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CORRELATION OF PRE-MORBID ALCOHOLISM AND CHANGES IN THE LEVEL OF BIOGENIC AMINE METABOLITES IN CEREBROSPINAL FLUID OF ACCUTE BRAIN INFARCTION PATIENTS

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SUMMARY

Background: *The disorder of biogenic amine metabolism (serotonin – 5-HT and dopamine – DA) is expected in the brain (neuron) damage caused by acute ischemia. It is known that long-term abuse of ethyl-alcohol damages the quality of neurons diffusely in the brain. Cerebrospinal fluid (CSF) and its biochemical content, 5-HT and DA, are reliable indicators of the vitality of neurons.*

The main objective of this research was to demonstrate that the elevated content of metabolites 5-HT and DA in the CSF in patients with acute brain infarction, who were pre-morbid alcohol-dependent patients, is additionally emphasized by diffusive damage of neuron vitality caused by alcoholism.

Subjects and methods: *Study sample consists of two groups - 50 alcohol-dependent patients with acute brain infarction under the age of 65 (group A) and 50 patients with acute brain infarction who were not alcohol-dependent (group B). All subjects underwent the same procedure - CSF was taken during admission to the hospital and history was obtained through anamnesis, heteroanamnesis and clinical examinations.*

Results: *Metabolism of DA and metabolic turnover of DA (3, 4 dihydroxyphenylacetic acid + homovanilic acid; DOPAC + HVA) was elevated in the liquor of both patient groups. The statistically significant difference between the groups was found in metabolic turnover of 5-HT ($p<0.05$), and metabolic turnover of DA ($p<0.001$).*

Conclusions: *The metabolic neuron disbalance, i.e. their pathophysiological-biochemical dysfunction as a result of acute brain infarction, is present in a higher degree in patients with pre-morbid long-term alcohol abuse.*

Key words: *erebrospinal fluid (CSF) - biogenic amines in CSF - dopamine in CSF - serotonin metabolites in CSF - alcoholism and brain infarction*

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INTRODUCTION

Alcoholism, as a long-term intoxication, causes significant lesions of neurons and non-neuron brain structures, which has been confirmed by neuroimaging (CT, MR, PET) and post-mortem proofs in many studies, not only in human material but also on experimental models. Alcoholism leads to huge organic brain damages, and also causes the diffusion atrophy of the brain mass - reduction of neuron quantum in the brain of all structures and regions (Nenadović 1993, Eckardt & Martin 1986). Research has confirmed that the established pathoanatomic substratum in the brain caused by alcoholism leads also to interactive pharmacodynamic effects in the CNS, which can especially be confirmed in the cerebrospinal fluid.

Wide range of modern exact visual diagnostic methods (CT, MR, SPECT, PET) together with laboratory biochemical researches enable exploration of the brain and its damage on a live human being in native conditions (Chick et al. 1989). Biochemical researches of liquor (internal brain microenvironment, CSF) enable confirmation of the changes of the liquor constituents such as serotonin and dopamine (Carlborg et al. 2010, Jokinen, Nordstrom & Nordstrom 2009, Kishida et al. 2007).

Apoplexy of the ischemic type or brain infarction is among the focal vascular CNS disorders, and occurs as a result of occlusion (thrombosis or emboli) of the cerebral blood vessel and the interruption of circulation in its irrigation region. Such disorder of the cerebral circulation drastically interferes with brain functions, since in those circumstances blood does not bring essential energy substrates (oxygen and glucose), as well as neurotransmitter precursors (many amino acids) into the brain (Baune et al. 2008).

When a larger cerebral artery is occluded the infarction acts similar to a tumour i.e. as a lesion which occupies space due to an edema development. The consequence of infarction has repercussions on the function of the damaged brain area, causing neurological deficit lasting more than 24 hours. The level of development and the scope of clinical features depend on the size of the blood vessel i.e. the volume of

the lesioned brain mass and the area of the infarction region. Biochemical CSF constituents reflect the internal brain microenvironment, both in the states of physiological homeostasis, and in the states of disordered brain metabolism, i.e. neurons. In other words, in the states of acute brain infarction, as well as in long-term alcohol abuse i.e. alcoholism.

Alcoholism, together with hyperlipidaemia and hypercholesterolemia, smoking, obesity, oral birth control, physical and mental inactivity are among the confirmed risk factors for brain infarction, the treatment and elimination of which is possible, but the effects of which can not be confirmed (Dyke et al.1984, Gorelick 1989).

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The main objective of this research was to demonstrate that the elevated content of metabolites 5-HT and DA in the CSF in patients with acute brain infarction, who were pre-morbid alcohol-dependent patients, is additionally emphasized by diffusive damage of neuron vitality caused by alcoholism.

SUBJECTS AND METHODS

The study was performed at the Department of Neuropsychiatry of the Health Center in Kosovska Mitrovica, during 2007-2009. Two following basic research methods were used for achieving the objective of the study:

- method of clinical and laboratory examination and;
- method of analyzing the obtained data and the results.

The main ethical principles stipulated in the text of the Helsinki Declaration published in 1975 and revised in 1983 regarding clinical and biological experiments were completely respected, especially those pertaining to clinical research on human beings.

Two groups of patients were formed, test group A (alcohol-dependent patients with brain infarction) and a control group B (non-alcoholics with acute brain infarction). Patients of both groups were hospitalized. Group A consists of 50 hospitalized treated alcohol-dependent patients between the ages of 46 to 65 of both sexes. Data regarding pre-morbid alcoholism for these patients was obtained through the hetero-anamnesis, and/or in some cases auto-anamnesis, but important fact was that for 33 (66%) of the patients, there were also medical files regarding alcoholism treatment prior to the occurrence of the cerebrovascular insult. Control group B was composed of 50 non-alcoholics with acute brain infarction also hospitalized between 46 to 65 years of age. Their mental status regarding alcoholism was checked through detailed hetero-anamnesis, 35 (70%) never drank alcohol, and 15 (30%) drank occasionally, more precisely, not more than a few times a year, even then in moderation. For the needs of this study, the age of patients was deliberately stratified above 45 years of age, in order to achieve the assumed damaging effects on neurons, caused by the long-term abuse of ethyl-alcohol, precise up to two decades in test group A.

The neurological status of all the tested patients was estimated on the basis of the Geismar and associates scale from 1976 (Geismar et al. 1976). Within the first seven days from the occurrence of the insult, computerized tomography of the endocranium was performed on all the group A and B patients, which confirmed the occlusion of some large arterial carotid blood vessels of the left or right hemisphere, indirectly by examining the infraction zone of low density.

Immediately upon hospitalization (usually already in the hospital admitting department) lumbar punctures were performed on all the test group A (alcohol-dependent patients with brain infarction) and a control group B (non-alcoholics with acute brain infarction) patients, and liquor was taken for basic and biochemical examination determined for this study.

For each patient of both test groups the time which elapsed between the insult and the extraction of liquor for biochemical examination was recorded, expressed in hours, and in cases when this time span was longer that 24 hours, such patients were not included in the study.

The protocol on biochemical research of liquor included the taking of CSF by means of lumbar puncture in patients of test group A and control group B. The samples of the cerebrospinal fluid were placed in plastic test-tubes with corks and were kept at a temperature of -70^0C. In the laboratory, biogenic amines (5-HT and DA) and their metabolites (5 hydroxyindoleacetic acid – 5-HIAA; 3, 4 dihydroxyphenylacetic acid – DOPAC; homovanilic acid – HVA), were determined in the liquor samples by applying the HPLC (high-performance liquid chromatography) method. A metabolic turnover of 5-HT is the ratio of concentration of 5-HIAA and 5-HT (5-HT/5-HIAA), and metabolic turnover of DA is the sum of the concentrations of HVA and DOPAC (DOPAC + HVA).

Demographic data for group A and B patients were processed and presented in the form of tables and graphs. Descriptive statistical parameters were applied for the demographic data by calculating the distribution of relative frequencies and arithmetic mean values, as well as standard error of the mean (SEM). Testing of the statistical significance in frequency size and levels of researched features was also used. Tests were also carried out regarding statistical significance of differences for small samples by applying Student's T-test. For nonparametric forms the χ^2 test was used. The Pearson test of correlation was used for different clinical diagnostics and parameters.

RESULTS

Table 1 shows that 11 (22%) of patients in the test group A were between the age of 46 to 55, and only 3 (6%) of patients in the control group B, while the remaining 39 (78%) of patients in group A and 47 (94%) of patients in group B were between the age of 56 to 65. We found statistically significant difference in the age distribution between the two groups (p=0.0437).

We found gender differences between the group as there were more men in the group A [41/50 vs 31/50, χ^2=8.064, df=2, p=0. 0177 (Table 1)].

Figure 1 shows the hourly distribution of time which elapsed between the occurrence of the ischemic cerebral insult and the extraction of CSF for biochemical examination. Within the first 4 hours from the occurrence of brain infarction, liquor was taken by lumbar puncture from 4 patients both in group A and group B. Within a time span of 5 to 8 hours liquor was taken for biochemical examination from 9 patients in group A and 6 patients in group B. During the period of 9 to 12 hours from the occurrence of the insult, liquor was taken from 15 patients in each of the two groups. In the case of 3 patients in group A and 7 patients in group B, 13-15 hours elapsed from the occurrence of apoplexy to the extracting of liquor by lumbar puncture for biochemical examination.

Table 1. Structure of patients of group A and B according to age and gender

Group	Age				p	Sex				p	Total	
	46-55		56-65			male		female				
	N	(%)	N	(%)		N	(%)	N	(%)		N	(%)
A	11	22	39	78	0.0437	41	82	9	18	0.0177	50	100
B	3	6	47	94		31	62	19	38		50	100

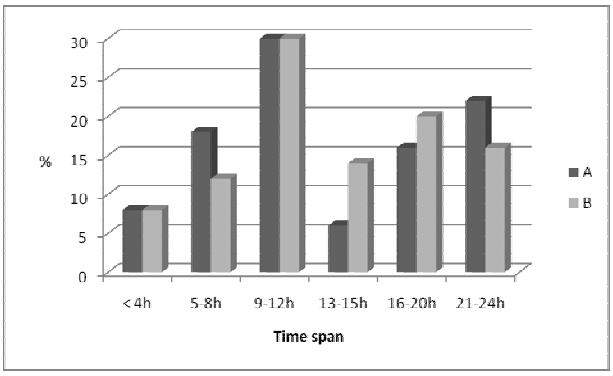

Figure 1. Time span from the occurence of brain infarction until the obtaining of liquor for analysing in test group A and control group B

Cerebrospinal fluid for biochemical examination was taken from 8 patients in group A and 10 patients from group B within 16-20 hours after the ischemic insult. A time span of 21-24 hours lapsed from the brain infarction to the extraction of liquor by lumbar puncture for biochemical examination in the case of 11 patients in the test group A, as well as 8 patients in the control group B.

There was no essential statistical significance regarding the time difference of extracting liquor for examination between the test and the control group patients (χ^2=2.896, df=5, p>0.05).

Patients of test group A and control group B included in this research have experienced cerebrovascular ischemic insult for the first time, i.e. brain infarction in the last 24 hours upon being admitted for treatment. Brain infarction in all patients of groups A and B was confirmed by a CT of the endocranium. By applying the Geismar scale (Geismar, Marquardsen & Silvest 1976), neurological motor deficiency in all group A and B patients was confirmed, either of the hemiparesis or hemiplegic type, right or left.

As can be seen from Table 2, at the time of admittance for treatment, 35 (70%) of group A patients and 36 (72%) of group B patients suffered from hemiparesis. At the time of admittance for treatment, 15 (30%) of group A patients and 14 (28%) of group B patients had hemiplegia. We didn't find statistically significant difference in the level of neurological motor deficiency between the two groups (χ^2=0.023, df=1, p = 0.878).

Table 2. Distribution of motor deficiency in test group A and control group B patients

Motor deficiency	Group			
	A		B	
	N	(%)	N	(%)
Hemiparesis	35	70.0	36	72.0
Hemiplegia	15	30.0	14	28.0
Total	50	100.0	50	100.0

χ^2=0.023, df=1, p= 0.878

Biochemical examination of the CSF of patients in test group A (50 alcohol-dependent patients with brain infarction) and 50 patients of group B (non-alcoholics with brain infarction), in this research the control group, was done in accordance with the HPLC method in order to establish the content of biogenic amines 5-HT and DA. Metabolic turnover of 5-HT in the liquor of test group A patients was confirmed by established concentrated level 5-HT of 69.9 ± 5.1 pmol/ml and content of 5-HIAA (metabolite 5-HT) of 96.8 ± 4.2

pmol/ml, and 5-HT turnover (5-HIAA/5-HT) amounts to 1.6. A 5-HT turnover of control group B was 0,9 since the concentrated content of 5-HT in CSF of these patients was 48.9 ± 4.8 pmol/ml, and concentrated content of its metabolite 5-HIAA 45.1 ± 9.3 pmol/ml (Table 3).

Table 3. Parameters of metabolism 5-HT(Serotonin) and DA(Dopamine)

	Group		p
	A	B	
Serotonin			
5-HT (pmol/ml)	69.9 ± 5.1	48.9 ± 4.8	>0.05
5-HIAA (pmol/ml)	96.8 ± 4.2	45.1 ± 9.3	>0.05
5-HIAA/5-HT (5-HT turnover)	1.6	0.9	<0.05
Dopamine			
DOPAC (pmol/ml)	98.0 ± 10.3	49.0 ± 5.0	>0.05
HVA (pmol/ml)	278.1 ± 11.4	141.0 ± 21.0	>0.05
DOPAC+HVA (DA turnover)	376.1 ± 12.8	190.0 ± 14.3	<0.05

A concentrated content of 5-HT and 5-HIAA in CSF of patients in test group A compared to the patients of control group B, showed statistically significant difference ($p<0.05$). High statistical significance of difference in metabolic turnover in CSF of test group A patients, compared to the control group was also confirmed.

DA metabolism shown by the content of metabolites of this amine, DOPAC (3, 4 dihydroxyphenylacetic acid) and HVA, in CSF, was higher in patients of test group A, compared to the DA content and its metabolites in CSF of control group B patients. DOPAC concentrated level was 98.0 ± 10.3 pmol/ml, established by biochemical examination of CSF according to the HPLC method, while in the liquor of patients in control group B it was 49.0 ± 5.0 pmol/ml. Content of HVA in CSF of test group A patients (alcohol-dependent patients) was 278.1 ± 11.4 pmol/ml, and with patients of the control group 141.0 ± 21.0 pmol/ml. Dopamine metabolic turnover (DA turnover, DOPAC + HVA) was 376.1 ± 12.8 pmol/ml for patients of test group A, and 190.0 ± 14.3 pmol/ml in CSF for control group B patients (Table 3).

The difference of concentrated content of DA metabolites as well as DA turnover in CSF of test group A patients, and control group B patients was statistically significant ($p<0.05$).

DISCUSSION

Research interest in this study was directed to the correlation and/or significance of pre-morbid alcoholism to the biochemically established changes in the level of biogenic amines (5-HT and DA) and their metabolic turnover [5-HIAA (5 hydroxyindoleacetic acid) / 5-HT; DOPAC (3, 4 dihydroxyphenylacetic acid) + HVA (homovanilic acid)] in CSF (Nenadovic 1993, Mrsulja et al. 1991, Nakayama et al. 2007).

It is assumed that alcoholism, as a long-term intoxication, causes lesions of neuron and non-neuron brain structures, which was confirmed by exact diagnostic methods and patho-anatomic proofs in numerous studies (Roy et al. 1991, Linson 1991). There are numerous studies which provide evidence not only on human material, but also on experimental models (Hutson & Curzon 1986, Nakayama et al. 2007, Samad & Haleem 2009). By establishing pathological substratum in the brain of the alcohol-dependent patients, researchers based their interest on the correlation and existence of cognitive disorders and damage (Nenadovic 1993, Chick et al. 1989). Direct toxic effect of ethyl-alcohol on human brain neurons (cytosol and membrane) has not been confirmed (Nenadovic 1993, Hibbeln et al. 1998).

It is well known that alcoholism, or more correctly alcohol abuse starts at a younger age, actually in the third life decade (Roy et al. 1991). Therefore, patients, who are alcohol-dependent patients, and who are engaged in our research are older than 45. It is assumed that alcoholism, i.e. at least two decades of abuse of alcoholic beverages, leads to damage of the morphology and metabolism in human brain neurons. The younger age structure of the patients in test group A correlates to the objective and methodology of this research, alcoholism being a "confirmed" risk factor for the occurrence of acute brain infarction (Gorelick 1989).

The assumption in this research is that there exists a provable disorder of content and metabolism of monoamines (5-HT and DA) in the liquor of those patients, compared to patients of the control group (Hibbeln et al. 1998, Heinz et al. 2002).

The objective of this research is based on diffuse damage of neuron and non-neuron brain structures, caused by the toxic effect of alcohol in a prolonged period of time (Chatterjee & Gerlai 2002, Linson et al. 1991). It is possible to prove the above stated in the

metabolism disorder on the level of the brain cell, which is possible to prove by CSF examination, as internal microenvironment of the brain. The intention of this research did not reach subtle evidence of certain finely altered constituents i.e. metabolites of biogenic amines in the liquor.

The results in Figure 1 are illustrative, and the reason why the samples were taken at different points in time is due to the fact that taking cerebrospinal fluid is a delicate operation, that requires the consent of the patient and / or family. Therefore the moments of taking the fluid varied.

Alcoholism causes most probably, during the years prior to the occurrence of brain infarction, profound damage of the overall vital neuron quality – in the entire brain. In this research it correlated with the neuro-biochemical result in liquor immediately after the occurrence of the brain infarction. Other authors have also proved, more than once, changes of molecular biochemistry, especially metabolic turnover of certain neurotransmitters in liquor (Nenadovic 1993, Bräutigam et al. 2002, Mrsulja et al. 1975, Brown et al. 1991). Changes of metabolism of DA and 5-HT are reliable, indisputable, indirect evidence of neuron lesion as a unit of brain integrum.

The research concept of this study corresponds to the findings of other authors that levels of serotonin metabolites 5-HIAA are higher in brain infarction patients, and also in alcohol-dependent patients (Heinz et al. 2002, Gopal et al. 2007, Samad & Haleem 2009).

Concentrated level of 5-HIAA and serotonin, especially metabolic turnover of 5-HT, is prominent in alcohol-dependent patients with brain infarction of test group A in this research, compared to control group B patients. It can be justly concluded that pre-morbid alcoholism plays a significant role in changes of the content and metabolism of 5-HT in the brain, and in this research cumulatively with acute brain infarction. In their researches other authors have established damage of the dopaminergic system with consequent disorder of content of DA and HVA on the brain substratum post mortem in persons with diffuse neuron damage (pre-senile dementia of Alzheimer's type, alcoholism etc.) (Hutson & Curzon 1986, Aklillu et al. 2009, Markianos et al. 2009). Within this study, a considerably higher level of DA metabolites, DOPAC and HVA, was established by biochemical examination in CFS of test group A patients - alcohol-dependent patients with brain infarction. The metabolic turnover of DA (DOPAC + HVA) was also elevated in CSF of alcohol-dependent patients - group A patients, compared to patients of control group B.

The increased level in liquor of biogenic amines, of 5-HT and DA especially, as well as their metabolites, obviously participate in neuronal damage caused by alcoholism pre-morbidly.

One of the major limitations of the study was that test and control groups were not matched according to age and gender. The main reason lies in the fact that the total population in the region of the research and in our country is relatively small, and therefore it is extremely difficult to provide a sufficiently representative sample for this specific research. Secondly, the procedure of taking CSF is extremely delicate, and so is storage and analysis of the cerebrospinal fluid.

To the best of our knowledge, this is the first study which analyzes the level of biogenic amines and its meaning in CSF of patients with brain infarction who were pre-morbid alcohol-dependants. Furthermore, the results of this research consolidate the opinions regarding the necessity of further development of neuroscientific interests (neurobiochemical research in psychiatry and neurology). Psychiatry in particular should, in adapting its further scientific and research efforts, justifiably deal with the chemical - molecular processes within the physiological metabolism in live neurons of the human brain. Also, the results of the presented research impose dilemmas or can be a starting point for new researches with the aim to exactly establish possible connection of the brain damage through material evidence of changed quantities and relation of their metabolites on one side, and distorted psychic balance, as a product of brain function in conditions of altered neuro-biochemistry on the other, and all in order to improve clinical practice.

CONCLUSION

Research results of this study prove diffusive neuron damage, i.e. their vital functional quality in patients who have abused alcohol for years and sustained brain infarction. In these patients a disorder of concentrated metabolite of 5-HT and DA content has been simultaneously established. This shows that alcoholism has an effect on the metabolic disbalance of neurons and leads to patho-physiological disorders in the metabolism and functioning of the brain in general.

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