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Serum and ascitic fluid serotonin levels and 5-hydroxyindoleacetic acid urine excretion in the liver of cirrhotic patients with encephalopathy

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ABSTRACT

Purpose: The excess and deficit of serotonin can be the cause of somatic and mental disorders. The aim of this study was to evaluate serotonin levels in blood and ascitic fluid as well as excretion of 5-hydroxyindoleacetic acid (5-HIAA) in urine in patients with hepatic encephalopathy (HE).

Material and Methods: The study included 75 alcoholic cirrhotic patients divided into 3 groups (HE_1, HE_2, HE_3), 25 patients each, with grade 1, 2 and 3 of hepatic encephalopathy according to West-Haven classification. The control group (C) included 25 clinically healthy volunteers. Venous blood and ascitic fluid were collected in fasting. On the same day a 24-hour urine collection was performed. Immunoenzymatic method was used to determine the serotonin level in serum and ascitic fluid, and 5-HIAA in urine (IBL-RE-59121, RE-59131).

Results: In the control group, mean serum serotonin level (ng/ml) was 155.5±38.1 and in the 3 study groups: HE_1 – 175.2±32.4 (NS), HE_2 – 137.2±28.6 (NS), HE_3 – 108.3±46.3 ($p<0.001$). Serotonin concentration in ascitic fluid was on the average about 25% of its level in serum. The excretion of 5-HIAA in urine (mg/24h) in all groups, was: C – 5.9±2.1, HE_1 – 5.8±1.8 (NS), HE_2 – 4.8±1.2(NS), HE_3 – 4.3 ±1.3 ($p<0.05$).

Conclusion: The results of our study indicate that serum and ascitic fluid level of serotonin and urine excretion of 5-HIAA depends on the grade of hepatic encephalopathy. In patients with severe hepatic encephalopathy serotonin concentration in blood is decreased which can affect some clinical manifestation of this disease.

Key words: Liver cirrhosis, hepatic encephalopathy, serotonin, 5-hydroxyindole acetic acid

INTRODUCTION

Serotonin is synthesized from exogenous L-tryptophan which is converted in the body into 5-hydroxytryptamine (serotonin), catalyzed by tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase [1]. Serotonin synthesis occurs in the gastrointestinal tract (GI), central and peripheral nervous system and in immune system cells [2-4]. GI is the largest source of serotonin. Approximately 90% of the total serotonin is found here and it is synthesized mainly in enterochromaffin cells (EC) and in enteric neurons of submucous and myenteric plexus layer [5, 6].

Serotonin is also a significant constituent of platelets which do not synthesize but take it up and store it [7]. Released serotonin is taken up from the extracellular space by the serotonin reuptake transporter (SERT). SERT is widely expressed in enterocytes, neurons of central and peripheral nervous system and in blood platelets [8], where serotonin is

metabolized and its main metabolites (5-hydroxyindoleacetic acid and 5-methoxyindoleacetic acid) are excreted in the urine [9]. Basic secretion of serotonin by EC cells in GI is high and it increases in response to pH changes, intestinal contents osmolality, intraintestinal pressure, toxins, some drugs and β-adrenergic and muscarine stimulation [10,11].

Serotonin homeostasis can change in different pathological conditions. Liver cirrhosis can be one of them particularly in the period of its failure and portal hypertension [12]. In cirrhotic patients, secondary changes appear in the GI that are determined as portal enteropathy [13]. In the altered intestinal wall both synthesis and metabolism of serotonin are disturbed. An example is serotonin secretion disorders in numerous GI acute and chronic diseases. The possibility of serotonin leakage to systemic circulation cannot be excluded either. It could be manifested by anxiety, sleep disorders and other emotional disturbances [14]. In these cases, liver should play the role of a filter where serotonin catabolization processes also take place. This mechanism fails in the case of liver diseases. In experimental studies on rats with toxic hepatocellular damage a marked increase of serotonin and 5-HIAA concentration was observed in brain structures which was probably caused by blood-brain barrier permeability [15]. However, it is not certain whether these products originated in brain or in the GI because the applied toxic agent (thioacetamide), similarly to cytostatics, can induce serotonin synthesis in EC cells. Regardless of the source of serotonin, the increase in its level can cause the central nervous system (CNS) symptoms similar to those occurring in patients with hepatic encephalopathy (HE).

The aim of this study was to determine the level of serotonin in serum and ascetic fluid and excretion of 5- HIAA in urine in patients with different stages of HE.

MATERIAL AND METHODS

Patients

The study included 75 alcoholic cirrhotic patients (active drinkers) with portal hypertension and ascites (grade B and C liver disease according to Child-Pugh classification [16]) and 25 healthy volunteers. *Tab 1.* demonstrates general characteristics of the enrolled subjects. All of the patients abused alcohol within the period of 8 to 20 years. The diagnosis was based on clinical examinations, imaging and laboratory investigations. Furthermore, all patients were subjected to neurological, psychiatric, psychological examination and in 52 patients to psychometric testing e.g. number connection test (NCT-A and NCT-B) and line tracing test (LTT) to determine the severity of the encephalopathy. Three groups of patients were distinguished (25 patients each) on the basis of 5-stage (0-4) West Haven criteria [17], fulfilling grade 1, 2 and 3 of HE (HE_1, HE_2, HE_3 – respectively).

A written consent was obtained from all the examined patients and the Ethics Committee of the Medical University in Lodz (Poland) approved the study protocol (RNN/22/05/ KB).

Laboratory test

The following laboratory investigations were repeatedly performed: blood cell count, bilirubin, ALT, AST, GGT, ALP, glucose, cholesterol, urea, ammonia, creatinine, GFR, prothrombin, INR, albumins, globulins and markers of viral hepatitis (HBsAg and anti-HCV).

Three days prior to the examination and on the day of the examination all the patients remained on the same diet i.e. Nutridrink (Nutricia) 3x400 ml (1800 kcal) and 1500 ml of mineral water.

Venous blood and ascitic fluid (during paracentesis) were collected at the same time, in fasting, and after centrifugation the material was stored at -70°C. At the same day 24-hour

Table 1. **Characteristic of the subjects enrolled on the study.**

Feature/parameters	Cirrhotic patients			Healthy subjects
	HE_1	HE_2	HE_3	
Age (years)	42.2±9.0	47.3±7.8	45.6±10.2	43.1±6.8
Gender (F, M)	F – 6 M – 19	F – 9 M – 16	F – 5 M – 20	F – 11 M – 14
Bilirubin [mg/dl]	6.8±5.4	9.4±8.6	9.0±6.6	0.7±0.2
Ammonia [μg/dl]	39.5±8.8	90.4±16.6	88.7±28.4	30.4±8.9
Albumin [g/dl]	3.6±1.1	3.2±1.8	3.3±1.6	5.4±0.6
AST [U/l]	86.5±66.8	72.4±61.0	70.0±68.2	21.0±4.8
ALT [U/l]	90.0±107.3	116.4±98.6	87.8±96.8	23.1±5.9
GGT [U/l]	118.3±78.7	114.6±90.4	132.0±98.2	26.0±6.1
ALP [U/l]	50.4±21.6	56.1±30.2	60.3±39.0	37.4±10.8
GFR [ml/min]	99.2±6.3	96.0±8.6	94.3±15.0	108.9±9.6

HE_1, HE_2, HE_3 – 1, 2, 3 degree of hepatic encephalopathy according to West-Haven Score;
AST – aspartate aminotransferase; ALT – alanine aminotransferase; GGT – gamma-glutamyl transpeptidase; ALP – alkaline phosphatase; GFR – glomerular filtration rate

urine samples were collected and stored at 4°C. At the end, the volume of urine was measured and the samples were frozen to -70°C.

Serotonin concentration in serum and in ascitic fluid was determined by immunoenzymatic method with IBL-RE59121 kit and the concentration and content of 5- HIAA in urine with IBL-RE59131 kit.

All the laboratory tests were conducted in the Laboratory of Military Teaching Hospital, Veterans Central Hospital (Lodz, Poland).

Statistical analysis

The non-parametric Mann-Whitney test was used to compare the serotonin concentration in serum and ascitic fluid between patients.

The non-parametric Kruskal-Wallis test was used to compare the amount of the excreted 5- HIAA in urine.

The verification of the significance of the differences in the results was performed at the level of p=0.05. Statistica (Version 7.1, Statsoft, Poland) and Excel (Version 2007, Microsoft Co.) systems were used for statistical evaluation.

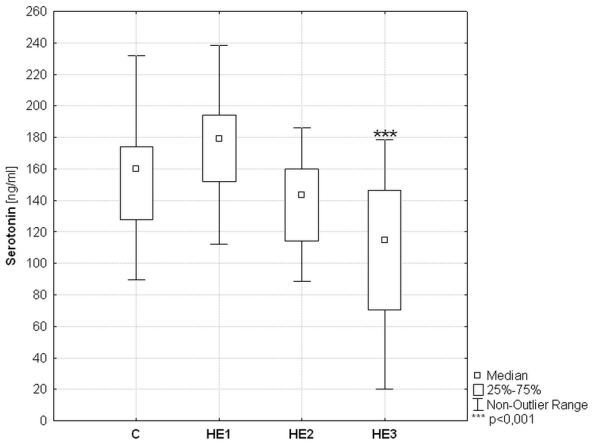
RESULTS

In the control group, the mean serum serotonin level was 155.5±38.1 ng/ml (*Fig. 1*). In the group of patients with moderate HE the results were similar to those obtained in the control group: HE_1 – 175.2±32.4 ng/ml ($p>0.05$) and HE_2 – 137.2±28.6 ng/ml ($p>0.05$). In HE_3 group, the serotonin level was significantly lower - 108±46.3 ng/ml ($p<0.001$).

The serotonin concentration in ascitic fluid was significantly lower than in serum in all groups: HE_1 – 31.5±15.1 ng/m, HE_2 – 25.0±11.4 ng/ml and HE_3 – 22.1±14.7 ng/ml; differences between the groups were not statistically significant (*Fig. 2*). In the control group the excretion of 5-HIAA in urine was 5.9±2.1 mg/24h. In patients with severe liver failure the urinary excretion of 5-HIAA was lower than in the control group and it was: HE_2 – 4.8±1.2 mg/24h ($p<0.05$) and HE_3 – 4.3±1.3 mg/24h ($p<0.01$) (*Fig. 3*).

The serotonin levels, both, in blood and in ascitic fluid, were inversely proportional to the severity of the disease. The same dependence was found in relation to the excretion of its metabolite (5-HIAA) in urine which is an indirect proof that homeostasis of serotonin in patients with liver failure is impaired.

Figure 1. **Serum levels of serotonin in healthy subject (C) and in cirrhotic patients with 1 (HE_1), 2 (HE_2), 3 (HE_3) degree of hepatic encephalopathy (according to West-Haven Score).**



DISCUSSION

HE is a complex neuropsychiatric syndrome characterized by disturbances in behavior and consciousness and by neurologic signs [18,19]. Ammonia is most frequently mentioned among substances playing a role in encephalopathy [20]. It disturbs

Figure 2. **Concentration of serotonin in the ascitic fluid in cirrhotic patients with 1 (HE_1), 2 (HE_2), 3 (HE^3) degree of hepatic encephalopathy (according to West-Haven Score); no statistical significance.**

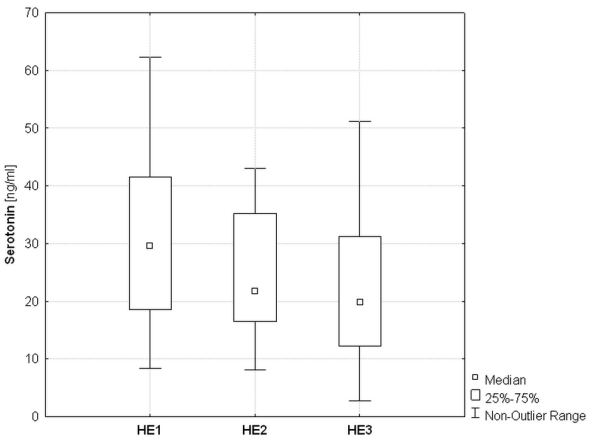
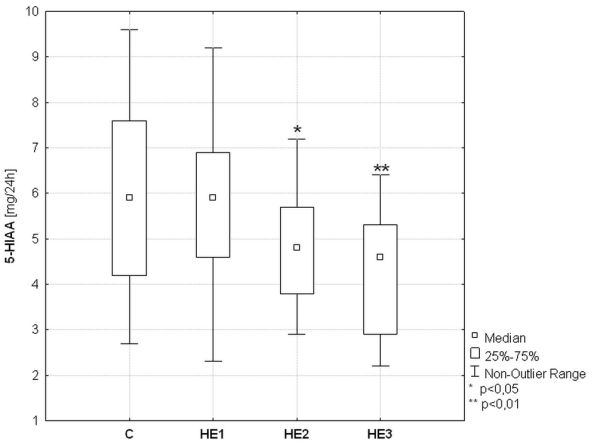


Figure 3. **Urinary 5-hydroxyindoleacetic acid (5-HIAA) excretion in health subject (C) and in cirrhotic patients with 1 (HE_1), 2 (HE_2), 3 (HE_3) degree of hepatic encephalopathy (according to West-Haven Score).**



brain tissue enzymatic processes, inhibits the activity of acetylocholine and dopamine and intensifies storage of false neurotransmitters. However, high level of ammonia in blood is not found in all patients with HE [21,22]. In these cases other compounds and metabolites can be the pathogenic factors - serotonin also among them [20,23,26].

The results of our present study confirm our earlier observations and indicate that serotonin homeostasis in patients with liver cirrhosis is impaired [21]. In moderate stages of liver dysfunction changes in serotonin level are minimal, but in severe stages with symptoms of HE blood serotonin level is decreased. It can be caused by reduced synthesis of serotonin both in GI and in liver. Rössle et al. [27] show that the half-life of tryptophan is two times longer in patients with liver diseases after oral tryptophan loading test than in healthy people. They suggest, that oral tryptophan loading test should be used for evaluating functional impairment of liver. Other authors show, that increased tryptophan blood level disturbs CNS functions causes chronic fatigue and sleep disorders [24,25,28]. It is possible especially in patients with liver cirrhosis and low protein level. Serum-free tryptophan can easily enter into brain through the blood-brain barrier. It should be noted that transformation from tryptophan to serotonin also takes place in brain [29]. Tryptophan- deficient or tryptophan-enriched diet can cause CNS functional disorders and symptoms such as chronic fatigue [30]. The tryptophan mechanism and serotonin synthesis in brain is still not fully understood, but their role in pathogenesis of HE is very likely. In alcoholic liver disease, serotonin deficiency may cause depression, while its excess may induce psychosis [31,32,33]. Symptoms of serotonin syndrome are observed in patients with liver cirrhosis and alcohol withdrawal delirium [34]. In our previous studies, there were no symptoms of delirium, but patients suffered from apathy and fatigue, which can be associated with decreased serotonin and increased melatonin blood levels [26]. At the same time, in a group of patients with severe stage of liver dysfunction, adequate decrease in excretion of the main metabolite of serotonin- 5-HIAA- was noticed. The above indicate impaired synthesis of this neurotransmitter. Low level of serotonin may be caused by alternative metabolic pathways of tryptophan, e.g by oxidization into oxindole [35] and kynurenine pathways [36].

Curzon's hypothesis suggests that elevated level of glycocorticosteroids, being the consequence of chronic mental or biological stress, activates hepatic pyrolase which instead of metabolizing tryptophan into serotonin enhances the conversion of tryptophan to kynurenine and to the resulting serotonin deficit [36-38]. Such process takes place in many tissues including those in CNS [39]. Kynurenine pathway metabolites are formed; kynurenic acid has neuroprotective properties whereas quinolinic acid increases neurotoxic effects. Another metabolite, 3-hydroxykynurenine, shows similar neurotoxic activity by inducing oxygen free radical generation [40,41].

Changes in serotonin homeostasis can cause many other disturbances in the body.

Possible serotonin leaking through intestinal wall into the blood stream should be taken into account. This mechanism is proved by the presence of serotonin in the portal vein and in ascitic fluid. Serotonin induces vasospasms through 5-HT1b and 5-HT2a receptors in hepatic vessels which can be an additional pathogenic factor of liver failure [40,41].

Furthermore, through 5-HT2a receptors, located in endothelial cells, serotonin enhances platelet aggregation which contributes to the portal systemic thrombosis, which frequently occurred in patients with portal hypertension [42,43].

The pathogenesis of HE is complex and the role of serotonin is still not defined precisely. Nevertheless, considering changes in the homeostasis of this neurotransmitter seems to be justified.

CONCLUSIONS

The results of our study indicate that serotonin level in serum and ascitic fluid, as well as 5-hydroxyindoleacetic acid in urine excretion depend on the grade of HE. In patients with severe HE, serotonin concentration in blood is decreased causing clinical manifestation of the disease.

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