

Value of Endothelial Dysfunction in the Pathogenesis of Portal Hypertension

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ABSTRACT

Background: Until now it was thought that the main cause of portal hypertension is a mechanical obstruction of blood flow in the portal vein due to proliferation of connective tissue in the liver parenchyma (irreversible factor). The role of reversible factors remained underestimated. These include the following components: tone of the blood vessels, blood rheology, micro thrombosis, swelling of the liver parenchyma. The tone of hepatic blood vessels is provided by a number of factors, among which the most important is nitrogen monoxide (NO) – natural vasorelaxing substance, produced by vascular endothelium. The role of endothelium-dependent factors in the pathogenesis of many chronic diseases is extensively studied for today, but the impact of endothelial dysfunction on liver hemodynamics and forming of portal hypertension is not clear yet.

Aim & Objectives: The aim of our research was to examine the condition of hepatic blood flow in patients with portal hypertension, which developed on the background of chronic alcoholic steatohepatitis (ASH) and liver cirrhosis (LC), depending on the degree of endothelial dysfunction in these patients.

Methods: Research was carried out at the Gastroenterological Department of Emergency Hospital in Chernivtsi. The study involved 63 patients with ASH, having signs of initial stage of portal hypertension (study group 1). Also 31 patients with LC with expressed portal hypertension were examined (study group 2). As controls 62 ASH patients without any signs of portal hypertension were examined (control group). Exclusion criteria in both groups were: old age, the presence of cardiac failure, kidney failure and other diseases in decompensate stage.

Results: Summarizing the results obtained in the research process, it should be noted that in all examined patients with chronic alcoholic hepatitis EGD and Doppler signs of preclinical stages of hyperkinetic type of portal hypertension were observed. Absence of cirrhotic changes of liver tissue in these patients is confirmed by histopathological study of liver biopsies.

Blood tests have revealed the decreasing of NO level (endothelial vasorelaxing factor) and depression of endothelium-dependent fibrinolytic activity of plasma. Doppler examination of the brachial artery patients of the 1-st group have revealed decreased endothelium-dependent vasodilation (EDVD) - $7,3 \pm 0,18\%$ (against $12,9 \pm 0,22\%$ in controls ($p < 0,05$)). Amongst 82 % patients of the 2nd group EDVD was only $5,4 \pm 0,15\%$, what is significantly less, than in 1-st and control groups ($p < 0,05$). Amongst 18 % patients of 2nd group during conducting of test for reactive hyperemia it was registered the paradoxical vasoconstrictive reaction, indicating a more expressed endothelial dysfunction. The analysis of the data determined the availability of reverse

correlation between the degree of portal hypertension and the level of NO in blood ($r=0.87$) and between the degree of portal hypertension. and EDVD index ($r=0,54$) with a high degree of probability, indicating the important pathogenetic role of endothelial dysfunction in the development and progression of portal hypertension.

Conclusion: There are manifestations of endothelial dysfunction in patients with portal hypertension, which have been developed on the ASH background. The gradual increasing severity of portal hypertension is observed in the same moment with the deterioration of the endothelium functional condition. The imbalance of endothelium-dependent vasoactive substances is an important part of potentiating of hepatic hemodynamic failure and formation of high pressure in the portal vein system in such patients..

Prospects for further investigations is the search for medications to correct endothelial dysfunction in order to improve results of treatment of patients with portal hypertension on the chronic alcoholic steatohepatitis background.

Keywords: Endothelial dysfunction, portal hypertension, chronic alcoholic hepatitis, liver cirrhosis.

Background

The actuality of this research is caused by significant growth of the incidence of portal hypertension in patients with diffuse chronic liver disease in recent years [1-5].

But, violation of hepatic hemodynamic in patients with chronic hepatitis and in patients with liver cirrhosis are significantly differentiated, due to difference of mechanisms of formation of portal hypertension on its various stages, as it is also confirmed the literature data [6]. So, at liver cirrhosis leading role in causing of increased pressure in the portal vein belongs to irreversible factor - violation of liver architectonics and disorders of systemic blood circulation, closing the pathogenetic circle. The violation of portal blood flow is partially caused by the regenerative nodules, compressing the portal vein branches. This should lead to the post sinusoidal portal hypertension. However, in case of liver cirrhosis hepatic veins wedge pressure (sinusoidal) and the pressure in the main trunk of portal vein are the same, but the stasis extends to branches of portal vein [7]. Sinusoids, apparently, cause the main resistance to the blood flow. Due to changes in the Disse space, caused by it's collagenisation, sinusoids become narrowed. It could be especially expressed in alcoholic liver damage, when the flow in sinusoids may be declined also due to hepatocytes swelling [8]. As a result, obstruction develops from the portal areas throughout to sinusoids to hepatic veins [9].

The growth of hemodynamic disorders in the liver cirrhosis cause the progression of portal hypertension, creating a threat of deadly complications – bleeding from esophageal varices [10]. Thus, the syndrome of portal hypertension acquires the primary role in the prognosis for the patient's life and puts on the first place therapeutic measures to prevent and stop the esophageal-gastric bleeding. However, the use as therapeutic as well as surgical methods of correction of portal hypertension at the stage of liver cirrhosis has a palliative character and does not allow to achieve satisfactory results, because the it leads to the deterioration of the blood supply of the liver and accelerates the development of hepatic encephalopathy [11]. So, it is necessary to

examine patients with chronic hepatitis carefully to reveal early signs of portal hypertension in them, for timely treating of hemodynamic disorders at the reversible stage of their development. Progress in understanding the mechanisms that hinder a free hepatic blood flow, opens new perspectives for the development of more effective therapeutic strategies. Due to the modern vision of the pathogenesis of portal hypertension the pressure level in portal vein is determined by three factors: the size of portal blood flow (increases not only because of splanchnic vasodilatation, but also because of increased angiogenesis in the liver and formation of arterio-venous anastomoses between the branches of hepatic artery and portal vein in the fibrous septum), vascular tone of the branches of portal vessels, and general intrahepatic vascular resistance [12]. From the above it follows that the pathogenesis of portal hypertension can not be reduced only to the difficulties of intrahepatic venous blood flow due to mechanical barriers, changes of liver architectonics and other local factors. The hemodynamic factors are also very important, and their reversibility determines the priority of this trend in the development of methods for therapeutic correction of portal hypertension.

Until now it was thought that the main cause of portal hypertension is a mechanical obstruction of blood flow in the portal vein due to proliferation of connective tissue in the liver parenchyma (irreversible factor). The role of reversible factors remained underestimated. These include the following components: tone of the blood vessels, blood rheology, micro thrombosis, swelling of the liver parenchyma. The tone of hepatic blood vessels is provided by a number of factors, among which the most important is nitrogen monoxide (NO) – natural vasorelaxing substance, produced by vascular endothelium [13-17]. The role of endothelium-dependent factors in the pathogenesis of many chronic diseases is extensively studied for today, but the impact of endothelial dysfunction on liver hemodynamic and forming of portal hypertension is not clear yet [18].

Aim & Objectives

The aim of our research was to examine the condition of hepatic blood flow in patients with portal hypertension, which developed on the background of chronic alcoholic steatohepatitis (ASH) and liver cirrhosis (LC), depending on the degree of endothelial dysfunction in these patients.

Methods

Research were carried out at the Gastroenterological Department of Emergency Hospital in Chernivtsi. The study involved 63 patients with ASH, having signs of initial stage of portal hypertension (study group 1). Also 31 patients with LC with expressed portal hypertension were examined (study group 2). As controls 62 ASH patients without any signs of portal hypertension were examined (control group). Exclusion criteria in both groups were: old age, the presence of cardiac failure, kidney failure and other diseases in decompensated stage. Characteristics of these groups is shown in the (table 1).

For the verification of the diagnosis liver biopsy, esophagogastroduodenoscopy (EGD), Doppler study of hepatic blood flow (using ultrasound and Doppler diagnostic system "En Visor HD" (Philips, USA)) were performed [19]. We've measured lumen diameters of portal vein in it's

broad segment, vena lienalis, hepatic veins 2-3 cm above the place of their confluence in the vena cava inferior. We've performed the calculation of the linear (V_{lin.}) and volume (Q) velocity of blood flow in vena lienalis, portal vein, vena cava inferior using pulse-wave sensor 2.5 MHz. Also we've calculated congestive (CI), portal-spleen venous (PSVI), spleen-vascular (SVI) and hepatic-vascular (HVI) indexes [20-25].

The presence of endothelial dysfunction was evaluated for content in the blood of stable metabolites of NO (nitrites, nitrates) (using Griss reagent), von Willebrand factor (vWF) level, changes in anticoagulant and fibrinolytic activity of endothelium (the content of antithrombin III (ATIII), summary (SFA), non-enzymatic (NFA) and enzymatic fibrinolytic activity (EFA) of blood plasma („Danush Ltd” reagents).

To determine the degree of manifestation of endothelial dysfunction we've studied index of the endothelium-dependent vasodilation (EDVD) of brachial artery. EDVD was evaluated according to Celermajer-Sorensen test(1992) using duplex Doppler ultrasound of brachial artery at rest and at the condition of reactive hyperemia [26].

The study started after patient stay in a horizontal position for 10 minutes. The diameter of brachial artery was measured by 10 MHz transducer in longitudinal section on 2-1,5 cm above the elbow bend before and after tests with reactive hyperemia through 30-90 seconds. The cuff of sphygmomanometer was imposed to patient's arm and pumped to 50 mm Hg more than his systolic blood pressure. The duration of occlusion phase was 5 minutes. The normal reaction of brachial artery was dilatation to 10% or more of the initial diameter on the reactive hyperemia background, smaller indicators or vasoconstriction regarded as abnormal.

Studies were performed in compliance with the Council of Europe Convention on Human Rights and Biomedicine and recommendations of the Committee on Bioethics at the Presidium of Academy of Medical Sciences of Ukraine. Statistical data processing was implemented in the application of "STATISTICA 6.0". After checking the normality of distribution and equality of variances in the samples we've calculated arithmetic average and its error ($M \pm m$). When checking the statistical hypotheses, null hypothesis was rejected at significance level less than 0.05. The reliability of differences of averages of independent samples was evaluated using Student's t-test by U. Gosset. The degree of correlation between pairs of independent signs was evaluated by Pearson's coefficient of correlation - r, which reliability was determined by comparing the calculated value of r with critical ones.

Results

According to the results of liver biopsy in patients with ASH, the histological features of portal hypertension have been revealed, even despite the absence of any cirrhotic signs. Liver biopsy have found the significant polymorphic cellular infiltration of portal tracts in these patients, that made up nearly a third of their volume. Here also was observed the hyperplasia of veins, swelling and sclerosis of stroma. Inflammatory lymphocytic infiltrates localized in portal tracts and in the center of hepatic lobules. Peryportal single, graduated or bridge-like portal-central necrosis of hepatocytes with focal or diffuse lymphoid-cell infiltration manifested somewhere.

EGD results revealed signs of the I-st stage of portal hypertension in 85,7% patients of 1st study group – rare blue veins, with diameter up to 2-3 mm, placed at the level of mucous membrane. Endoscopic signs of II-III stage of portal hypertension were determined in all patients of 2nd study group.

At ultrasonography in patients of 1-st group polymorphic Echographic picture was determined: the parenchymal structure was diffusely heterogeneous (foci of heterogeneity without clear contours); echogenicity was increased evenly or unevenly; the size of the liver was increased not only by right, but also left parts; the capsule differentiated less bright than normal; lower edge rounded, obtuse angles; sound conductivity was reduced; "thinning" or poor visualization of the diaphragm contour. The contours of liver were smooth and clear. In contrast, in patient with LC ultrasound visualized inequality of liver contours, a significant increase in its size and foci of parenchymal heterogeneity were clearly defined boundary. There was also the phenomenon of "dorsal attenuation" and "clipped" vascular network. All patients have had splenomegaly, but there were not any signs of ascites.

Assessment of Doppler examination data revealed that in patients of the 1st group Vlin. parameters of portal vein were increased for 16.6% with a slight increase in its diameter compared with controls ($p < 0.05$). Q in portal vein was higher than normal value for 27,4 %. The diameter of vena lienalis and Q in vena lienalis were in the upper limit of normal. CI was slightly increased, but the difference with control was not significant statistically ($p > 0,05$). PSVI was decreased for 13 %, but HVI and SVI were increased for 14,2 % and 18,7 % correspondently ($p < 0,05$). Besides, ultrasound examination visualized such additional signs of portal hypertension: porto-systemic collaterals in 9 (33,3 %) patients; thickening of gallbladder wall – in 20 (74 %); moderate splenomegaly – in 11 (40,7 %) patients. Amongst 2nd group it was registered reliable increasing of PV diameter (for 35,3 %) and increasing of VL diameter (for 52,5 %); grows of CI for 21,2 %, grows of HVI – for 28,9 % and SVI – for 45,5 %; also – decreasing of Vlin. parameters in portal vein comparing with 1-st group (48,1 %) and control group (for 31,5 %) ($p < 0,05$). Q in portal vein was depressed (for 38,3% comparing with 1-st group and for 11,3% comparing with control group ($p < 0,05$)) and Q in vena lienalis increased (for 52,2% comparing with 1-st and control groups ($p < 0,05$)). In 100% of examined patients porto-systemic and spleen-renal collaterals have been visualized in 32,5% cases we've observed a steady thickening of vascular walls of portal vein branches.

In patients of the 1-st group it was revealed significant decreasing of NO level ($p < 0,05$) comparing with control (for 27,4 %). In patients of the 2nd group it was revealed much more significant decreasing of NO level, comparing with 1-st (for 25,7 %) and control groups (for 46,9 %) ($p < 0,05$). Similar trends were recorded also in the investigation of hemorheological properties of the endothelium: reliable decreasing of SFA: in the 1-st group - for 25,1 % comparing with controls ($p < 0,05$), in the 2nd group – for 39,5 % and for 19,2 % comparing with controls and 1-st group correspondently ($p < 0,05$). The data of NFA and EFA in the 1- st group were less, than in control group (26,9 % and 24,6 % correspondently) ($p < 0,05$), in the 2nd group – they were less, than in control group (45,8 % and 37,6 % correspondently) and less, than in 1-st group (25,8 % and 14,5 % correspondently). In the patients of 1-st group ATIII level and vWF level changed relative to the control group slightly ($p > 0,05$). In the patients of 2nd group ATIII level was decreased (for 3,6 % and for 3,1 % relative to the control and 1-st groups correspondently) and vWF level was increased for 19,3 % and for 15,9 % relative to the control and 1-st groups correspondently) (table 2).

Doppler examination of the brachial artery patients of the 1-st group have revealed decreased EDVD - $7,3 \pm 0,18\%$ (against $12,9 \pm 0,22\%$ in controls ($p < 0,05$)). Amongst 82 % patients of the 2nd group EDVD was only $5,4 \pm 0,15\%$, what is significantly less, than in 1-st and control groups ($p < 0,05$). Amongst 18 % patients of 2nd group during conducting of test for reactive hyperemia it was registered the paradoxical vasoconstrictive reaction, indicating a more expressed

endothelial dysfunction. The analysis of the data determined the availability of reverse correlation between the degree of portal hypertension and the level of NO in blood ($r=0.87$) and between the degree of portal hypertension and EDVD index ($r=0.54$) with a high degree of probability, indicating the important pathogenetic role of endothelial dysfunction in the development and progression of portal hypertension.

Summarizing the results obtained in the research process, it should be noted that in all examined patients with chronic alcoholic steatohepatitis EGD and Doppler signs of preclinical stages of hyperkinetic type of portal hypertension were observed. Absence of cirrhotic changes of liver tissue in these patients is confirmed by histopathological study of liver biopsies.

The presence of initial signs of portal hypertension in examined patients is consistent with recent literature data, describing hemodynamic mechanisms of increased pressure in the portal vein system on the background of chronic inflammatory diseases of the liver at the pre-cirrhotic stage [7; 9; 12].

The reasons for the endothelial dysfunction progression in patients with chronic hepatitis are: systemic inflammatory reaction, dysbiosis and endotoxemia, violation of metabolic liver function. They form a closed pathological system, the main target of which is vascular endothelium, including there sinusoids of liver reticuloendothelial system.

Thus, endothelial dysfunction accompanied with NO deficiency is characterized by violation of endothelium-dependent relaxation of blood vessels and by increasing of endothelium adhesivity, what ultimately leads to spasm, thrombosis, formation of liver tissue hypoxia and progression of fibrosis, increased pressure in the portal vein system [13-16].

Endothelial dysfunction may be an independent cause of poor circulation in the tissue as often provokes angiospasm or thrombosis of blood vessels (that, in fact, observed in some forms of ischemic heart disease). On the other hand, violation of regional blood flow (ischemia, venous congestion) can also lead to endothelial dysfunction. On the other hand, violation of the regional blood flow (ischemia, venous congestion) can also lead to the endothelial dysfunction [17].

The question about the place of nitrositive stress in the pathogenesis of chronic inflammatory diseases remains controversial: is it a cause or a consequence of the of endothelial dysfunction formation. Clarification of this point is fundamental to understanding and further study of chronic pathological processes.

Conclusion

There are manifestations of endothelial dysfunction in patients with portal hypertension, which have been developed on the ASH background. The gradual increasing severity of portal hypertension is observed in the same moment with the deterioration of the endothelium functional condition. The imbalance of endothelium-dependent vasoactive substances is an important part of potentiating of hepatic hemodynamic failure and formation of high pressure in the portal vein system in such patients.

Prospects for further investigations is the search for medications to correct endothelial dysfunction in order to improve results of treatment of patients with portal hypertension on the chronic alcoholic steatohepatitis background.

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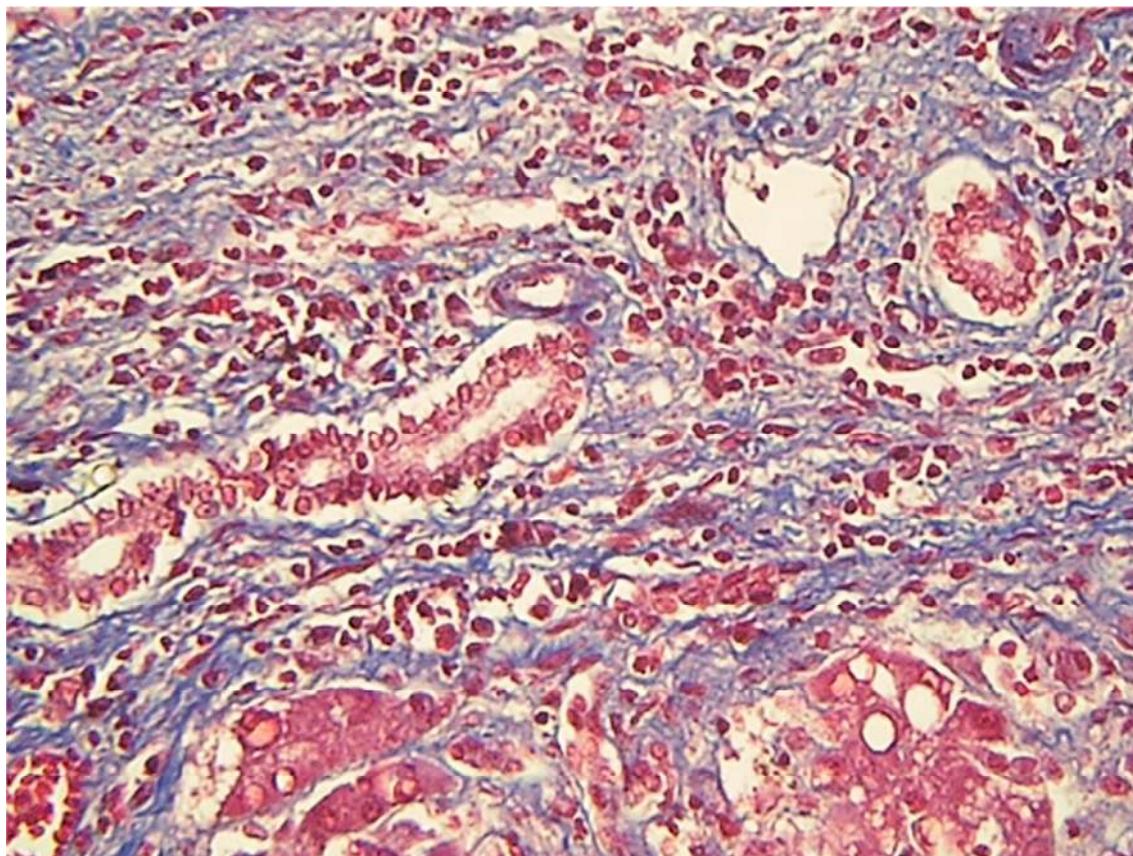


Figure 1: Patient R.P.M., 44 y., Case history № 3463. Alcoholic steatohepatitis. Fragment of the portal tract. Expressed edema and lymphocytic infiltration. Coloring with water blue chromotrope by N. Z. Slinchenko method. Ob. 40^x. Oc. 10^x.

Table 1: Groups characteristics

Group	Total amount	Gender		Age, years	Duration of the disease
		Male	Female		
Control group	62	45	17	40±2,5	8,3±5,5
Study group 1	63	44	19	41±3,5	8,7±4,2
Study group 2	31	21	10	40±4,5	3,2±1,5

Table 2: The indicators of functional activity of endothelium in the examined patients

The indicators	Groups		
	Control group, n=62	Study group 1, n=63	Study group 2, n=31
NO, mmol/l	15.25±0,29	11.07±0.33*	8.22±0.43*/**
AT III, %	95.12±0,37	94.64±0.33	91.70±0.70*/**
vWF, %	102.0±0,76	104.92±1.15	121.64±2.28*/**
SFA mcg asofybrine/1 ml /hour	11.15±0,23	8.35±0.23*	6.75±0.16*/**
NFA mcg asofybrine/1 ml /hour	2.49±0,11	1.82±0.05*	1,35±0.09*/**
EFA mcg asofybrine/1 ml /hour	8.66±0,13	6.53±0.14*	5.40±0.12*/**

Notes:

* - The difference is credible compared to the control group;

** - The difference is credible compared to the first group.

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