufficiency of interferon but non - adequate response to IFN. Also, conjugated with polyethylene - glycol (Pegasys, Pegiliran interferon, peginterferon alpha 2b) is produced has the prolonged half - life (approximately 8 to 12h after administration) and it will be administered once per week. It is approved for the treatment of chronic hepatitis C in adults as well as in combination with oral vidarabin in patients untreated with IFN- or who have relapsed following INF \( \alpha \) - therapy (3). The recommended dosage for IFN alpha 2a and 2b for treatment chronic HCV is 3,000,000 units 3x weekly in i.m. or iv. injections. For IFN alphacon 1 is recommend - ed the dosage of 9 mcg 3x weekly for the first treatment of IFN.

**Beta IFN**

Treatment: multiple sclerosis, neoplasm of basal cells.

**Gamma IFN**

Treatment: chronic granulomatosis, tumor of kidney, leishmanias (5,8,9).

**Conclusion**

Nowadays by the application of the recombinant tech - niques are produced highly purified recombinant alpha IFN (recombinant alpha 2a and alpha 2b). Besides the type of the recombinant alpha IFN we have also lymphoblast type which is got by the stimulation lymphoblast cell lines by virus. It is significant to emphasise also the combined therapy of IFNs with some antiviral drugs, cytostatic or immunomodulators. So, for example in the choice of therapy of HCV the determination of genotype is also significant. For the determination therapeutic model for example the duration of combined therapy IFN and Ribavirin in genotype 1 is 48 weeks and the non - 1 genotype is 24 weeks. The treatment for the six months exclusively for therapy IFNs alpha 2a and 2b although several studies emphasise that the treatment for the period of 1 year or more also can be efficiency. The studies show that the effect of combine therapy by IFN and Ribavirin 40 - 50%; is weaker in the case of genotype 1 (30%) and better in other genotypes. FDA approves the combine therapy in June 1998. For patients with chronic form of HCV which earlier where on "single" therapy by IFN. The preliminary results with PEG interferon show the success in the treatment and to 55%. The application of IFN in the therapy in children requires the maximum caution. The pregnancy represents contraindication in the therapy with IFN. The application of IFN by itself or combined in the treatment both of viruses and malignant diseases has the final aim to achieve the maximum thera - peutic efficiency in the median of survival and quality of life of such patients.

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BASIC CHARACTERISTICS OF INFORMATION SYSTEM OF HEALTH INSURANCE IN FB&H

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Abstract

Due to the territorial and administrative division in the war period, information system of health protection after the war was divided in two systems, what matched organisation of health insurance in that period. Those information systems were incompatible, developed on different, both, hardware and software. Therefore, Ministry of Health, within the project "Basic hospital services", financed through the World Bank loan, applied new, common information system in health insurance. Goal of this paper is to present basic features of information system of health insurance in FB&H, as well as the way of its functioning in respect to other institutions included in the system, respective data bases, sites of entering and updating data, while using data available with Federal Bureau of Health Insurance.

Keywords: characteristics, information system, health insurance of FB&H

Introduction

System of health insurance in FBIH, built in accordance with the Law on Health Insurance (FBIH Official Gazette no. 30/97 and 7/02), have established three types of health insurance: obligatory, extended and voluntary health insurance. Sources of realization of basic rights of the insured person are provided from obligatory health insurance and from the other sources foreseen by the law (taxes, donations, budget etc.). Health insurance on cantonal level are collectors of the revenues stemming from obligatory health insurance. Cantonal fond of health insurance are financing health protection and realization of other rights from obligatory health insurance by means of making agreements on procurement of services of health insurance to insured persons. Some cantonal fond of health insurance are making special agreements about health insurance programs according to the level of health protection (primary, specialized, consultative or hospital) on the basis of (temporary) standards of health protection. Some other cantonal fond of health insurance retained system of contracting based on the lump sum of wages for the employees, material costs and major investments. Cantonal fonds are settled in cantonal centres, having their branch offices in each municipality of the canton.(1,2,3,4) Within the system of health insurance, there is, apart from cantonal fonds, Federal fond of Health Insurance which coordinates common services for all cantonal fonds, services of expert groups of the Federal Ministry of Health related to the process of drafting and adopting legal provisions, as well as rendering some other services related to health insurance. Furthermore, federal Solidarity Fund was established (within the frame of Federal Bureau of Health Insurance) in order to ensure equal rights to all insured persons on the territory of FBIH. Organisational structure of health system in FBIH and position of health insurance within the health sector is shown in the diagram below.

Methodology and Purpose of the Paper

Purpose of this work is to present basic features of health information system in FBIH, along with its functions related to the institutions linked to the system, data registered within the system, sites of data entering and updating. This work is based on method of descriptive analysis.

Characteristics of the System

Institutions included

Institutions that are included in information system are:

- Cantonal agencies of health insurance, including branch offices in each municipality of FBIH
- FBIH Ministry of Health
- FBIH Agency for Public Health
- FBIF Agency for health insurance and reinsurance
- All cantonal health ministries
- Al cantonal agencies for public health
Equipment and software are identical in each institution within the system. IBM produces all computers and printers, network facility is 3 COM, PC operative system is Windows 2000 and database is Oracle 8i. Applicative software is developed in Dolphi development tool. Agencies for health insurance are filling data into the system and use them for running the system of health insurance. Other institutions are provided with adequate equipment and software in order to gain indicators from health insurance agencies in electronic version, by modem. This link is shown in following diagram.

**Registry Keeping**

System register following data: basic data, documentation, revenues and expenditures within the health insurance system. There are following registers:

1. **Revenues register**, which includes:
   - Deductions for health institution
   - Donations
   - Budget allocations
   - Cashiers and other payments
   - Payments from abroad

2. **Expenditures register** includes:
   - Costs of drugs within the canton

3. **Basic registers, code book and indicators register** include:
   - Costs of treatment in health institutions in other cantons
   - Costs of treatment abroad
   - Costs concerning orthopaedic tools
   - Refunds for lost wages
   - Reimbursement for sick leave
   - Refunds of travel costs
   - Payment of funeral costs
   - Refunds of personal paying

**Sites of data entry and updating**

Updating of common codebooks is carried out on federal
Updating of data regarding insured persons and insurers is done on the municipal level (along with issuance and verification of certificates).

Updating of data regarding health institutions, doctors and doctoral teams is done on the cantonal level (along with financial data regarding revenues and expenditures). Below follows the scheme of registration according to the site.

Processes in system
Diagram presented below displays relations between Agency for Health Insurance and other actors in process of health insurance. The square in the centre of diagram presents activities of the Agency, while circles present external entities, which are participating in process of health insurance. Arrows indicate relations between process and external entities. All functions of the health insurance system could be divided into several logical units, depending on which data certain functions are processing. There are seven of those units:

- Registration of basic registers
- Registration of code books - nomenclatures
- Registration of documentation
- Registration of financial transactions
- Design of indicators and reports
- Input and output of data
- Conversion of older data bases

These components are having different scope and require different amount of work on different levels of organisation of the agency. Following diagram shows links between the registries within the single unit of organisation that carries out data entry and updating.

General mode of functioning of system
Entire system functions as a whole, which is realized in following manner:

- Structure of data base in entire system is standardised
- Clients' applications in entire system are standardised
Security Aspects

Method of authorization
In order to let client's application access the basis, one uses Oracle method of verification. User mentions the name, password and name of the server as he applies.

Services for export and import of data are using Windows 2000 verifications. "Call-back" option within the operative system is used in order to further increase safety. Each unit of organisation, that establishes the connection with the other, has its own user's name, password and phone number (for sake of "call-back" option) in that other unit of organization. When one who initiates the transfer establishes the link with the receiver of data, link is cut after the checking of the password and then receiver calls initiator on already defined phone number. This procedure prevents any breaking into the system.

Solutions for registration of accesses to the system and changes of database
Every table contains field with the date and time of the change of each syllable and identifier of user who changes data. Besides, log file registers all accesses and actions within the course of working. There are three possible levels of registration into the log files, depending on initial parameters of applications. Level of registration is highest during initial period, since it needs to cover all actions and fix all mistakes in the course of work. Level of registration decreases when system reaches level of stable usage. Log files also register all mistakes in work with applications, beside registration of actions.

a) Solutions for limitation of user's rights
System features several groups of rights, named, in the terms of Oracle technology, ROLES. Administrators of
the bases can add new users as well as add new group of working rights with the data to these users. Besides, each user could be allowed or forbidden to work with certain menus of clients’ applications. Applications built into the applicative software are in charge for these operations. Passwords over the basis (and clients’ applications) are given by the administrator of the basis. Each canton appoints particular person in charge for this matter, either as an employee or as an honorary worker.

b) Solutions for filing and recovery of data
Filing and back up of data is taped into the tape unit on the server. Back up of statistics is done according to the needs and on the basis of decision of the administrator in charge for back up of data. That process enables filing of temporary data on the tape and reverting that data into the status and state it had before break up of the system. The administrator initiates back up itself, directly over the server of the database.

Applications

Clients’ applications
Applications of clients are classified according to their general functionality. Those applications are unique in entire system, same as the database itself. There are three applications:

- Main application, used for work with all data on the places where this data is updated
- Application for export and import of data; and
- Application for conversion of data from existing into the new system

Clients’ applications communicate with the same database way it is presented in the following picture.

Service applications
The task of service applications is to automate actions in order to decrease the need for users’ interaction. These applications are located on the servers with modems, so they are designed as a service of operative system. When data for transfer are prepared and packed in appropriate format, service application takes the lead and sends the data to some other computer. (4,5)

Discussion

Due to the territorial and administrative division in the war period, information system of health protection after the war was divided in two systems, what matched organisation of health insurance in that period. Those information systems were:

- system with Inter-cantonal Agency of Health Insurance, which covered territory where Croats were majority population; and
- system, which functioned on the territory where Bosniaks were majority.

Beside these two major systems, there were also applicative solutions developed by local software enterprises in order to fill the gaps within the health insurance system in the regions with Bosniak majority. These two information systems were mutually fully incompatible. Developed on different, both, hardware and software they have been an obstacle for unification and standardisation of health insurance system as it was defined by law, since legal system required cantonal organisation of health insurance.

Therefore, Ministry of Health, within the project "Basic hospital services", financed through the World Bank loan, applied new, common information system in health insurance. That new system is applied in nine out of ten cantonal agencies, as well as in the Federal Bureau of Health Insurance.
Conclusions

Today in Federation of Bosnia and Herzegovina exists only one information system of health insurance which is used by almost all cantonal fonds of health insurance as well as Federal fond of health insurance. Characteristics of this system, presented in this work, show as that using this system can make datas more available easier for use and, generally, more useful.

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Abstract

In order to achieve the multi-claim products required for the dental care category, it is necessary for the formulator to use a variety of different ingredients. This places a number of demands on the development process. Innovations in the areas of pharmaceutical technology have contributed to the formulation of the products having superior efficacy as well as other attributes that may contribute to clinical response and patient acceptability. Improved clinical efficacy and tolerability, along with conditioning signals, should encourage patient compliance with oral hygiene further complementing professional efforts directed at disease prevention. The most effective way of preventing the development of dental disease is in controlling the production of dental plaque. It is formed by microbial action. The removal of plaque from the teeth and related areas is essential for the maintenance of a healthy mouth.

In this paper we have presented the main components of toothpastes and mouthwashes. For the active ingredients, their supposed effect as therapeutic agents is also explained.

Key words: formulation, ingredients, toothpastes, mouthwashes

Introduction

The most effective way of preventing the development of dental disease is in controlling the production of dental plaque. Plaque is a soft thin layer which deposits on teeth, gums and all appliances fitted in the mouth. It is formed by microbial action. Dietary sugars, in particular sucrose, contribute to the formation of plaque and their presence increases the rate of formation and thickness of plaque. The removal of plaque from the teeth and related areas is essential for the maintenance of a healthy mouth (1).

In this paper we have presented the main components of toothpastes and mouthwashes. For the active ingredients, their supposed effect as therapeutic agents is also explained.

Ingredients of toothpastes and mouthwashes

A toothpaste in defined as a semi-solid material for removing naturally occurring deposits from teeth and is supposed to be used simultaneous with a toothbrush. A mouthwash is defined as a non-sterile aqueous solution used mostly for its deodorant, refreshing or antiseptic effect. Mouthwashes or rinses are designed to reduce oral bacteria, remove food particles, temporary reduce bad breath and provide a pleasant taste.

Mouthwashes (mouthrinses) are generally classified as either cosmetic or therapeutic or a combination of the two. Cosmetic rinses are commercial products that remove oral debris before or after brushing, temporary suppress bad breath, diminish bacteria in the mouth and refresh the mouth with a pleasant taste. Therapeutic rinses often have the benefits of their cosmetic counterparts, but also contain an added active ingredient, (for example fluoride or chlorhexidine), that help protect against some oral diseases.

The amount of the different ingredients in mouthwashes varies from product to product. Some practically have the same composition as toothpastes, although they do not contain abrasives. Distinct from toothpastes most mouthwashes contain alcohol, as a preservative and a semi-active ingredient. The amount of alcohol is usually ranging from 18 - 26 %.

Abrasives

Abrasives are the substances that are used for abrading, grinding or polishing. They remove substances adhering to the surface of the teeth without scratching it and bring out their natural luster.

One of the major properties of the abrasive is hardness. The degree of abrasivity depends on the hardness of the abrasive, the morphology of the particles, and on the concentration of abrasive in the paste. As the hardness of the enamel on the tooth surface is 6-7 on the Moh’s scale, the hardness of an abrasive should be 3 or less. For practical purposes, the particle size should be 20µm or less; if it is
more than this they may damage the tooth surface and gums. The abrasives found in toothpastes are often not as hard as the enamel, but as hard or harder than the dentine. Abrasives are most often found as crystals, small and smooth particles are preferred to avoid tooth wear. Needled and rod-shaped particles must be avoided (2).

Although many methods have been suggested for measuring the abrasive effect powders incorporated in toothpastes, the RDA method (Radioactive Dentine Abrasion) is the most widely accepted in the world today. In this method, an extracted human tooth is irradiated to convert the 31P in its dentine to 32P. The tooth is then put into an abrasion testing machine together with an abrasive and the abrasion of 32P is measured using a radioactivity counter. The pH of abrasives range from weakly acidic to weakly alkaline and they should be white powders which are insoluble in water, flavourless and odourless. The following substances are widely used abrasives, which satisfy these conditions:

**Calcium carbonate (CaCO₃)**
A fine, white, odourless, microcrystalline powder, practically insoluble in water (3). This abrasive has been used for a very long time. Its abrasiveness is generally higher than that of calcium phosphate. There are two types—a heavy and precipitated type. The raw material for the former is limestone and for the latter calcium hydroxide.

**Calcium phosphate, dibasic; Calcium phosphate, di-basic, dihydrate** (CaHPO₄, CaHPO₄ x 2H₂O)
There is a dihydrate form and an anhydride form. As the anhydride form is harder than the dihydrate form, it is not often used by itself. The dihydrate form has a mild abrasive effect and feels good on use. It is neutral in pH and has good compatibility with other ingredients. However, when it is in toothpaste for a long period of time, it loses its abrasiveness, changes to the anhydride form and makes the toothpaste go hard. For this reason a magnesium salt or other stabiliser is added (2).

**Silica, silica hydrate** (SiO₂, SiO₂ x nH₂O)
The main ingredient of the silica used in abrasives is high purity amorphous silicon dioxide and there are varieties of different types whose properties vary with the method of production. Silica is very suitable for use in toothpastes containing fluoride because no insoluble salt is formed when it reacts with fluoride. As its refractive index is lower than that of other abrasives, silica can be used to make clear gel toothpastes.

**Other abrasives**
Aluminium hydroxide is also used as an alternative to calcium phosphate, dibasic, because it is cheaper. Other abrasives such as calcium pyrophosphate, insoluble sodium metaphosphate, magnesium carbonate and aluminium may also be used for special types.

**Binders**
Binders are used to prevent the separation of powder and liquid ingredients and give an appropriate degree of viscoelasticity and form to the toothpaste. They can prevent the toothpaste from drying out by binding water. Also, they have an influence on the dispersion, foaming, rinsing and other qualities of the toothpaste in the oral cavity. The most widely used binder at present is sodium carboxymethylcellulose (CMC).

Carboxymethylcellulose is physiologically inactive, it dissolves in water, it is very compatible with other ingredients, highly stable and relatively low in price. There are many types of CMC having a variety of different characteristics stemming from different degrees of hydroxy group substitution and polymerisation, so it is necessary to select the most appropriate one for the purpose in mind. Other known cellulose derivatives include methylcellulose, hydroxyethylcellulose and hydroxypropylcellulose. Examples of other binders used are polysaccharides such as sodium alginate, carrageenan and xanthan gum; synthetic polymers like sodium polyacrylate and inorganic clay minerals as bentonite and laponite.

**Sodium alginate**
It is obtained from algae belonging to the Phaeophyceae, mainly species of Laminaria (4). It consists chiefly of the sodium salt of alginic acid. A white or pale yellowish-white powder which is odourless or almost odourless and tasteless. Slowly soluble in water, forming a viscous, colloidal solution; practically insoluble in alcohol and in ether. Sodium alginate has little surface activity and its emulsifying power is achieved by increasing the viscosity of the aqueous phase. It is used as a suspending and thickening agent and in the preparation of water-miscible pastes, creams and gels. According to the viscosity required, from 1 to 10 % is used in the preparation of pastes and creams.

**Carrageenan**
A dried aqueous extract from species of Chondrus, Gigartina, Eucheuma or other members of the families Gigartinaeae, Solieriaeae, Hypneaeae and Fucellariaceae. A white to yellowish coarse or fine, almost odourless powder with a muclaginous taste. Soluble 1 in 100 of water at 85°C. It disperses more readily if first mixed with alcohol. It is used as an emulsifying, suspending and thickening agent in formulations of toothpastes, creams and emulsions. Carrageenans are galactans or polymers of D-galactose, are heavily sulfated, and are anions with multiple electrolytes of molecular weight ranging from 105 to106. All carrageenans have a linear structure of (AB)n type, with alternating 1,3 and 1,4 bonds. Classically, seven types of carrageenans are distinguished as a function of the nature of the sequence. These are 1, κ, λ, μ, ν, θ, ζ carrageenans (5).
Develops on certain species of Brassicaceae where, by using the vegetable substrate, it produces a gummy exudate: xanthan "gum", a high-molecular-mass anionic polysaccharide. It exists as the sodium, potassium or calcium salt (6).

Industrially, this "gum" is produced by a bacterial culture on correctly buffered and aerated media containing carbohydrates with Xanthomonas campestris. Upon completion of fermentation, the polymer is recovered by precipitation with isopropanol, filtered, dried, and crushed (7). It is a cream-coloured powder. Soluble in hot and cold water, xanthan gum forms aqueous solutions of which the viscosity remains practically unchanged by temperature changes, as well as pH changes.

The behaviour of these solutions is of the pseudoplastic-type: decrease in viscosity proportional to shearing and instant recovery of the initial viscosity upon discontinuation of shearing. Incompatibilities are rare (borates, hypochlorites, peroxydes, free radical generators). The gum is compatible with most salts, with moderate surfactant concentrations, and with most preservatives; it tolerates alcohol concentrations up to 50% percents. Compatible with most vegetable hydrocolloids, it does not form gels by itself; but it forms thermally reversible gels. It is devoid of toxicity. Xanthan gum is used as a stabiliser, binder (thickener), and emulsifier.

Humectants

They prevent loss of water, and subsequent hardening of the paste in the tube or when it is exposed to air. They also provide creamy texture. These are short-chained polyalcohols such as glycerol, sorbitol (highly concentrated aqueous solution), propylene glycol and polyethylene glycol.

Solvents

Water is the most common solvent used in toothpaste. It dissolves the ingredients and allows them to be mixed. Alcohol is used in mouth rinses (mouthwashes) as a solvent and taste enhancer.

Foaming agents

The functions of foaming agents are to disperse the toothpaste throughout the oral cavity in order to enhance the cleaning effect and, acting as a surfactant, clean away the dirt inside it. Also, by means of their volume of foam, they give a feeling of thickness, and satisfaction. Surfactants having excellent foaming, dispersion, suspension, permeation, cleansing and hard water resistance qualities as well as no toxicity or irritation, are selected for foaming agents.

Surfactants lower the surface tension of the liquid environment in the oral cavity so that the substances in the toothpaste/mouthwash can contact the teeth more easily. They penetrate and dissolve plaque. This makes it easier to clean the teeth. The foaming effect produced by the surfactants is also beneficial in cleaning the teeth, and contributes to remove debris and gives a feeling of cleanliness. Another function of the surfactant is in dispersing the flavours in the toothpaste/mouthwash. Because, they go into the mouth, attention is also paid to taste and smell. The one most frequently used at present is sodium lauryl sulfate; other examples are sodium lauryl sarcosinate, sodium alkylsulfo succinate, sodium cocomonomoglyceride sulfonate and sucrose fatty acid esters.

Sodium lauryl sulphate (SLS)

A mixture of sodium alkyl sulphates, consisting mainly of sodium dodecyl sulphate. It is a white or pale yellow powder or crystals with a slight characteristic odour. Freely soluble in water; partly soluble in alcohol (6). It exhibits high affinity for proteins and is a strong denaturing agent. Incompatible with cationic materials and with acids below pH 2.5. Sodium lauryl sulphate may be irritant to the skin and mucosa. It may also damage the mucosal mucin layer by denaturing its glycoproteins (8). The epithelium will then be more exposed for irritants and this can result in aphthous ulcerations in some patients. It has also been claimed that there is a connection between the use of toothpaste or mouthwash containing SLS and an increased frequency of recurrent aphthous ulcers (RAU) in some patients. A product without SLS may thus be recommended for patients with RAU (8). The adverse effects of SLS have resulted in the development of toothpaste and mouthwashes with alternative surfactants such as sodium lauryl sarcosinate, sacamidopopybetaine. Common for these surfactants are that they are less irritating to the oral mucosa. It is effective in both acid and alkaline solution and in hard water. Also, it has antimicrobial activity due to its ability to interfere with membranes and a variety of biologic processes in microorganisms.

Flavouring agents

They get rid of the unpleasant smell and taste of the other raw materials and give a cold, refreshing taste. Combina-
tions of water-insoluble essential oils, such as spearmint, peppermint, eucalyptus and menthol are often used as flavouring agents in toothpastes and mouthwashes. The flavouring agents are solubilised and dispersed through the paste or liquid via the surfactant.

Sweeteners

Sweeteners also improve the taste of toothpastes and mouthwashes and give them a mild and sweet taste. The most common used sweeteners are sodium saccharin, sorbitol and glycerol. Xylitol is a sweetener that is also claimed to provide anti-caries activity.

Colouring agents

Most toothpastes and mouthwashes contain colour-substances which give them an attractive appearance. The colour-substances are classified by the Colour Index (CI), published by the Society of Dyers and Colourists and the American Association of Textile Chemists and Colourists, or by a system called the FD&C Colours. Titanium dioxide is often added to toothpastes to give them a white colour.

Preservatives

Preservatives prevent the growth of micro-organisms in toothpastes and mouthwashes. Mostly, they include sodium benzoate, methylparaben and ethylparaben.

Pharmaceutical agents

One or more therapeutic agents are usually added to toothpastes and mouthwashes. Most toothpastes today contain fluorides to prevent caries. Recently there has been a development of different toothpastes with additional purposes, such as stain and calculus removal, and prevention of gingivitis, sensitive teeth and gum problems. In the following text the different pharmaceutical therapeutic agents are categorised according to their claimed effect.

Anticaries agents

Fluoride

Fluoride is considered to be the most effective caries-inhibiting agent, and almost all toothpastes today contain fluoride in one form or the other. The most common form is sodium fluoride (NaF), but mono-fluoro-phosphate (MFP) and stannous fluoride (SnF2) are also used. The fluoride amount in toothpaste is usually between 0.10-0.15 %. Fluoride is most beneficial when the mouth is not rinsed with water after tooth brushing. In this way a bigger amount of fluoride is retained in the oral cavity. Toothpastes are the main vehicle for fluoride. The combined therapeutic and cosmetic mouthwashes usually so contain fluoride, but in a non-therapeutic dose. However, there are fluoride-rinses with higher fluoride concentrations.

The mechanism by which fluoride prevents caries is not clearly understood. It is known that the fluoride ion (F-) can replace the hydroxyl ion (OH-) in hydroxyapatite, the major crystalline structure of enamel. The substituted crystal, called fluorapatite, is more resistant to acids, such as those produced by plaque bacteria, than the original hydroxyapatite (9).

As the tooth develops and enamel is formed, ingested fluoride is incorporated into the enamel. Therefore, because enamel develops its outer layer first, more fluoride can be expected to be deposited on the outer layers as compared to the inner layers. It is this surface enamel layer containing fluoride that imports caries resistance to a tooth. The incorporation of fluoride into enamel can be represented as a chemical reaction:

\[
\text{Ca}_{10}(\text{PO}_4)_{6}\text{(OH)}_2 + F^- \rightarrow \text{Ca}_{10}(\text{PO}_4)_6\text{F}_2 + 2\text{OH}^-
\]

Hydroxyapatite  Fluorapatite

It is also suggested that fluoride has anti-bacterial actions. In an acidic environment, if fluoride is present, hydrogen fluoride (HF) is formed. HF is an undissociated, weak acid that can penetrate the bacterial cell membrane. The entry of HF into the alkaline cytoplasmic compartments results in dissociation of HF to H+ and F-. This has two separate, major effects on the physiology of the cell. The first is that the released F- interacts with cellular constituents, including various F-sensitive enzymes. The second effect is an acidification of the cytoplasmic compartment caused by the released protons. Normally protons are pumped out of the cell, but fluoride inhibits these processes. The decreased intracellular pH will make the environment less favourable for many of the essential enzymes required for cell growth (10).

As the most important anti-caries effect is claimed to be due to the formation of calcium fluoride (CaF2) in plaque and on the enamel surface during and after rinsing or brushing with fluoride. CaF2 serves as a fluoride reservoir. When the pH drops, fluoride and calcium are released into the plaque fluid. Fluoride diffuses with the acid from plaque into the enamel pores and forms fluorapatite (FAP). FAP incorporated in the enamel surface is more resistant to a subsequent acid attack since the critical pH of FAP (pH=4.5) is lower than that of hydroxyapatite (HA) (pH=5.5). Fluoride decreases the demineralisation and increases the remineralisation of the enamel between pH 4.5-5.5, and hence the demineralisation period is shortened (10).

Xylitol

A polyhydric alcohol (polyol) related to the pentose sugar, xylose. White crystals or crystalline powder. Very solu-
ble in water; sparingly soluble in alcohol (6). It has a sweet taste and produces a cooling sensation in the mouth. Xylitol cannot be fermented by oral microorganisms. It is considered to be a cariostatic agent since it can inhibit the carbohydrate metabolism in different oral microorganisms. Xylitol seems to be unique among the sugar alcohols in its inhibitory effect on glycolysis. The inhibitory effect on glycolysis has been related to the uptake of xylitol via a constitutive fructose specific PTS (phosphotransferase system) and subsequent intracellular accumulation of xylitol-5-phosphate. Such a mechanism leads to reduced acid formation from glucose, and a reduction in the *Streptococcus mutans* content in both plaque and saliva (11).

**Calcium / Phosphate**
Calcium and phosphate supplementation in a toothpaste or mouth rinse will increase the concentration of these ions in the oral cavity. In this way they improve remineralisation and increase fluoride uptake (12).

**Sodium bicarbonate**
Several studies have shown that bicarbonate is one of the salivary components that potentially modifies the formation of caries. It increases the pH in saliva, and in this way creates a hostile environment for the growth of aciduric bacteria. Sodium bicarbonate can also change the virulence of the bacteria that cause tooth decay. Animal studies have shown that toothpastes containing sodium bicarbonate reduce the amount of both *Streptococcus sobrinus* and *Streptococcus mutans*, and this may reduce caries. Studies on human show a statistically reduction in number of mutants streptococci. Sodium bicarbonate can also prevent caries by reducing enamel solubility and increase remineralisation of enamel (13).

**Anti-plaque agents**

**Sodium lauryl sulphate**
It has been shown that the enzymes glucosyltransferase and fructosyltransferase are incorporated in an active form into the pellicle; and by synthesising glucan in situ from sucrose, can provide a surface for colonisation by *Streptococcus mutans*. These enzymes can be inhibited by SLS. Such inhibition can clearly retard the regrowth of plaque (14).

**Triclosan**
Triclosan is a non-ionic chlorinated phenolic agent with antiseptic qualities. Triclosan has a broad-spectrum efficacy on Gram-positive and most Gram-negative bacteria. It is also effective against mycobacterium and strictly anaerobic bacteria, and against the spores and fungi of the *Candida* species. The mechanism of its antiseptic action is by acting on the microbial cytoplasmic membrane, inducing leakage of cellular constituents and thereby causing lysis of the micro-organisms. In spite of its activity in vitro, clinical plaque studies have revealed only moderate levels of antiplaque activity.

Evidence has accumulated to suggest that triclosan itself does not produce optimal plaque inhibitory effects without the addition of other chemicals which increase its antibacterial effect. Most commonly used are copolymer PVM/MA [Poly(Methylvinylether/Maleic anhydride)] and zinc citrate. They enhance surface retention of triclosan (15). An antiseptic has to be retained in the oral cavity for a certain amount of time in order to have antiplaque activity. The retention sites for triclosan are not yet established, but the teeth and the micelles in saliva are suggested. Triclosan also has antiinflammatory effect by acting on the eicosanoid-cascade. Triclosan inhibits both cyclooxygenase (COX) and lipoxygenase (LOX), and thereby inhibits the production of prostaglandins and leukotrienes. Clinical studies also indicate that triclosan reduces oral mucosal irritation caused by sodium lauryl sulphate (16,17).

**Metal-ions**
The most widely used metal-ions in dental preparations are zinc (Zn<sup>2+</sup>) and stannous (Sn<sup>2+</sup>). These metals have the ability to limit bacterial growth, inhibit plaque formation, inhibit the glycolytic sequence in oral anaerobic bacteria, and to restrict the ability of plaque bacteria to convert urea to ammonia (14). They can also inhibit some bacterial enzymes. It is also possible that they can reduce the bacteria's ability to colonise the tooth surfaces.

a) **Stannous-ions**
Stannous-ions are added to toothpastes and mouthwashes in the form of stannous fluoride or stannous pyrophosphate. Stannous fluoride was frequently used as a vehicle for fluoride in dental preparations. At present time it is rarely used, although extensive research during the last two decades has established that stannous fluoride possesses several interesting properties. It has been claimed that stannous fluoride is more effective in caries inhibition than sodium fluoride and monofluorophosphate. This is probably because stannous fluoride has additional properties compared with other fluoride vehicles. However such differences are not always statically significant in small-scale studies (18). Mouth rinses containing stannous fluoride have been found to reduce the relative amounts of *Streptococcus mutans* and *Streptococcus sanguis* in plaque, to reduce the population of *Streptococcus mutans* in saliva and to increase the salivary levels of *Lactobacilli* (14).

The stannous fluoride treated enamel becomes hydrophobic, a property which may contribute to the antiplaque effect of stannous fluoride, since hydrophobic surfaces are less easily colonised by bacteria (18). The cariostatic protection provided by stannous fluoride is dependent on a deposition of CaF<sub>2</sub> reservoir on the tooth surface. Both the antiplaque effect and the inhibition of acid formation by stannous fluoride are most likely caused by the oxidation of thiol groups which stannous fluoride is known to per-
form. Stannous ions may inhibit bacterial glycolysis because the enzymes depend on the thiol group for their biological activity (18). The antiplaque effect of SnF can clearly also contribute to the cariostatic activity.

b) Zinc-ions
Zinc is added to toothpastes and mouthwashes as zinc chloride or zinc citrate. Zinc is a relatively non-toxic, non-cumulative essential trace element. Zinc inhibits the PTS pathway of glucose uptake by Streptococcus mutans, Streptococcus sanguis and Actinomyces naeslundii, and the metabolism of glucose to lactic acid. The effects of zinc are believed to be intracellular, resulting from the inhibition of sulphhydryl enzymes, specifically enzyme I in the phosphotransferase transport system and aldolase and glyceraldehyde dehydrogenase in the glycolytic pathway. Zinc also inhibits the trypsin-like protease activity of Porphyromonas gingivalis and of Capnocytophaga gingivalis (14). The role of zinc in plaque inhibition or as a calculus inhibitory agent when used in toothpastes has been established by a number of workers (14). It is shown that surfactants enhance the plaque-inhibitory role of zinc (19).

Essential oils
Essentials oils of thymol, menthol, eucalyptol and methyl salicylate are thought to have anti-bacterial activity by altering the bacterial cell wall. Mouth rinses containing these active ingredients have been reported to reduce plaque and gingivitis significantly.

Chlorhexidine
Chlorhexidine formulations are considered to be the "gold standard" antiplaque mouthwashes due to their prolonged broad spectrum antimicrobial activity and plaque inhibitory potential (20). The mechanism of action of chlorhexidine is related to a reduction in pellicle formation, alteration of bacterial absorption and/or attachment to teeth, and an alternation of the bacterial cell wall so that lysis occurs (9). Chlorhexidine is effective against both Gram-positive and Gram-negative bacteria, but has most effect against Gram-positive bacteria. Chlorhexidine is bacteriostatic at very low concentrations, especially against Streptococcus mutans. It also has effect against fungi, but non or little effect against spores. It also has effect against some viruses. Chlorhexidine is retained in the oral cavity for 24 hours by binding to phosphate, sulphate and carboxyl groups in bacteria, plaque, saliva and on the enamel surface. The anti-bacterial action is due to a disturbance of the transport through the cell membrane and of the bacterial metabolism, and by causing leakage through the cell membrane. Its antiviral effect is caused by interaction with the viral protein cap. Local side effects of chlorhexidine including disturbance of taste and staining of teeth, tongue and restorative materials have tended to restrict its use to only a short term (20).

Anti-calculus agents
These agents act by delaying dental plaque calcification, thereby promoting plaque removal with normal tooth brushing (21). Of the anti-calculus agents, the crystal growth inhibitors have been most extensively tested clinically.

Pyrophosphate
Pyrophosphate has introduced in toothpastes to inhibit the formation of supragingival dental calculus (21). Pyrophosphate is added as tetrasodium pyrophosphate, tetrapotassium pyrophosphate or disodium pyrophosphate. It has been shown that pyrophosphate has high affinity to hydroxyapatite (HA) surfaces, probably by an interaction with Ca²⁺ in the hydration layer. By interacting with HA and the enamel surface, pyrophosphate reduces their protein-binding capacity. It also has the ability to inhibit calcium phosphate formation. It is therefore conceivable that pyrophosphate introduced in the oral cavity through toothpastes may affect pellicle formation. However, the P-O-P bond of pyrophosphate is known to be susceptible to enzymatic hydrolysis by plaque and salivary phosphata ses, and the effect may thus be of limited duration in the oral cavity (14). Consequently, the tartar control toothpastes that contain pyrophosphate as a calculus inhibitor also incorporate phosphates inhibitors that prolong the activity of pyrophosphate in the mouth.

Studies have indicated that fluoride in combination with PVM/MA Copolymer gives a significant protection of pyrophosphate against phosphatases (22). The clinical consequences of a poorly formed or partly missing pellicle are not known. Suggested consequences are abrasion of teeth, increased demineralisation, and hypersensitivity of teeth (14).

Zinc-ions
Zinc has anti-calculus effect due to its anti-plaque properties, but in addition it is thought to influence calculus formation by inhibiting crystal growth.

Anti-dentine hypersensitivity agents
Although the condition is referred to as "dentine hypersensitivity" it isn't really the dentine that is sensitive. The sensitivity of dentine is caused by fluid-filled tubules in communication with the pulp (14).

Potassium salts
Potassium ions are thought to act by blocking action potential generation in intradental nerves (23). It is claimed that potassium salts in dental preparations increase the concentration of potassium ions around the pulpal nerves, and thereby depolarises the nerve. This can inhibit a nerve response from different stimuli.
**Anti-aphtous agents**

**Aminoglucosidase and glucose oxidase**
Enzymatic toothpastes and mouthwashes do not contain surfactants like SLS because the surfactant can denature the enzymes. SLS may induce adverse effects in oral soft tissues and increases the frequency of ulcers in patients suffering from recurrent aphtous ulcers (RAU). The ulcers were generally reported to be smaller and less painful, to have a shorter healing time and the frequencies of aphtous ulcers episodes were decreased (24).

**Whitening agents**
Whitening toothpastes do not lighten the colour of the tooth structure; they simply remove surface stains with abrasives or special chemical or polishing agents, or prevent stain formation.

**Abrasives**
An abrasive is required for the effective removal of a discoloured pellicle. Abrasives provide a significant whitening benefit, particularly on smooth surfaces, but are of limited use for areas along the gum line and interproximally. Some whitening toothpastes contain coarse abrasives that can damage the dental tissue.

**Dimethicones**
Dimethicones are versatile substances that ranges from low molecular weight polydimethylsioxane fluids to high molecular weight polymers that are gum-like in nature. They cause a smooth surface on the tooth that prevents stain formation.

**Papain**
Papain is a sulfhydryl protease consisting of a single polypeptide chain, extracted from the Carica papaya plant (4). It is able to hydrolyse peptid bonds, and can also catalyse the transfer of an acyl group. It is used in toothpastes as an non-abrasive whitening agent.

**Sodium bicarbonate**
It is known that toothpastes containing high concentrations of sodium bicarbonate are more effective in removing intrinsic tooth stain than those not containing sodium bicarbonate (25).

**Anti-halitosis agents**

**Zinc-ions**
Bad breath or halitosis originates mainly from the oral cavity. The unpleasant smell is due to the retention of anaerobic, Gram-negative bacteria. These bacteria use sulphur-containing amino acids as substrates in their production of volatile sulphur-containing compounds (VSC). VSC have a distinctly unpleasant odour even in low concentrations. Zinc inhibits the production of VSC in the oral cavity by interacting with sulphur in the amino acids or their metabolism. Zinc can be retained in the oral cavity for approximately 2-3 hours after tooth brushing by binding to acidic substances on the oral mucosa, in the saliva or on bacterial surfaces.

**Conclusion**
In order to achieve the multi-claim products required for the dental care category, it is necessary for the formulator to use a variety of different ingredients. This places a number of demands on the development process. Innovations in the areas of pharmaceutical technology have contributed to the formulation of the products having superior efficacy as well as other attributes that may contribute to clinical response and patient acceptability. Improved clinical efficacy and tolerability, along with conditioning signals, should encourage patient compliance with oral hygiene further complementing professional efforts directed at disease prevention.
References


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CONTRIBUTION TO THE KNOWLEDGE OF POSITION, FLOW AND ARTERIAL DISTRIBUTION OF CEREBRAL BLOOD VESSELS IN FOETUSES 4 TO 9 MONTHS OF AGE

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Abstract

We studied cerebral blood vessels in 25 fetuses of gestational age 16-36 weeks and in 10 cadavers of still-born babies by injection-corrosive method. In the early fetal life, arteries are thin with the straight flow, which is directly connected with the brain development. Progressive changes are observed in all the three cerebral arteries in 28-week old fetus, which straight flow becomes more and more tortuous. As in the 32nd week the brain develops faster and gyri and sulci are being formed, the arteries assume wavy flow and number of their rami increases. In a still-born baby, arteries are of rather bigger caliber; they branch abundantly; and due to their relatively broad cerebral sulci, it can be said that their flow is partly tortuous. Our results show evidently that position, flow and relation of cerebral arteries change concurrently with the brain development and appearance of cerebral gyri and sulci.

Key words: fetus, brain, arteries, veins

Introduction

Fetal cerebral blood flow is fragile and partially myelinated brain with high content of water is very difficult to handle. According to Kier (1), lack of joint radiographic and anatomical studies in fetal period might be the result of technical problems. Newton & Potts (2), emphasize that normal perinatal angio-structure is more understandable and pathogenesis of certain congenital anomalies can be elucidated if data on prenatal growth and topographic change of different arteries are known. These authors conducted a large study of cerebral arterial system in fetus, including methods such as vascular injections, casting, dissection, and radiographic and anatomical analyses. The material subjected to our study consisted of aborted human fetuses, ranging in gestational age between 10 and 36 weeks; age was ascertained by measuring occipitotemporal diameter and cranio-coccygeal length. Numerous fetal blood vessels correspond to adult configuration already by the end of the first trimester. Hoyt (3) reports that increase in cranium is not followed by the growth of hemispheres, so that gyri separated by temporary sulci are formed on hemispheres. These temporary sulci disappear during the fourth month of intrauterine life, probably as the result of somewhat faster growth of cranium. Increase of width of cerebral hemispheres and cerebrum changes

Material and methods

We studied cerebral blood vessels in 25 fetuses of gestational age between 16 and 36 weeks and in 10 cadavers of still-born babies by injection- corrosive method. All research described in the submitted publication involving human subjects and material derived from human subjects complied with ethical principles outlined in Helsinki Declaration. In making a successful preparation by this method a good condition of blood vessels is an important factor, thus the preparation started within 36 hours from the moment of death. Period longer than 36 hours considerably reduces value of the preparation. We accessed aorta arch blood vessels in the group of fetuses and still-born babies by opening the anterior thoracic wall. The initial parts of the right and left common carotid artery were prepared by careful dissection. During the preparation we took care to preserve the venous elements. In certain number of preparations, we injected plastic mass into the cerebral venous system of a still-born baby through internal jugular vein. The method consists of two phases, injection and corrosion phase. After carotids were approached, the ligature was made in blood vessels which were not necessary for stuffing. We inserted injection needles with a rounded point into the arterial lumen, which we fixed afterwards. 12% vinyl chlorid acetonic solution dyed with acid-resisting dyes was used as the injection mass. The stuffed preparations were placed into broad glass containers with cold water in order to harden the plastic mass. Preparations were immersed in technical HC1 after 24 hours. Soft tissues were degraded by acid, and blood vessels casts remained where plastic mass was applied. Aft-
er 7 - 10 days, the preparations were washed with mild stream of water in order to remove soft tissue residues. Such received preparations were analyzed for developmental changes, flow and position of cerebral arteries.

Results

In injection-corrosion preparations of 20 weeks old fetuses carotid siphon is more open. Almost vertical flow of anterior cerebral artery is noticed. Branches of anterior cerebral artery flow in a straight line without significant bending. The middle cerebral artery is well visible. Its first segment, pars sphenoidalis, is directed on the bias upwards and laterally. On the left side we observed trifurcation of the first segment into periinsular branches which show a slight outwards convexity. On the right side, the first segment is directed more aslant upwards and laterally than on the left side. On insula level, the middle cerebral artery ramifies, so that width of the lumen reduces progressively as the rami occur. Cortical terminal rami of the middle cerebral artery display a straight flow. Anterior cortical branches of the middle cerebral artery are shorter while the parietal branches are considerably longer (Fig. 1). In 24 weeks old fetus carotid siphon is plane. Middle cerebral artery is no more vertical but displays a slight bending in genu corporis callosi area. The initial part of middle cerebral artery assumes more horizontal flow. Peripheral branches of anterior and posterior arteries have slightly wavy flow, while branches of middle cerebral artery are scanty (Fig. 2). In fetus of 28 weeks of intrauterine life, progressive changes are observed in all of the three cerebral arteries, whose straight flow becomes more and more tortuous. Initial part of middle cerebral artery is placed almost horizontally, and increased distance of periinsular arteries from anterior cerebral artery is observed. In preparations of 32 weeks old fetus, pericallosal artery has a more expressed angle, so that all three parts of this artery are well visible. Arteries have tortuous flow, and abundance of blood vessels is observed. Cortical branches of the anterior cerebral artery pass over the upper edge of hemisphere toward the middle cerebral artery; convergence of cortical branches of all the three cerebral arteries can also be observed (Fig. 3).

Corrosive preparations of still-born babies display rather long tortuous arteries, and their branching pattern is similar to that of adult persons. Cortical rami are distributed

Fig. 1 Corrosive preparation of 20 weeks old fetus. Ventral and lateral aspects of the preparation
1. internal carotid artery
2. carotid siphon
3. anterior cerebral artery (precommunical part)
4. anterior cerebral artery (postcommunical part)
5. middle cerebral artery (cortical part)
6. middle cerebral artery (sphenoidal part)
7. posterior cerebral artery
8. basilar artery

Fig. 2 Corrosive preparation of 24 weeks old fetus. Anterior and lateral aspects of the preparation
1. internal carotid artery
2. carotid siphon
3. anterior cerebral artery
4. middle cerebral artery
5. posterior cerebral artery
6. basilar artery

Fig. 3 Corrosive preparation of 32 weeks old fetus. Ventral and lateral aspects of the preparation
1. internal carotid artery
2. carotid siphon
3. anterior cerebral artery
4. middle cerebral artery
5. posterior cerebral artery
6. basilar artery
in such a way that one part of rami leaves the place of vascularisation of the particular area, while the other part of rami approaches that place of vascularisation, so that the territories of vascularisation overlap. Carotid siphon displays a sharp bend (Fig. 4). By injecting the plastic mass into the jugular vein we obtained corrosive preparations that display the venous system of still-born babies. Dural sinuses are well observed, also superficial and deep veins of the brain. The lower anastomotic vein (Labbe), which connects transverse sinus with superficialis meddle cerebral vein is observed. Well displayed are prefrontal parietal and occipital veins, as well as abundance of vein vessels around the foramen magnum (Fig. 5)

Discussion

In monitoring developmental changes in arteries of fetuses 4-9 months of age we paid a great attention to the position, shape, flow and ramification of main cerebral arteries. By the injection-corrosive method in fetuses 4-9 months of age we received relevant data about the position, flow, relations, variations and anastomoses between separate cerebral arteries. It should be taken into account that fetal vessels are thin and fragile, which was manifested by frequent intracerebral and intracranial extravagations of our injection material, regardless of the fact that we injected the material under low pressure and slowly in phases. In early fetal life arteries are thin and have a straight flow, which is directly connected with development of the respective cerebral structures. Cerebral arteries of fetuses 28 weeks of age display progressive change on all three cerebral arteries which straight flow becomes more and more tortuous. As in the 32nd week of intrauterine life, the brain develops faster and gyri and sulci are being formed, the arteries assume wavy flow and number of their branches increases. In a still-born baby, arteries are of rather bigger calibre; they branch abundantly; and due to their relatively broad cerebral sulci, it can be said that their flow is partly tortuous.

More literature about embryonic development of blood vessels was found in works of Streeter (6), Padget (5), Kaplan (7), while literature about fetal blood vessels is scant. Newton and Potts (2) emphasize that should the data on prenatal growth and topographic changes of different arteries be known, normal perinatal angio-architecture would be more understandable and pathogenesis of certain congenital anomalies may be illuminated. These authors prepared a great study of cerebral arterial system in fetuses, including vascular injections, casting, dissection, and radiographic and anatomic analyses amongst their methods. In the conclusion to their studies, the authors report that numerous fetal arterial schemes already suppose adult configuration at the end of the first trimester. Comparing our findings on fetal blood vessels with those of
the other authors who were partially occupied with these issues, we can say that they do not deviate from those of Streeter (6) and Hoyt (3). Van Overbeeke (8), and Icardo (9) report that as cerebral hemispheres grow over thalamus and midbrain, so the tree of posterior cerebral artery moves more backwards and thus vascularises ever increasing number of visible structures, what is also in accordance with our findings.

**Conclusion**

Our findings clearly show that changes appear in position, flow and relation of cerebral arteries concurrently with the development of adjacent cerebral structures and occurrence of gyri and sulci, what concurs with rather scant literature published in this field.

**Fig. 5** Corrosive preparation of a still-born baby. Cerebral veins and sinus durae matris. Lateral aspect of the preparation.

1. superior sagittal sinus
2. inferior sagittal sinus
3. straight sinus
4. transverse sinus
5. sigmoid sinus
6. inferior anastomotic vein (Labbé)
7. prefrontal veins
8. frontal veins
9. parietal veins
10. occipital veins
11. internal jugular vein

**References**


ENDOMETRIAL CANCER EPIDEMIOLOGY AND PREVENTION IN FEDERATION OF BOSNIA AND HERZEGOVINA, B&H

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Abstract

In Federation of Bosnia and Herzegovina during 2002 a total of 67 cases of endometrial cancer (ICD 10th Revision Code C54) were registered among female population older then 15 years (1 per 10 000 population). Nine women were diagnosed with non specific malignant uteri neoplasia (C55) - without clarifying if that was cervix or corpus uteri located cancer, but assumption is that these cancers are actually endometrial cancer. Majority of cases are older then 50 years, 48 of them (71.6%), while 29 (28.4%) are from 15 to 49 years old. During 2000 about 189 000 new endometrial cancer cases were reported with 44 700 endometrial cancer deaths in the World. In this paper we presented geographical distribution of cases registered in FB&H, as well as leading risk factors, protective factors and prevention and possibilities for screening methods.

Key words: endometrial cancer, incidence, incidence rate, risk factors and prevention, FB&H

Introduction

Incidence

During 2000 about 189 000 new cases of endometrial cancer with 44 700 deaths of the same disease were reported all over the World. Incidence rates are almost three times higher in developed comparing to developing countries. Endometrial cancer is on the fifth place of all female common cancer locations. The highest endometrial cancer incidences in Europe are registered in Check Republic and Slovakia, and the lowest is in Greece. Endometrial cancer incidence in developed world reported significant increase during 1970s after introduction of subsistent estrogen treatment, but in 1980s that trend stabilized.

Risk factors

All factors related to exposure to estrogens have increasing risk of development of endometrial cancer. The most predominant are:

- menarche before 12th year or menopause after 50th year of life,
- infertility,
- adiposity,
- animal fat predominant in diet,
- tamoxifen therapy,
- estrogenic substitute treatment without progesterone has 10 times risk increase which remains even 10 years after discontinuation of treatment,
- combined substitute hormone therapy slightly increase endometrial cancer risk,
- breast cancer or ovarian cancer (joint risk factors),
- pelvic radio treatment,
- Diabetes mellitus type 2.

Genetic factors are involved in about 6% of total number of endometrial cancer cases. High endometrial cancer risks have women carrier of mutations linked to heredity non lipozal colon cancer (HNPCC).

Endometrial cancer in Federation of Bosnia and Herzegovina

In Federation of Bosnia and Herzegovina (one of the two entities of Bosnia and Herzegovina) during 2002 a total of 67 cases of endometrial cancer (ICD 10th Revision Code C54) were registered among female population older then 15 years (registered rate 0.68 per 10 000 population) and 9 women were diagnosed with non specific malignant uteri neoplasia (C55) - without clarifying if that was cervix or corpus uteri located cancer, but assumption is in that case that these cancers are actually endometrial cancer. Majority of cases are older then 50 years, 48 of them (71.6%), while 29 (28.4%) are from 15 to 49 years old. According to the data gathered from Woman's health services during 2002 endometrial cancers were on the 4th place (Table 1.) with total of 67 cases treated. The highest number of cases was registered in Tuzla and West Herzegovina Cantons, eleven cases each, while the highest incidence rate was in West Herzegovina - 3.23/10000. De-
tailed geographical distribution in FBiH was presented in Table 2. It is relevant to mention that Cancer register for FB&H has been in a process of re-establishing after more than 12 years. Extended data on any cancer related diseases are not yet easy to obtain due to the high internal and external migration still in place in Bosnia and Herzegovina, and duplication or even triplication notification of any cancer case. New register, unlike the old one, will have mandatory unique identifier for each notification, and it will be supported by a computer processed data base, so notification multiplication will be avoided.

**Protective factors and prevention**

Some factors can help in endometrial cancer protection such are regular intake of oral contraceptives and soy and fiber rich diet. Maintaining of normal weight, optimal intake of oral contraceptives and hormone substitutes, knowledge of risk factors and early symptoms (e.g. abnormal hemorrhage from uterus) is very important among women in menopause.

According to the American Cancer Society (ACS) Guidelines for Early detection of endometrial cancer from 2003 main recommendation for women in postmenopausal age at average risk is to informed about endometrial cancer risks and symptoms as well as to see their physician after any unusual uteri hemorrhage.

**Screening methods for diagnostics and prevention**

The most common diagnostics procedure in diagnosing endometrial cancer is biopsy. Positive predictive value of endometrial biopsy among women at average risk and asymptomatic clinical signs according to the world-wide experience shows low, while it is high among women at specific risk (risk factors found in anamnesis). That is the main reason why it will be reasonable that that cohort should be considered for screening methods of this kind.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Cancer location</th>
<th>Number of Cases</th>
<th>Incidence rate (per 10000 population)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cervical Cancer (C53)</td>
<td>166</td>
<td>1.69</td>
</tr>
<tr>
<td>2</td>
<td>Cervical Cancer in situ (D09)</td>
<td>160</td>
<td>1.63</td>
</tr>
<tr>
<td>3</td>
<td>Breast Cancer (C50)</td>
<td>153</td>
<td>1.55</td>
</tr>
<tr>
<td>4</td>
<td>Endometrial Uteri Cancer (C54)</td>
<td>67</td>
<td>0.68</td>
</tr>
<tr>
<td>5</td>
<td>Ovarian Cancer (C56)</td>
<td>43</td>
<td>0.44</td>
</tr>
<tr>
<td>6</td>
<td>Breast Cancer in situ (D05)</td>
<td>25</td>
<td>0.25</td>
</tr>
<tr>
<td>7</td>
<td>In situ other gynecological cancers (D07)</td>
<td>21</td>
<td>0.21</td>
</tr>
<tr>
<td>8</td>
<td>Vulvo-vaginal Cancer (C51 -52)</td>
<td>12</td>
<td>0.12</td>
</tr>
<tr>
<td>9</td>
<td>Non specific m. uteri neoplasia (C55)</td>
<td>9</td>
<td>0.09</td>
</tr>
</tbody>
</table>

**Table 1.** Leading gynecological cancers/malignant neoplasia in Federation of B&H during 2002 according to provides services in Womans health care dispensers

<table>
<thead>
<tr>
<th>Canton</th>
<th>Number of Cases (C54 + C55)</th>
<th>Incidence rate (per 10000 population)</th>
<th>Female population (&gt;15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Una Sana Canton</td>
<td>2 + 0</td>
<td>0.18 + 0</td>
<td>113,115</td>
</tr>
<tr>
<td>Posavina Canton</td>
<td>0 + 0</td>
<td>-</td>
<td>20,058</td>
</tr>
<tr>
<td>Tuzla Canton</td>
<td>11 + 7</td>
<td>0.31 + 0.32</td>
<td>216,142</td>
</tr>
<tr>
<td>Zenica Doboj Canton</td>
<td>9 + 1</td>
<td>0.56 + 0</td>
<td>162,000</td>
</tr>
<tr>
<td>Bosnian Podrinje Canton</td>
<td>3 + 0</td>
<td>1.81 + 0</td>
<td>16,608</td>
</tr>
<tr>
<td>Middle Bosnia Canton</td>
<td>9 + 0</td>
<td>0.95 + 0</td>
<td>94,608</td>
</tr>
<tr>
<td>Herzegovina Neretva Canton</td>
<td>10 + 0</td>
<td>1.01 + 0</td>
<td>98,756</td>
</tr>
<tr>
<td>West Herzegovina Canton</td>
<td>11 + 0</td>
<td>3.23 + 0</td>
<td>34,000</td>
</tr>
<tr>
<td>Sarajevo Canton</td>
<td>7 + 1</td>
<td>0.39 + 0.06</td>
<td>179,519</td>
</tr>
<tr>
<td>Herzeg Bosnica Canton</td>
<td>5 + 0</td>
<td>1.27</td>
<td>39,317</td>
</tr>
<tr>
<td><strong>Total FB&amp;H</strong></td>
<td><strong>67 + 9</strong></td>
<td><strong>0.68 + 0.09</strong></td>
<td><strong>984,163</strong></td>
</tr>
</tbody>
</table>

**Table 2.** Geographical distribution of Endometrial Cancer (C54) and Non specific Malignant Uteri Neoplasia (C55) cases and Incidence rates in Federation of Bosnia and Herzegovina
Transvaginal ultrasound (TVU) is use as a non invasive screening method for detection of endometrial changes. However, it has not yet determined what endometrial width has high sensitivity and high specificity. Endometrial width varies depending on pre- or post-menopause, eventual substitute hormone therapy (estrogen versus combine), tamoxifen treatment... Because of all of the above mentioned transvaginal ultrasound does not meet sufficient specificity level to become a successful screening method. International collaborative group for heredity non lipozal colon cancer (HNPCC) recommends colonoscopy and curettage, transvaginal ultrasound and determining of CA-125 in sera for women at high risk once a year after they rich 30. For women which are not in reproductive age and being operated because of a colon cancer should be offered to simultaneous hysterectomy and oophorectomy. Evidences on positive influence of screening methods for women with HNPCC and detection of endometrial cancer in an early stage for longer survival age all over the world are not confirmed, especially comparing to an early diagnosed symptomatic endometrial cancer. Our country currently is undertaking a reform of a health care sector. Transition from health care system based on primary health care is reforming to a family medicine care and protection. This reform should be take place by 2010, so it is planned that health protection and prevention measures in woman's health protection will meet similar measures recommended in other countries (annual gynecological examination, biopsy and endometrial cytological analysis with transvaginal ultrasound among women at higher risk, ...) If all screening and diagnostics techniques and methods be undertaken on time, incidence and especially mortality rate of endometrial cancer should decline with time.

References

GROWTH HORMONE (GH): USAGE AND ABUSE

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Abstract

Growth hormone is essential for body growth but it also modulates metabolic pathways as well as neural, reproductive, immune, cardiovascular, and pulmonary functions. Numerous beneficial effects of growth hormone have led to its expanded therapeutic use in both children and adults. There are several officially approved applications of human growth hormone and many more proposed applications that resulted from huge number of clinical studies on GH therapy.

Growth hormone abuse includes improper or excessive use. Over the last decade GH has become one of the most commonly abused drugs in sport due to the fact that its administration is currently undetectable. Enormous doses that are injected and frequent simultaneous abuse of other substances such as other anabolic steroids (testosterone) lead to frequent side effects that may be fatal.

In spite of numerous beneficial effects of growth hormone the true physiological impact of GH replacement therapy on various metabolic parameters may be confounded by the dose and route of administration of GH so accurate physicians’ monitoring during GH therapy is needed.

Key words: growth hormone, therapeutic use, abuse

Introduction

Growth hormone (GH) is a polypeptide hormone secreted by the pituitary gland in a pulsatile manner every two hours with a mean daily secretion of 0.5 mg. The predominant form has a molecular weight of 22kDa and half-life in plasma of 15-20 min after secretion or intravenous injection (1). After subcutaneous or intramuscular injection, blood concentration of GH reach the peak between 1 and 3 h after injection and drop to undetectable levels 24 h after administration. When administered by mouth GH is completely digested to its constituent amino acids (2). After secretion GH may be found in the system circulation in the “free” state or bound to GH binding protein (GHBP). Growth hormone receptors are present on all cells in the body. One GH molecule binds two receptors and leads to their dimerisation. This process is essential for initiation of intracellular signaling (3). The circulating GH is removed from the blood stream through receptor-mediated degradation, predominantly in the liver and kidney. The liver and kidney internalize GH receptor complex and completely degrade it to its basic amino acids. Only small quantities of GH appear in the urine (1).

Growth hormone secretion is controlled by two hypothalamic peptides: growth hormone releasing hormone-GHRH and somatostatin (SS) which are final mediators of numerous neuroendocrine, metabolic, nutritional and immune influences on GH secretion (4). The precise mechanism that mediates the effects of physiological factors on GH secretion in humans may be difficult to determine. The most important stimuli of GH secretion in humans are sleep, exercise and stress (5). The sleep-related GH secretion occurs most during the phase of deep slow-wave sleep in the early hours of sleep. In circumstances where sleep pattern is disturbed GH secretion is impaired. Results of numerous studies showed that acute physical exercise increase GH secretion within 10-20 minutes of aerobic exercise, peak at the end of exercise and remain elevated for approximately one to two hours (6). Ageing is associated with decreased concentrations of GH. In man, each decade of increasing age is associated with a 14% decline in 24 hour GH production rates (7). The total amount of GH secreted during 24 h in normal adults over the age of 65 is, in the majority of cases, concurs with people with organic GH deficiency secondary to pituitary pathology. There is, thus, evidence of the development of functional GHD with increasing age, the so called “somatopause”. The majority of middle-aged and elderly normal subjects may be considered incompletely GHD.

Growth hormone is essential for body growth but as normal growth occurs over relatively short period of time and GH secretion continues through life it is not surprising that GH has many other functions that regulate body composition, fluid homeostasis, glucose and lipid metabolism, bone metabolism, exercise performance and cardiac function (8). These actions improve the quality of life of adults and confer beneficial effects when used appropriately.

Growth hormone increases protein synthesis (9). Studies of Florini and al. (10) showed that GH increased the number and activity of ribosomes and increased RNA polymerase in rat muscle suggesting that GH may control synthesis at the translation level. Growth hormone may also reduce urea synthesis increasing the availability of nitrogen for protein synthesis. When GH is replaced protein synthesis rates in the postabsorptive and postprandial state...
are increased which leads to protein accretion and increase in lean body mass (LBM). The effects of GH on postabsorptive glucose metabolism are more subtle. Though muscle utilisation of glucose is already low the further suppression of glucose uptake is typically seen after GH exposure. Large dose of GH were reported to decrease postabsorptive glucose output acutely, compatible with increased glucose uptake while in vitro experiments have shown increased gluconeogenesis (11). It is still beyond doubt that GH contributes to the overall insulin resistance of type 1 diabetes and also acts as initiator of the vicious circles leading to acute metabolic derangement (12).

**Figure 1.1.** Growth hormone molecule bound to two growth hormone receptors (Biotechnology: Science, Engineering and Ethical Challenges for the 21st Century 1996: Joseph Henry Press)

Growth hormone stimulates lypolysis. Pulsatile and continuous administration of more moderate amounts of GH 70-400 micrograms to healthy postabsorptive humans reveals clear dose-dependent stimulation of lypolysis, circulation levels of free fatty acids (FFA) and glycerol and increased lipid oxidation rates (13).

All these metabolic effects influence body composition increasing LBM and decreasing fat body mass (13). Several independent studies showed that GH is capable of affecting cardiac function by modulating preload, after load and contractility through direct and indirect mechanism (14). Some studies showed that administration of GH and IGF I reduce peripheral vascular resistance (15). Studies on animals showed that GH directly stimulates myocyte growth (14).

**Growth hormone use**

These numerous beneficial physiological effects of growth hormone led to extended application of the hormone in both children and adults.

The therapeutic application of human growth hormone was first demonstrated 45 years ago in the treatment of pituitary dwarf. At first, the quantities were limited and originated from cadaver pituitaries. Ever since, the number of proposed applications of human growth hormone has grown. Biosynthetic GH initially became available for prescription use in the United States in 1985. Data indicated that the first therapeutic application in 1985 was followed by clinical complications. Three young men developed Creutzfeldt-Jakob disease caused by contaminated material (16). Today, recombinant GH with amino acid sequence identical to GH of human pituitary origin is produced by several pharmaceutics companies. Growth hormone preparations that contain minimum impurities, are apparently safe and readily available.

The officially approved uses of human growth hormone vary from country to country, but generally include:

**in pediatric conditions:**

- **children with growth hormone deficiency or insufficiency**
  This is the most important indication for GH therapeutic application (17). The cause for the GH insufficiency is particularly important in determining appropriate treatment. Because of its pronounced anabolic effects, GH is contraindicated in children with an active malignant condition.

- **Turner syndrome**
  Growth hormone may be used in the cases of short stature induced by Turner syndrome (18). Because the growth in these patients varies the decision whether and when to treat with GH should be made on the basis of each patient's height and growth velocity.

- **poor growth due to chronic renal insufficiency (CRI)**
  Growth delay in children with CRI, resulting from numerous physiological derangements including acidosis, secondary hyperparathyroidism, malnutrition or zinc deficiency, is an important indication for GH use (19). Also, there are some pediatric conditions for which GH therapy is under investigation:

  - **Idiopathic, genetic or primordial short stature** in children who are more than 2.5 standard deviations below mean. Numerous clinical trails have documented the capacity of GH to induce growth acceleration in such cases but several reports suggest that it may enhance pubertal development and may reduce the duration of growth during puberty (20).

  - **constitutional delay of growth and development**
    This clinical state is characterized by normal prenatal growth followed by growth deceleration during infancy and childhood. This causes significant psychosocial adolescent stress. Of note, the use of GH in these cases will result in permanent closure of epiphysis precluding further growth (20).
- **intrauterine growth retardation and Russell-Silver syndrome**
  The children whose growth has not caught up by the age of 4 may benefit from GH therapy, as some studies have suggested (21).

- **skeletal dysplasia's**
  Growth hormone therapy was tried in several skeletal dysplasia's associated with abnormally short stature (22). Much of the experience in treating these conditions was gained in management of achondroplasia.

- **osteogenesis imperfecta**
  Some studies showed that this condition may be effectively treated with GH. In particular, with such treatment patients may experience improved bone mineralization and improved growth (23).

- **Prader-Willi syndrome**
  Preliminary findings suggest that GH treatment in some patients with Prader-Willi syndrome accelerates growth, reduces hyperphagia, appreciably affects lipolysis and decreases obesity (24).

- **Down syndrome and the other syndromes associated with short stature**
  Because short stature is a characteristic of many syndromes GH therapy has been attempted in several conditions including Down syndrome (25), Fanconi syndrome, Bloom syndrome.

  **in adults (who have completed their statural growth)**

In spite of these beneficial effects AACE Guidelines for growth hormone use, list approved use of GH in adults only in cases of:

- **adult growth hormone deficiency (GHD)**
  This is the most important indication for GH replacement in adults (20). Importance of GHD in adulthood as a disease first became apparent in the late 1980s. Studies estimated total of 35000 adults with GHD in USA and approximately 6000 new cases that occur each year. GHD adults have several abnormal clinical features including increased adiposity, reduced muscle strength and exercise capacity, and impaired psychological well-being. Some of these features are normal signs of aging so certain specific biochemical criteria remain necessary for diagnosis.

- **AIDS related wasting**
  HGH may also play a role in immune reconstitution (26) and body composition improvement in AIDS patients. In addition to these generally accepted therapeutic applications of human growth hormone many proposed applications have not been established as yet. The anabolic actions of human GH made it attractive as a potential agent for catabolic problems in wide range of clinical conditions including:

  - severally catabolic patients in an intensive care environment (27)
  - chronic catabolic states (16)
  - burns (28)
  - cystic fibrosis (29)
  - inflammatory bowel disease (30)
  - infertility short-gut syndrome (30)
  - obesity (31)

**GH Therapeutic use in Cardiovascular Medicine**

A number of animal studies have shown that therapeutic use of GH could be beneficial in experimental model of heart failure with growth promoting and positive inotropic actions. Studies by Duerr and Yang (32) demonstrated beneficial effects of exogenous administration of insulin-like growth factor (IGF-I) and GH in the rat model of post-infarction heart failure. Some studies showed remarkable enhancement of cardiac function following GH therapy in GHD patients with severe dilated cardiomyopathy (33). Following these findings, several investigations by independent groups have confirmed the efficacy of GH in more advanced stages of heart failure. Large placebo controlled trails to confirm these data are currently ongoing. Recent evidence also showed that GH secretion may be impaired in patients with less advanced stages of heart failure.

**Growth Hormone Abuse**

Growth hormone abuse includes improper or excessive use (34). There are a lot of scientific debates on what type of GH applications may be labeled as abuse. Over the last decade GH has become one of the most common drugs in elite sport. For the first time it was discovered in possession of cyclists during Tour de France in 1998 (35). Since that period the abuse of recombinant human growth hormone in sports is considered to be a widespread phenomenon. Its popularity lies in the fact that it possesses marked ergogenic properties. Although growth hormone is listed as prohibited class E substance in Appendix A to the Olympic Movement Anti Doping Code no official test for the detection of GH abuse is implemented (35). Several tests are currently under study. Although the abuse has been reported in competitive athletes, the largest groups by far that abuse GH are competitive and recreational body builders. In both groups, usually, GH is self-administered without any medical supervision (35). Enormous doses injected and frequent simultaneous abuse of other substances such other anabolic steroids (testosterone) lead to frequent side effects which may be fatal (36).
Application of human GH to increase height in children who already attained normal height should also be considered abuse (16). Another common form of abuse of human GH is to reverse the effects of aging as it’s considered to be the “fountain of youth”. There are a lot of advertisements in printed media and on the internet which promote the use of growth hormone or agents touted as increasing human growth hormone levels. Although anabolic effects and changes in body composition have clearly been associated with the application of human GH, little or no evidence exists of important positive functional effects on the process of aging in elderly people (34). Finally, the decision to treat healthy older people with GH or GH secretagogues will inevitable evoke certain ethical considerations.

**Conclusion**

In spite of numerous beneficial effects of growth hormone the true physiological impact of GH replacement therapy on various metabolic parameters may be confounded by the dose and route of administration so accurate physicians’ monitoring during GH therapy is needed.

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**References**


(6) Had'ovi} A., Naka{-I}indi} E., Ku{-ukali}-Selimovi} E., Avdag} N., Za}iragi} A. The level of physical activity and the growth hormone response to acute physical exercise. Bosnian J. Basic Medical Sciences. 2004: 3:47-49.


Abstract

Falls are the leading accidental cause of death among elderly people in their homes. Falls and their consequences are the primary reason in 40% of admissions to hospitals for people older than 65 years. The study population consisted of 77 randomly selected patients of both genders older then 65 years. Each patient was tested in his/her home and was completely informed about the methodology and the goals of investigation. Based on the exclusion criteria, three patients were excluded from the study, which means the investigation was conducted on 27 males (35.06%) and 50 females (64.94%) with the average age being 71.23 ± 5.63 years.

For each patient, a specially prepared questionnaire about risk factors was filled in. The sum of affirmative answers represented a relative index of fall risk. All patients were evaluated through Folstein's Mini-Mental State Examination Test that is suitable for on-sight use in patient's home. The score value over 20 excludes dementias, delirium, schizophrenia and affective disorders.

Considering the values of the risk factor, scores obtained by the questionnaire and MMSE test scores, statistically significant differences were found between males and females (p < 0.005, respectively p < 0.01), “fallers” and “non-fallers” (p < 0.001, respectively p < 0.01), while considering the relation to the way of living (alone or with family), there were no statistically significant differences (p > 0.05).

Key words: risk factors of fall, elderly people, Mini-Mental State Examination Test.

Introduction

Rapid industrialization and urbanization, as well as a rapid improvement in technology, have put elderly people on periphery of the interest in health institutions. One of the most important problems in this population group is fall, i.e. the consequences of falling. The outcome of falls in the elderly is devastating. Fall is the leading cause of death resulting from different kinds of injures in people older than 65 years, while in people older than 75 years, almost 70% of interventions in emergency centres are related to fall (1). Normal postural control include the control of a relative position of body parts directed by skeletal muscles in relation to gravitation and one opposed to other (2). Falls are more frequently the result of a complex interaction between damaged body functions and surrounding elements. A twisted plank does not represent a problem for a normal, healthy person while the changes in body balance, muscle tonicity and cognition typical for elderly people may be sufficient to cause falling and a hip fracture (3).

The aim of paper

The aim of paper is to explore the most common risk factors that predispose falling in the elderly and to conduct Folstein's Mini-Mental State Examination Test.

Subjects and methods

The conducted study is clinical, and it contains manipulative, prospective and control research. The research included 80 randomly chosen patients of both genders and older than 65 years. The criteria for including into the study were: age over 65 years and mobility with or without aids (cane). The exclusion criteria were death of the subjects or occurrence of some disease that could influence the functional ability of the subjects during the period of study. All the subjects have been examined by the physical therapist. For each patient, a specially prepared questionnaire about risk factors was filled in (Appendix 1). The questionnaire contains 22 questions that are scored dichotomically (“yes” or “no”). The sum of affirmative answers represents a relative index of fall risk. This estimation is based on investigators’ observation, patients’ answers and the data from the accessible medical documentation. All the patients were evaluated by Folstein's Mini-Mental State Examination Test (a test of cognitive functions) - Appendix 2. This test was adjusted to the conditions at home. The standardization of test has shown that the score over 20 excludes dementia, delirium, schizophrenia and affective disorders (5).

The results were statistically processed with SPSS program, Version 9.0, and shown in the form of tables and graphs. For each clinical test, distribution, frequency and the measures of central tendency, specificity, sensitivity, positive and negative predictive value, the percent of falsely positive and negative results. With the t test, it has been established that the examined variables significantly differ statistically. With the aim of establishing the in-
dividual variables that predict the fall, a logistic regression analysis has been conducted, and dependent variable was represented by the information about fall (0 - no fall, 1 - with fall).

**Results**

Out of 80 subjects, 3 (3.75%) were excluded from the study: one subject had MMSE test score less than 20, one died, and one had a stroke. The study included 77 subjects, 27 males (35.06%) and 50 females (64.94%). The distribution of examined subjects by gender is shown in Graph 1. The average age was 71.23 ± 5.63 years (range from 65 to 90 years): for females 71.22 ± 5.80 years (range from 65 to 90 years), and for males, 72.67 ± 5.26 years (range from 65 to 82 years). The mean values of age, body weight, and blood pressure are shown in Table 1. Table 1. The mean values of age, weight, height, and blood pressure in subjects

Two subjects (both women) had no any kind of specialist's training, 30 subjects completed primary school only (38.96%), 34 subjects had intermediate specialist's training (44.16%), and 11 subjects had advanced or university-level specialist's training (14.29%). The level of education in relation to gender is shown in Table 2.

In the study period, 21 subjects (27.27%) fell two or more times, while the same number reported about only one fall. Out of 27 males, 4 subjects reported about fall (14.81%) where 1 lives alone and 3 with family. Out of 50 females, 17 reported about fall (34%) where 9 live with family and 8 alone. The data about fall related to gender and the way of living (alone or with family) are given in the Tables 3 and 4.

The mean values of the parameters (age, weight, systolic and diastolic blood pressure related to the information about fall) are given in Table 5. Table 5. The mean values of age, weight, systolic and diastolic blood pressure in relation to the information about fall

### Table 1. The mean values of age, weight, height, and blood pressure in subjects

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>72.67 ± 5.26 years</td>
<td>71.22 ± 5.80 years</td>
</tr>
<tr>
<td>Weight</td>
<td>79.11 ± 9 kg</td>
<td>72.82 ± 12.61 kg</td>
</tr>
<tr>
<td>Height</td>
<td>177.07 ± 7.86 cm</td>
<td>162.9 ± 14.89 cm</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>149.8 ± 21.5 mmHg</td>
<td>154.5 ± 25.7 mmHg</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>85.2 ± 9.9 mmHg</td>
<td>87.7 ± 11.9 mmHg</td>
</tr>
</tbody>
</table>

### Table 2. The level of education in relation to gender

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No education</td>
<td>0 0</td>
<td>2 4</td>
<td>2</td>
</tr>
<tr>
<td>Primary school</td>
<td>2 7.41</td>
<td>28 56</td>
<td>30</td>
</tr>
<tr>
<td>Intermediate Specialist's Training</td>
<td>16 59.26</td>
<td>18 36</td>
<td>34</td>
</tr>
<tr>
<td>Advanced or University-level Specialist's Training</td>
<td>9 33.33</td>
<td>2 4</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>27 100</td>
<td>50 100</td>
<td>77 100</td>
</tr>
</tbody>
</table>

### Table 3. Distribution of subjects by the way of living and gender

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lives alone</td>
<td>5 18.52</td>
<td>18 36</td>
<td>27</td>
</tr>
<tr>
<td>Lives with family</td>
<td>22 81.48</td>
<td>32 64</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>27 100</td>
<td>50 100</td>
<td>77 100</td>
</tr>
</tbody>
</table>
The mean values of risk factor gained through questionnaire and MMSE Test results are given in Tables 6-8. Regarding the values of the risk factor scores gained through questionnaire and MMSE Test, there are statistically significant differences between males and females, as well as between the subjects who fell and did not fall, while related to the way of living (alone or with family) there are no significant differences (Tables 9-11).

Discussion

In the old age, the psychophysical abilities of humans gradually decrease and the ability to adjust to endogenous and exogenous strains declines as well. In 1987, Harada et al. (6) have established that in the USA, 9.5 million persons have difficulties in performing the activities of everyday life, and 59% (5.6 million) is older than 65 years. In the age group from 65 to 74 years, each 10th person has difficulties, and in the age group of 75 to 84 years, it is every 4th person. For the persons older than 85, this ratio is 3 out of 5 persons.

The study included 77 randomly chosen subjects of both genders and average age 71.73 ± 5.63 years (range 65 to 90 years). Each patient was questioned in his/her own home and was completely introduced to the methodology and the aims of research. The perceptual presence of females was 64.94% (50 subjects), and males 35.06% (27 subjects), which is in accordance to the demographic data about this population group in accordance to our conditions (1). In other studies, the gender structure was different, so Harada et al. (6) observed that females represented 87% of subjects in their research group.

Regarding the age difference, there was no statistically significant difference related to gender. Although the average values of systolic and diastolic pressure in females and males were different clinically, that difference was not statistically significant. The measured values were a bit higher than the normal values, especially in females, which indicate an inadequate medical treatment.

For the body weight parameter, a significant difference

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Fall</td>
<td>4</td>
<td>14.81</td>
<td>17</td>
</tr>
<tr>
<td>Without fall</td>
<td>23</td>
<td>85.19</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 5. The mean values of age, weight, systolic and diastolic blood pressure in relation to the information about fall

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Fall</th>
<th>No fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>72.38 ± 5.98 year</td>
<td>71.48 ± 5.52 year</td>
</tr>
<tr>
<td>Weight</td>
<td>75.57 ± 13.45 kg</td>
<td>74.82 ± 11.25 kg</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>152.1 ± 22.4 mmHg</td>
<td>153.1 ± 25.1 mmHg</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>88.3 ± 13.4 mmHg</td>
<td>86.3 ± 10.4 mmHg</td>
</tr>
</tbody>
</table>

Table 6. Mean values of risk factor and MMSE Test results by gender

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factor score</td>
<td>2.59 ± 3.35</td>
<td>5.32 ± 3.71</td>
<td>4.36 ± 3.80</td>
</tr>
<tr>
<td>MMSE Test</td>
<td>28.2 ± 1.8</td>
<td>25.7 ± 2.2</td>
<td>26.6 ± 2.4</td>
</tr>
</tbody>
</table>

Table 7. Mean values of risk factor and MMSE Test results by the way of living

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Lives alone</th>
<th>Lives in a family</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factor score</td>
<td>4.87 ± 3.75</td>
<td>4.15 ± 3.83</td>
<td>4.36 ± 3.80</td>
</tr>
<tr>
<td>MMSE Test</td>
<td>27.2 ± 2.0</td>
<td>26.3 ± 2.5</td>
<td>26.6 ± 2.4</td>
</tr>
</tbody>
</table>

Table 8. Mean values of risk factor and MMSE Test results by the information about fall

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Fall</th>
<th>Without fall</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factor score</td>
<td>8.33 ± 3.07</td>
<td>2.88 ± 2.87</td>
<td>4.36 ± 3.80</td>
</tr>
<tr>
<td>MMSE test</td>
<td>25.5 ± 2.5</td>
<td>27.0 ± 2.3</td>
<td>26.6 ± 2.4</td>
</tr>
</tbody>
</table>
has been observed between males and females, which confirms a constitutional, i.e. anthropologic difference. However, the process of osteoporosis in females must not be excluded since none of the examined females was using means to prevent osteoporosis.

Considering education, i.e. the level of education, a statistically significant difference is evident between males and females (p < 0.001). Even 56% of females from the examined group had only primary education related to 7.41% of males. The lower level of education in females belonging to the group of elderly people can be observed in the context of social, cultural and sub-cultural circumstances in our geographic areas where it was common that female children were less often sent to school. This was especially present in rural areas where most of our subjects came from. The primary education, in the time when the subjects had to go to school, was not compulsory, so the number of those that had intermediate, advanced or university-level specialist's training was smaller. Thus, for example, 4% of females had advanced or university-level specialist's training in comparison to 33.33% of males from the study group.

Between the subjects who live alone and the subjects who live with family there was no statistically significant difference related to the age group, body weight and the values of systolic and diastolic blood pressure. The results of this study are different from the data in other literature since only 29.87% of the subjects lived alone, while that percent in other studies was two to three times higher (7, 8). The discrepancy can be explained through cultural and economic differences of our society since it is usual in our geographic areas that the younger generations live with parents, which is different from the trend in Western countries, and the lack of the place for living partially imposes such a solution. Furthermore, in our geographical areas there are no conditions that would make the life alone of the elderly easier, which is different from the Western countries. It could be expected that the percentage of the older persons who live alone would be even smaller, but the war contributed migrating of younger generations that led to the increasing in the number of the elderly who live alone.

The mean risk factor score and MMSE Test were significantly statistically different in relation to gender and the data about fall, which is opposite to the data about the way of living. It is interesting that the average score of MMSE Test of the subjects in this study is quite higher than that in the other studies that used this criterion (5, 9, 10, 11, 12, 13) (after adjusting it to the age). Since the test score depends on education, and in the mentioned studies this parameter was not used, it is not possible to make an adequate comparison, and it can only be supposed that the level of education was lower in those studies.

Table 9. Testing the significance of difference of the individual tests between males and females

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>t-test value</th>
<th>Levels of freedom</th>
<th>Stat. significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factor score</td>
<td>3.183</td>
<td>75</td>
<td>p &lt; 0.005</td>
</tr>
<tr>
<td>MMSE Test</td>
<td>4.989</td>
<td>75</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Table 10. Testing the significance of difference of the individual tests between subjects who live alone and with family

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>t-test value</th>
<th>Levels of freedom</th>
<th>Stat. significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factor score</td>
<td>0.761</td>
<td>75</td>
<td>N.S. p &gt; 0.05</td>
</tr>
<tr>
<td>MMSE Test</td>
<td>1.486</td>
<td>75</td>
<td>N.S. p &gt; 0.05</td>
</tr>
</tbody>
</table>

Table 11. Testing the significance of difference of the individual tests between subjects who fell and who did not fall

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>t-test value</th>
<th>Levels of freedom</th>
<th>Stat. significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factor score</td>
<td>7.298</td>
<td>75</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>MMSE Test</td>
<td>2.476</td>
<td>75</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>
Conclusions

1. The study included 77 subjects: 27 males (35.06%) and 50 females (64.94%). The average age was 71.23 ± 5.63 years (range from 65 to 90 years): for females 71.22 ± 5.80 years (range from 65 to 90 years), and for males 72.67 ± 5.26 years (range 65 to 82 years).

2. Considering the age difference, there were no significantly statistically differences related to gender. Although the mean values of systolic and diastolic blood pressure were clinically different, that difference was not statistically significant. The measured values were a bit higher than the normal values, especially in females, which indicate an inadequate medical treatment.

3. Considering education or the level of education statistically significant difference is evident between males and females (p < 0.001): Even 56% of females from the examined group have a primary education only related to 7.41% of males, while 4% of females had advanced or university-level specialist’s training in comparison to 33.33% of males in the research group.

4. Between the subjects who live alone (29.87%), and the subjects who live with family (70.13%) there were no statistically significant difference related to the age group, body weight and the values of systolic and diastolic blood pressure.

5. In the study period, 21 subjects (27.27%) fell two or more times, while the same number reported about only one fall. Out of 27 men, 4 subjects reported about fall (14.81%) where 1 lives alone and 3 in a family. Out of 50 women, 17 reported about fall (34%) where 9 live with family, and 8 alone. 44% of women who live alone fell two or more times in the period of study compared to 20% of men who live alone.

6. Between the subjects who fell and who did not fall there were no statistically significant difference considering age, body weight and the values of systolic and diastolic blood pressure.

7. Considering the risk factor score values, which have been obtained through questionnaire and the MMSE Test results, there were statistically significant differences between males and females (p < 0.005, i.e. p < 0.01), as well as the subjects who did not fall (p < 0.001, i.e. p < 0.01), while in the case of the way of living (alone or with family) there were no statistically significant differences (p > 0.05).
QUESTIONNARE

Name and Surname:
Age: Gender:
Body weight: Body height: Blood pressure:
Lives alone: Lives in a family:
Leading diagnosis (the disease group):
Note:

RISK FACTORS

1. For performing two or more of basic life activities (cooking, washing, clothing, walking, feeding,…), help is needed
   YES NO
2. For performing of two or more of manipulative activities (shopping, making phone calls, money handling, taking of medicines,…), help is needed
   YES NO
3. S/he had a fracture or disease of a hip, knee or foot joint
   YES NO
4. S/he has a visible consequences of the above mentioned conditions on the same joints
   YES NO
5. S/he uses walking aids (cane, walker, …)
   YES NO
6. Physical activity limited to basic home activities
   YES NO
7. Describes oneself as timid person
   YES NO
8. Complains about vertigo
   YES NO
9. Complains about difficulties in balance control
   YES NO
10. Has complaints that could be connected to postural hypotension
    YES NO
11. Did s/he fall once or two times this year?
    YES NO
12. Did s/he fall more than two times this year?
    YES NO
13. Was care necessary after the fall?
    YES NO
14. Was there a fracture as a consequence of those falls?
    YES NO
15. Is s/he afraid of falling in general?
    YES NO
16. Is s/he afraid of falling at home (bathroom, kitchen…)?
    YES NO
17. Is s/he afraid of falling outside home (public transportation vehicles, stairs, street…)?
    YES NO
18. Does s/he avoid leaving home because of that fear?
    YES NO
19. Does s/he have 3 or more health difficulties that require a regular medical control?
    YES NO
20. Do those health difficulties require home visits of medical workers?
    YES NO
21. Is there any of the following diseases that influence falling:
    - neurological (i.e. cancer, peripheral neuropathy, multiple sclerosis, lupus, …)
    - cardiovascular (i.e. postural hypotension)
    - musculoskeletal (i.e. total prosthesis of joints implanted)
    - sensory (i.e. sight impairment)
    - other (i.e. amputation, parkinsonism, Alzheimer's)
    YES NO
22. Does s/he take medicines dangerous from the aspect of falling
    - hypotensive agents
    - neuroleptics
    - hypnotics/ anxiolitics
    - antiarbitrmetics
    - antiparkinsonian drugs
    - analgesics/ anti-inflammatory drugs/ antirheumatic agents
    - vasoregulatory agents
    YES NO

Total number of affirmative answers___
FOLSTEIN MINI MENTAL STATE EXAMINATION

What is today's date? Maximum score = 5
What season is this? Score __
What year is this? Score __
What month of the year is this? Score __
What day of the week is this? Score __

Where are we now (street address)? Maximum score = 5
What floor are we on now? Score __
What country are we in? Score __
What town/city are we in? Score __
What is the name of this part of the city/town? Score __

Explain to the subject that you are going to name 3 objects that s/he has to memorize. Then say "glass, blanket and pencil" loud and clearly making a pause of 1 second between each word. After you utter all the three words ask the subject to repeat them. If s/he cannot do so, repeat the procedure and ask the subject to repeat. If s/he again is not able to do so, repeat the procedure one more time. Score of 3 points is reserved if the subject repeats the names of all the three objects after you uttered them the first time. Score 2 and 1 is reserved if the subject repeats the words correctly after your second, i.e. third repetition. If the subject is not able to repeat the names after the third repetition, the score is 0. Maximum score = 3

The subject is asked to start subtracting 7 from 100. Interrupt him/her after The five correct subtractions have been made (93, 86, 79, 72, 65). For each correct result, one point is given. If the subject is not able or does not want to perform the task, ask him to spell a 5 letter word backwards (for example Tuzla - ALZUT). The number of correct letters is also the number of points given. Maximum score = 5

Ask the subject to repeat the previously memorised 3 words (glass, blanket, pencil). Each correct word carries one point. Maximum score = 3

Show a watch and a pencil. Ask the subject what they are. Each correct answer carries one point. Maximum score = 2

Ask the subject to repeat the following sentence: "No ifs, ands or buts". Only one try is allowed. The score is 0 or 1. Maximum score = 1

Give a clean piece of paper (A4 format) to the subject. Ask him/her to listen to the following instruction: Take the paper in your right hand, fold it and give it back to me. For each completed instruction out of 3, one point is given. Maximum score = 3

Write the sentence "Close your eyes." on a piece of paper and in letters big enough. Tell the subject: "Read it and do it." If after the reading s/he closes eyes, the score is 1. Otherwise, it is 0. Maximum score = 1

Give the subject a pencil and a piece of paper and ask him/her to write a sentence. Score 1 point if the sentence contains a subject, verb and object and makes sense. Ignore spelling errors and handwriting. Maximum score = 1

On an empty piece of paper draw two intersecting pentagons. The sides should be around 2,5 cm long. Ask the subject to copy the drawing. If s/he succeeds, the score is 1. All the 10 angles and intersection of the two figures should be visible. Ignore the rotation of the drawing and the influence of tremor on the appearance of lines. Maximum score = 1
Folstein’s Mini Mental State Examination results in accordance to age and education:

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<th>30-34</th>
<th>35-39</th>
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References

Abstract

Fluoxetine is used in treatment of depression caused by a variety of different factors and from year to year new indications are being added, especially in conditions followed with strong bouts of pain. Additional fluoxetine based therapy that is known to help in improvement of mental state and mood stabilization can significantly increase analgesic effects.

Analgesic effects of fluoxetine as well as of fluoxetine in combination with morphine were analyzed on albino mice of both genders. The sense of pain was induced by thermal stimulus by the method of hot plate. Analgesic effect was measured 30, 60, 90 and 120 minutes after a single i.p. administration of fluoxetine in following dosages: 5, 10 and 20 mg/kg. The control group was treated with 0.1 ml/10 g physiological solution. Test group injected with morphine s.c. (7 mg/kg) was used to observe the effect of fluoxetine in combination with morphine.

Fluoxetine applied in 5mg/kg dosage causes increased pain reaction 60 and 90 minutes (p=0.049 and p=0.002) (t-test) following application when compared with corresponding values of control group. When fluoxetine is applied in 10 mg/kg dosage duration of pain reaction is significantly increased after 30 (p=0.01), 60 (p=0.001) and 90 minutes (p=0.026), when compared to the control group. When fluoxetine is applied in 20 mg/kg dosage duration of pain reaction is increased 60 and 120 minutes (p<0.001) after application when compared to the control group. After application of fluoxetine (5 mg/kg) in combination with morphine, reaction time to pain is significantly extended (p<0.001) 60, 90 and 120 minutes after application when compared to the control group injected exclusively with morphine.

Fluoxetine causes analgesic effect in all three applied dosages as well as it significantly increases analgesic effect when applied in 5mg/kg dosage in combination with morphine.

Key words: analgesia, hot plate, fluoxetine.

Introduction

Pain is defined by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage". Psychological influences have a very important role in definition and description of pain, causing an effect on both pain perception as well as reaction to the pain (1). Antidepressants are used widely to treat symptoms other than depression, many of which fit into a general category of pain. Adjuvant analgesics are described as any drug that has a primary indication other than pain but can be analgesic in some conditions. The primary role of antidepressants is when pain relief with conventional analgesics is inadequate or when pain relief is combined with intolerable or unmanageable adverse effects. A secondary role of antidepressants in treating chronic pain is their use in addition to conventional analgesics. This can be particularly effective with cancer where there is presence of pain at multiple pain sites, some nociceptive and some neuro-pathic (2,3,4).

Fluoxetine is an effective and a highly selective inhibitor of serotonin reuptake at the presynaptic neuronal membrane. Fluoxetine-induced inhibition of serotonin reuptake causes increased synaptic concentrations of serotonin in the CNS, resulting in numerous functional changes associated with enhanced serotonergic neurotransmission. When serotonin is released from nerve endings its activity at the receptor is ended by an action of a specific pump mechanism by which serotonin is taken into the membrane. Fluoxetine blocks this action of reuptake of serotonin into the membrane by above mentioned pump mechanism (5,6,7).

Serotonin plays a very important role in regulation of pain perception i.e. the mechanism of analgesia because it is a very important transmittor in pain-supressing systems. Decreasing the serotonin concentration increases nociceptive stimuli sensitivity (8,9).
Materials and methods

Analgesic effects of fluoxetine as well as of fluoxetine in combination with morphine were analyzed on albino mice of both genders, weighing 25-37 g. The mice were randomized in six groups with six mice in each group. The sense of pain was induced by thermal stimulus by the method of hot plate. The temperature of the plate was constantly 55°C during the experiment. Analgesic effect was measured 30, 60, 90 and 120 minutes after a single i.p. administration of fluoxetine in following dosages: 5, 10 and 20 mg/kg. The control group was treated with 0.1 ml/10 g physiological solution. Test group injected with morphine s.c. (7 mg/kg) was used to observe the analgesic effect of fluoxetine in combination with morphine.

The study has been conducted with the consent of Ethical Committee and in accordance to provisions of the Declaration of Helsinki.

Results and discussion

Fluoxetine applied in 5mg/kg dosage causes increased pain reaction 60 and 90 minutes (p=0.049 and p=0.002) (t-test) following application when compared with corresponding values of control group. When fluoxetine is applied in 10 mg/kg dosage duration of pain reaction is significantly increased after 30 (p=0.01), 60 (p=0.001) and 90 minutes (p=0.026), when compared to the control group. When fluoxetine is applied in 20 mg/kg dosage duration of pain reaction is increased 60 and 120 minutes (p<0.001) after application when compared to the control group. After application of fluoxetine (5 mg/kg) in combination with morphine, reaction time to pain is significantly extended (p<0.001) 60, 90 and 120 minutes after application when compared to the control group injected exclusively with morphine.

It can be observed from obtained results that analgesic effect is shown in all three applied dosages of fluoxetine. The strongest analgesic effect is expressed 90 minutes after the application of 5 mg/kg dosage. The analgesic effect with the longest time duration is obtained with 10 mg/kg (90 minutes) dosage, this dosage is also the fastest acting (30 minutes). Fluoxetine significantly increases analgesic effect when applied in combination with morphine.

Fluoxetine exhibits analgesic activity in some analgesic test systems when administered alone in animals, but the lack of such effects observed in other test systems suggests that demonstration of analgesic activity may be test-dependent. Fluoxetine has potentiated opiate agonist-induced analgesia in most but not all studies, possibly as a result of the drug's ability to enhance serotonergic neurotransmission (10).

Conclusions

Fluoxetine shows analgesic effect in all three applied dosages and the best analgesic effect is obtained with 10 mg/kg dosage. Fluoxetine in 5 mg/kg dosage significantly increases analgesic effect when applied in combination with morphine.
References


Instructions for preparation of manuscripts to be published in extenso in Bosnian Journal of Basic Medical Sciences

Submission: Only papers written in correct English are considered. Manuscripts must be typewritten in triplicate (with three sets of illustrations of which ones is an original), double spaced on one side of the paper with a 2.5 cm wide margin on top, bottom and both sides, accompanied by the identical file on a diskette.

Original research papers: Submitted manuscripts should be fully documented reports of original research. They must contain significant and original observations to be critically evaluated.

Short communications: These manuscripts should not exceed 2 printed pages (i.e. 5 manuscript pages), including an abstract essential references and not more 3 tables or figures. Short communications should represent complete, original studies and should be arranged in the same way as full length manuscripts.

Leading articles and editorials are solicited by the Editorial Board with the aim to bring to the general readership pressing topics in life sciences and related environmental and bioethical dilemmas.

Review articles and viewpoints: Authors who wish to contribute a manuscript to this category are encouraged to contact the Editor-in-Chief. Reviews should be focused on topics of current interest. View-points should offer a more personalized perspective on a topic that will be of interest to the general readership. All contributions to those categories will be subject to editorial review.

Commentaries and letters to the Editor concerning work published in the journal may also be submitted. They should not exceed 2 manuscript pages including one table or figures.

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Preparation of manuscripts

First page - A concise but informative full title of the article. Avoid abbreviations and colloquialisms.

Second page - A condensed title (running title) of no more than 70 letters and spaces. Name(s) of author(s). Write first names in full. Complete address of the laboratory (institution) for each author.

Third page - footnotes to the title, if any. List of any non-standard abbreviations.

Fourth page - An Abstract (no more than 250 words) must be divided into four separate sections: background and purpose, materials and methods, results and conclusions, and should be a factual condensation of the entire work including a statement of its purpose, a clear description of the findings and finally a concise presentation of conclusions.

Content of manuscript

Number the remaining pages consecutively and type the author’s(s) last name(s) at the top of each page. Write in the first person (except summary) and the active voice whenever possible.

Keep the INTRODUCTION brief, stating clearly the purpose of the article and its relation to other papers on the same subject. Do not give an extensive review of literature.

Provide enough information in the MATERIAL AND METHODS section to enable other investigators to repeat the experiments.

Report RESULTS clearly and concisely. Do not present the same results in tables and illustrations. Exceptionally, results and discussion can be combined in a single section.

In the DISCUSSION interpret the results, state their meaning and draw conclusions. Do not simply repeat the results.

Start each section on a separate sheet.
SUPPLEMENTS TO MANUSCRIPT

Footnotes: Avoid footnotes

Tables and illustrations: Tables and illustrations (both numbered in Arabic numerals) should be prepared on separate sheets. Tables require a heading, and figures a legend. Only good drawings and original photographs can be accepted: negatives or photocopies cannot be used. On the back of each illustration, indicate its number, the author’s name, and “top”. Colour illustrations can now be integrated within the text and are reproduced at the author’s expense. Each table and illustration must have all the necessary information to be understood independently of the text.

Submit a drawing twice the final size. Lettering and identifying marks should be clear and eligible after reduction. We prefer either an original drawing in India ink on white drawing or tracing paper or an electronic printout. Submit glossy prints of good quality. Write lightly in pencil the author’s name and figure number (indicate top) on the back of each illustration.

Abbreviations: Introduce an abbreviation only when the same term occurs three or more times.

References: References are to be cited on a separate page at the end of the article, numbered according to the citation order from the text, and marked by Arabic numerals in brackets. A citation in the text is marked by the reference related number. All authors are mentioned, if six or less, while if more than six, only three first ones are cited adding et al. Type references on a separate sheet. Cite in sequence authors names and initials, title of article, name of journal, year of publication, volume number of journal, and first and last pages. The surnames of the authors followed by initials should be given. There should be no punctuation other than commas to separate the authors. Abbreviate journal titles according to the International List of Periodical Title Word Abbreviations, Current Contents or according to Index Medicus. Material submitted for publication but not yet accepted should be noted as unpublished data and not included in the reference list.


Establishing

Tuzla-Farm exists since 1989. It was established as TuzlaMarketing with 100% private capital funds and as such exist until 2000. In year 2000, one part of company has been separated and renamed to Tuzla-Farm which is more specific for the activities that company deal with. Daughter companies of Tuzla-Farm are also four independence pharmacies - Pharmacies «Alma».

Activities

Import and retail sale with:

- medicines
- auxiliary medical products
- and vitamins
- baby care products and food
- pharmaceutical chemicals
- medicinal herbs and tea
- serums and vaccines
- surgical dressings and medical disposables
- medical instruments and equipment
- laboratory reagents, tests and equipment
- cosmetics
- dialysis equipment, disposables and dialysis solutions
- registration and marketing, market researches and evaluation for foreign medicines producers

Our resources

Our opinion is that our workers are the most important resource of the company. Tuzla-Farm has high qualified, trained and experienced personal which has a strong support of distribution system. That gives Tuzla-Farm possibility for continues supply of pharmacies, hospitals and laboratories.

Current number of employees is 45.

Business administration: 10
Warehouse: 25
Sales personal: 5
Delivery personal: 5

Goals

Our goals are to maintain high level of service and further expansion of our customer net. With use of modern and new information technologies we want to provide high level quality supply to our customers.

Capacity

- Wholesale pharmacy has storage facilities, offices and other space in total area of 515 m2 and depo of 250 m2. All space are 100% in company's private property and they fully comply with legislative and safety regulations. (flammable substances, termolabile substances etc.)
- Head office dispose with office space of app. 350 square meters. All space is also in 100% company's private property. All offices have modern computer equipment which are connected in company's network system.
- "Alma" pharmacies are daughter company of Tuzla-Farm. They represent four independent pharmacies which are placed in territory of Tuzla canton.
- Transportation

Optimally coordinated transportation consists of:

5 freight vehicles – for daily delivery
3 personal vehicles – for daily visits and marketing of our products

Transportation pool is fully renewed in 2002.

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APOTEKE SARAJ EVO oglas 1/1
CMYK (2)
oglas Bosnalijek CMYK (1)