

## HYPERDYNAMIC CIRCULATION AND SEROTONIN LEVELS IN PATIENTS WITH LIVER CIRRHOSIS

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**Summary:** Patients with liver cirrhosis and portal hypertension develop hyperdynamic state of circulation. Recent studies indicate that nitric oxide, prostaglandin's, endothelin-1 and serotonin are of major importance in the pathogenesis of portal hypertension and other hemodynamic complications. In our study we estimated the levels of serotonin in sera and 24h urine in 20 patients with liver cirrhosis, using original solid phase extraction procedure. Concentration of serotonin in sera was ranged from 18 to 270 nmol/L and in 24h urine ranged from 97 to 238 nmol/L/24h. In comparison with reference values (determined in range from 280-1300 nmol/L in sera and determined in the range from 157.8-1035.4 nmol/L/24h in urine), a significant fall of serotonin concentration in sera, and urine was noted. A statistically significant correlation between serotonin levels in sera and platelet count was found ( $p = 0.017$ ). Colour Doppler ultrasonography and peroral fiberpanendoscopy was applied in the whole series to evaluate degree of portal hypertension. The average diameter of portal vein was 16 mm (SD=1.36) and mean blood flow velocity in portal vein was 12 cm/s (SD=1.12). Splenomegaly was approved in the whole group and the mean splenic craniocaudal distance was 17.75 mm (SD=1.65). Esophageal varices grade I-IV were detected in 8 (40%) of our patients and remaining 12 (60%) were grade III-IV. In cirrhotic liver, the spleen congestion, result from platelet degradation. Serotonin, release in to spleen sinusoidal spaces, induces strong vasoconstriction respons in portal venous circulation, which contributes to maintain portal hypertension. However, we consider that the decrease of serotonin levels in sera lead to development of hyperdynamic state of circulation.

**Key words:** hyperdynamic circulation, serotonin, liver cirrhosis

### Introduction

Patients with liver cirrhosis and portal hypertension develop a hyperdynamic state of circulation, with high cardiac output, increased blood volume, reduced systemic vascular resistance, and they are prone to arterial hypotension. The increased hepatic and collateral resistances as well as portal blood flow maintain portal hypertension (1). This irregularity is in the base of other complications, including ascites and renal failure, and it has been related to peripheral arterial vasodilatation, mainly occurring in the splanchnic circulation (2).

The pathogenesis of the hyperdynamic state of circulation and arterial vasodilatation are still unclear. According to the vascular overfilling hypothesis, a still unknown signal(s) coming from the diseased liver prompts the kidney to retain sodium. The retained sodium expands plasma volume, leading to increased cardiac output and reduced systemic vascular resistance. On the contrary, the peripheral arterial vasodilatation hypothesis suggest that arterial vasodilatation mainly occurring in the splanchnic circulation, leads to a reduction in effective arterial blood volume, arterial hypotension, activation of the main endogenous vasoconstricting and sodium retaining systems, and the development of sodium and water retention, ascites, and hepatorenal syndrome (2).

Now, it is believed that nitric oxide (NO), prostaglandin's, endothelin-1(ET-1) and serotonin are of tremendous importance in the develop a groupment of portal hypertension and the other hemodynamic and renal complications of cirrhosis (3, 4).

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## Material and Methods

We estimated the serotonin levels in a group of 20 patients with liver cirrhosis and portal hypertension, 12 (60%) patients had alcoholic liver cirrhosis and 8 (40%) of remaining patients were treated for post viral hepatitis cirrhosis.

In evaluation of portal hypertension real-time, duplex Doppler and color Doppler ultrasonography on Toshiba Core Vision SSA-350A with 3,75 MHz sector duplex probe were used. Diameters of the portal and lienal veins were measured in standard procedure with the study of hemodynamic parameters of portal circulation: direction, flow rate and diameter of portal vein. Esophageal and gastric varices were diagnosed by peroral fiberpanendoscopy, using an Olympus GIF XQ 10 endoscope.

Serotonin was measured in sera and 24h urine, which was collected in 2 L bottles, with 30 mL 6 mol/L HCl. Serotonin was extracted from sera and urine, using original solid phase procedure given by »Bio-Rad Laboratories GmbH München« (5). The HPLC system consisted of AS-100 HRLC Automatic Sampling System, with Rheodine 7125 valve, Bio-Rad 1350 pump and Bio-Rad 1640 electrochemical detector. Chromatographic data were calculated using »Chrome Line V 4.20 b« HPLC software, delivered by »Bio-Rad Laboratories GmbH München«. Chromatographic separation had been done on reversed phase C-18 column delivered with original mobile phase from »Bio-Rad Laboratories München«. Detection was electrochemical on potential +0,60 V (6).

The platelet count was determined by Coulter method on »Coulter Onyx« blood cell counter (7).

## Statistical analysis

Statistical analyses were performed using Basic Statistic Software. Correlation between the serotonin levels and platelet count was sought by calculating the Spearman's test.

## Results

Colour Doppler sonography was applied to achieve better diagnostic sensitivity of portal hypertension. The average diameter of portal vein was 16 mm (SD=1.36) and mean blood flow velocity in portal vein was 12 cm/s (SD=1.12). Splenomegaly was approved in the whole group and the mean splenic craniocaudal distance was 17.75 mm (SD=1.65).

Esophageal varices grade I II were detected in 8 (40%) of our patients and remaining 12 (60%) were grade III IV.

Concentration of serotonin in sera was ranged from 18 to 270 nmol/L. In comparison with reference values (determined in range from 280 1300 nmol/L)

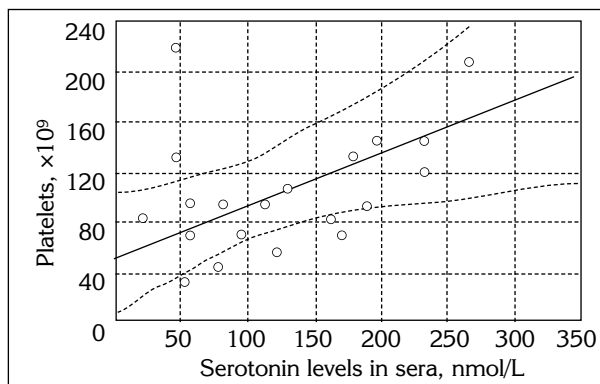


Figure 1. Serotonin levels in sera in correlation with platelet count

(8), a significant fall of concentration of serotonin in sera was noted.

Concentration of serotonin in 24h urine was ranged from 97 to 238 nmol/L/24h. In comparison with reference values (determined in the range from 157.08 1035.4 nmol/L/24h) (8), a significant fall of concentration of serotonin was noted in 24h urine.

A statistically significant correlation between serotonin levels in sera and platelet count was found ( $p = 0.017$ ) (Figure 1).

## Discussion

NO and prostaglandin are endogenous vasodilators produced by vascular endothelial cells. Patients with cirrhosis have increased plasma levels of NO and its metabolites, increased NO in exhaled air, increased nitric oxide synthase (NOS) activity in neutrophils and monocytes, and greater than normal forearm vasoconstriction in response to the NOS inhibitor N<sup>G</sup>-monomethyl-L-arginine (L-NMMA) (9 11).

In the cirrhotic liver, a reduced release of NO by sinusoidal endothelial cells contributes to increased hepatic resistance (12). On the other hand, an increased NOS activity in the splanchnic circulation seems of great importance in determining the increased splanchnic blood flow, which contributes to maintain portal hypertension and plays a major role in the pathogenesis of hemodynamic and renal complication of cirrhosis (13).

Recently, increased prostacyclin (PGI<sub>2</sub>) activities have been observed in systemic circulation and portal vein segments of the portal hypertensive rats. Also, prostaglandin was found to be involved in the modulation of collateral vascular tone in the portal hypertensive rats and could modify the vasoconstrictive effect of vasopressin (14, 15).

Endothelins (ETs) are a group of three related peptides of 21 amino acids (ET-1, ET-2, and ET-3) first described as potent vasoconstrictors (16). It has

been that endothelin ET-1 found to evoke potent and long-acting vasoconstriction on systemic and several regional circulations (17).

Now great attention has been paid to the role of ET-1 in hepatic circulation, particularly in relation to portal hypertension (18). ET-1 was found to induce hepatic vasoconstriction and subsequently increase intrahepatic resistance (17). The spleen is one of the major sites of ET-1 release in cirrhotic patients. Endothelial cells of the splenic sinus and possibly B lymphocytes in the germinal center and marginal zone of lymphoid sheaths and follicles seem to be sites of ET-1 production in the spleen (19).

In acute and chronic hepatic insufficiency changes in serotonin system occur, contributing to the development of portal hypertension, hepatic encephalopathy, and hyperdynamic circulation (20).

Serotonin induces strong vasoconstriction response in portal venous circulation via  $5\text{-HT}_2$  receptors. It has been evidenced that isolated mesenteric vein in patients with portal hypertension hypersensitively reacts to 5-HT. After application of serotonin inhibitors (ketanserin and ritanserin), portal pressure is decreased in patients with liver cirrhosis, confirming the importance of serotonin in pathogenesis of portal hypertension (21).

Hepatic encephalopathy is followed by changes in the neurotransmission of serotonin, including the catabolic enzymes, receptors and metabolites (22). Plasma and brain levels of aromatic amino acid tryptophan, which is a direct serotonin precursor, are elevated (23). Increased activity of monoamine oxidase A and B (MAO-A and MAO-B) and higher level of 5-hydroxyindolacetic acid (5-HIAA) have been evidenced in brain tissue from people who died of liver cirrhosis and hepatic encephalopathy (24, 25). Diminished density of postsynaptic  $5\text{-HT}_2$  receptors in the brain of patients with liver cirrhosis suggests that hepatic encephalopathy results from serotonergic synaptic deficiency (25).

Low concentrations of serotonin, as a circulating vasoconstrictor contributes to development of hyperdynamic syndrome with vascular dilatation in systemic, pulmonary and splanchnic vascular network (26). Low values of circulating serotonin in liver cirrhosis most probably result from impaired platelet count and/or fall of intraplatelet 5-HT, due to reduced inflow and retention of 5-HT in platelets (27, 28).

Marasini et al. (27) described a significant fall of serotonin in platelets of 14 patients with liver cirrhosis,

although levels of free circulating plasma serotonin were similar to those in controls. No correlation between level of circulating and intraplatelet serotonin was established in studied groups, and serotonin values did not correlate with platelet count, serum albumin, ammonia, transaminases, immunoglobulins, creatinin or cholestatic parameters. These authors suggest that the level of intraplatelet serotonin is an important index of hepatic deficiency, since it correlates with the degree of hepatic insufficiency.

Laffi et al. (28) gave the evidence of significant reduction of substances that are deposited in thick granules (adenosine triphosphate and 5-HT) and in alpha granules ( $\beta$ -thromboglobulin and platelet factor 4) in patients with liver cirrhosis, in comparison with controls. It is supposed that platelet disorder in deposition of substances mentioned above, in patients with liver cirrhosis, is in relation to platelet activation, condition defined as platelet exhaustion. Liver cirrhosis is characterized by reduced serotonin inflow into platelets, as a contributing factor of diminished intraplatelet serotonin. It has been assumed that metabolic factors or altered serotonin receptors reduce serotonin inflow into platelets.

In our study, a significant fall of serotonin levels has been confirmed in the sera and 24h urine. Statistically significant correlation was established between serotonin levels in sera and platelet count ( $p = 0.017$ ). These findings confirm that platelet metabolic disorder significantly influences alteration of serotonin system.

Concentration of circulating serotonin in liver cirrhosis can be influenced by other factors, as portosystemic collaterals, altered serotonin catabolism due to elevated activity of monoamine oxidase and disturbed metabolism of tryptophan, as serotonin precursor (29).

In the study of Beaudry et al. (30) levels of plasma unconjugated and conjugated serotonin in 30 patients with liver cirrhosis were significantly lower than in controls. Correlation between serotonin levels and severity of liver cirrhosis was not established. The authors suggest that the fall of 5-HT levels in hepatic cirrhosis may result from its impaired production.

In the cirrhotic liver, the spleen congestion, result from platelet degradation. Serotonin, release in to spleen sinusoidal space, induces strong vasoconstriction response in portal venous circulation, which contributes to maintain portal hypertension. However, we consider that the decrease of serotonin level in sera lead to development of hyperdynamic circulation.

## HIPERDINAMSKA CIRKULACIJA I NIVO SEROTONINA KOD PACIJENATA SA CIROZOM JETRE

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*Kratak sadržaj:* Pacijenti sa cirozom jetre i portnom hipertenzijom razvijaju hiperdinamsku cirkulaciju. Novije studije ukazuju da azot oksid, prostaglandini, endotelin-1 i serotonin imaju najvažniju ulogu u patogenezi portne hipertenzije i drugih hemodinamskih komplikacija. Određivan je nivo serotonina u serumu i 24h urinu kod 20 pacijenata sa cirozom jetre, koristeći originalan postupak ekstrakcije na čvrstoj fazi. Koncentracija serotonina u serumu iznosila je od 18 do 270 nmol/L a u 24h urinu od 97 do 238 nmol/L/24h. U poređenju sa referentnim vrednostima (koje su determinisane u opsegu od 280 do 1300 nmol/L za serumski serotonin, a za urinarni od 157,8 do 1035,4 nmol/L/24h) nađeno je značajno sniženje koncentracije serotonina u serumu i urinu. Postoji statistički značajna korelacija između nivoa serotonina u serumu i broja trombocita ( $p = 0,017$ ). U proceni stepena portne hipertenzije primenjena je kolor Doppler ultrasonografija i peroralna fiberpanedoskopija. Prosečni dijametar portne vene iznosio je 16 mm (SD= 1,36) a srednja brzina protoka krvi u portnoj veni iznosila je 12 cm/s (SD=1,12). Splenomegalija je dokazana kod svih pacijenata a prosečni kraniokaudalni promer slezine iznosio je 17,75 mm (SD=1,65). Variksi jednjaka I II stepena uočeni su kod 8 (40%) dok je 12 (60%) pacijenata imalo varikse III IV stepena. U cirozi jetre, kongestija slezine, dovodi do razgradnje trombocita. Serotonin, oslobođen u sinusoidalne prostore slezine, indukuje snažan vazokonstriktorni odgovor u portnoj venskoj cirkulaciji, koji doprinosi održavanju portne hipertenzije. Sugerirše se da sniženje nivoa serotonina u serumu utiče na nastanak hiperdinamske cirkulacije.

*Cljučne reči:* hiperdinamska cirkulacija, serotonin, ciroza jetre

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