Oxidative stress as one of the pathogenetic mechanisms of hepatopulmonary syndrome in patients with liver cirrhosis


Abstract. Background. Hepatopulmonary syndrome is one of the most dangerous syndromes in case of liver cirrhosis. Consequently, it is important to determine the role of oxidative stress, acid-base balance and ventilation-perfusion disorders as factors of hepatopulmonary syndrome development in cirrhotic patients. Materials and methods. We included 44 cirrhotic patients with hepatopulmonary syndrome verified according to the patented Method for diagnosing degrees of hepatopulmonary syndrome severity in patients with liver cirrhosis. In addition to the traditional examinations, we determined the gas composition parameters in venous blood, acid-base balance parameters, catalase activity and malondialdehyde level in all the patients. The received material was processed on a personal computer using Excel 2010, Statistica 6.0, RStudio v. 1.1.422 and R Commander v. 2.4-4. Results. Analysis of blood gas parameters revealed the reliability of the difference for PCO₂ (p = 0.03) depending on the class of liver cirrhosis severity. Also, with the liver cirrhosis severity increase, the malondialdehyde level increased, and catalase decreased. Moreover, significant inverse relationships between malondialdehyde content and PCO₂ (p = 0.039), HCO₃ (p = 0.039), TCO₂ (p = 0.036), Beb (p = 0.049), BEecf (p = 0.043) were found resulting in hypocapnia and partially compensated metabolic acidosis. The found direct correlation between malondialdehyde level and AaDO₂ (p = 0.044) indicates the arteriovenous pulmonary shunts, ventilation-perfusion disorders. The absence of catalase content changes can obviously be explained by the fact that its activity can be partially compensatory maintained. Conclusions. In patients with hepatopulmonary syndrome, the peroxidation activity enhances with an increase in the liver cirrhosis severity, resulting in the redox homeostasis imbalance, leading to the ventilation-perfusion disorders and partially compensated metabolic acidosis. Keywords: liver cirrhosis; hepatopulmonary syndrome; intrapulmonary vasodilatation; oxidative stress; metabolic acidosis

Introduction

Liver cirrhosis (LC) is a chronic diffuse disease of the liver with a structural rearrangement of its parenchyma in the form of nodular transformation and fibrosis due to disruption of the exchange between the hepatic sinusoids (lined with endothelium located on the space of Disse’s connective tissue, which hepatic stellate cells have, the activity of which increases under the influence of reactive oxygen species (ROS) and other metabolically active substances) [1, 2] and the adjacent liver parenchyma (hepatocytes located on the other side of the space of Disse), which leads to liver cell necrosis, the appearance of shunts between the portal and central veins bypassing hepatocytes with the portal hypertension development and liver failure. Physiologically in the body, there is a balance between the content of free radicals and the activity of the antioxidant defense system, because cells have the effective systems to control the content of intracellular ROS. Since the liver is an organ with very intensive metabolic and synthetic functions, it is one of the first to be exposed to ROS [1]. According to research results, hepatopulmonary syndrome (HPS) is one of the most threatening syndromes in cirrhotic patients, occurring in 4.0–47.0 % of cases [3, 4].

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It is characterized by the uncoordinated ventilation perfusion with impaired arterial oxygenation, caused by the intrapulmonary vascular dilatation [3, 5, 6]. The vascular component is characterized by diffusely or locally dilated pulmonary capillaries and, less often, pleural, and pulmonary arteriovenous anastomoses. As a result of a low ventilation-perfusion ratio caused by capillary dilatation (ventilation with excessive perfusion) and anatomical shunting through direct arteriovenous shunts (perfusion without ventilation), severe hypoxemia occurs, causing the reduction of the life quality and its duration in cirrhotic patients. Taking into account the unfavorable prognosis and high mortality in LC and HPS (2 times higher as compared to patients with LC without HPS) [7], it is necessary to verify the links of pathogenesis, in particular, one of the most important, but fragmentarily studied — the state of redox homeostasis, ventilation-perfusion relations, and violations of acid-base balance (ABB), to optimize the treatment according to the obtained results.

**The purpose** was to find out the role of the oxidative stress influence, disturbances of acid-base balance and ventilation-perfusion relations as factors of the hepatopulmonary syndrome occurrence in patients with liver cirrhosis.

**Materials and methods**

After receiving the written consent, approved by the local bioethics committee, to conduct the examination in accordance with the principles of the Declaration of Helsinki of Human Rights, the Convention of the Council of Europe on Human Rights and Biomedicine, and the relevant laws of Ukraine, 1,713 patients with preliminary stratification by the J.G. Turcotte — R.N. Pugh score (class A included 12, class B — 17, class C — 15 people) [8, 9]. The inclusion criteria were the presence of LC and HPS in the liver; standard bicarbonate (SBC), which evaluates the content of HCO₃ in the blood in standard conditions; base excess in blood (BE); pH of blood (pH); respiratory index (RI) as one of the indicators of multiple organ failure; and values of PO₂ and PCO₂ adjusted for temperature (PO₂ t and PCO₂ t, respectively).

The following indicators were used to assess the state of the buffer systems and determine the cause of the shift in the ABB: bicarbonate (HCO₃), which makes it possible to estimate the real content of bicarbonates in the patient’s blood serum; standard bicarbonate (SBC), which evaluates the content of HCO₃ in the blood in standard conditions; base excess in blood (BE); pH of blood (pH); respiratory index (RI) as one of the indicators of multiple organ failure; and values of PO₂ and PCO₂ adjusted for temperature (PO₂ t and PCO₂ t, respectively).

Indicators of redox homeostasis were determined by the activity of catalase (CAT), which converts hydrogen peroxide (H₂O₂) into water and molecular oxygen and is one of the most important “protective” substances of the body against oxidative stress, using a technique based on the ability of H₂O₂ to form a stable colored complex with molybdenum salts. The intensity of the color of molybdenum peroxide compounds depends on the amount of H₂O₂ in the solution. CAT, decomposing H₂O₂, reduces the color intensity in the sample [6].

In order to determine the content of thiobarbituric acid products, among which the most common one is malondialdehyde (MDA), formed in the process of enzymatic lipid peroxidation (LPO), mostly arachidonic and linoleic acids, under the influence of ROS, a technique based on the activation of LPO by ferrous ions (Fe²⁺) to the amount that is registered spectrophotometrically was used. At high temperature in an acidic medium, MDA reacts with 2-thiobarbituric acid, forming a colored trimethine complex with an absorption maximum at 532 nm [10].

The actual material was processed on a personal computer in Excel 2010, Statistica 6.0, RStudio v. 1.1.442 and R Commander v. 2.4-4 using descriptive statistics. Each parameter was tested for distribution normality. To compare samples with a normal (Gaussian) distribution, the Student’s t-test was used, comparison of three groups was made by ANOVA (with Tukey’s post hoc). To compare samples with a non-normal (non-Gaussian) distribution, Mann-Whitney criterion was used, comparison of three groups was made by Kruskal-Wallis (with Dunn’s post hoc). To identify and evaluate the relationships between quantitative indicators, a correlation analysis was performed using the parametric correlation method with the determination of Pearson linear correlation coefficient (R (P)) in case of a normal distribu-

Gastroenterologia, ISSN 2308-2097 (print), ISSN 2518-7880 (online) Vol. 58, No. 1, 2024
A correlation between MDA content and pH, PO\(_2\), and decompensation, depriving the organism of protection against the harmful effects of ROS (Table 2).

Evaluation of the redox system state demonstrated that in LC patients with a serum increase in MDA, the content of CAT decreases, which is accompanied by an increase in the LC severity according to Child-Turcotte-Pugh criteria. This confirms the strengthening of the oxidative stress effect on the body that at the stage of LC compensation slightly stimulates the activation of the antioxidant protection system, followed by its gradual suppression at the stages of LC sub- and decompensation, depriving the organism of protection against the harmful effects of ROS (Table 2).

Table 2 shows the correlations between the content of CAT, MDA, and parameters of blood gas composition.

According to the results, we did not find statistically significant correlations between the content of CAT and blood gas composition parameters (p > 0.05). For SBC, BB correlations with MDA are close to statistically significant (0.05 < p < 0.06), i.e., with an increase in MDA, there was a downward trend in the content of the body’s buffer systems. Perhaps, with a larger sample, these connections would also become significant. A significant inverse relationship was observed between MDA and pH, PO\(_2\), PCO\(_2\), and BB in LC patients with a serum increase in MDA, the content of CAT decreases, which is accompanied by an increase in the LC severity according to Child-Turcotte-Pugh criteria. This confirms the strengthening of the oxidative stress effect on the body that at the stage of LC compensation slightly stimulates the activation of the antioxidant protection system, followed by its gradual suppression at the stages of LC sub- and decompensation, depriving the organism of protection against the harmful effects of ROS (Table 2).

Table 3 shows the correlations between the content of catalse, malondialdehyde and blood gas composition parameters.
found between MDA and PCO₂ (R = −0.32, p = 0.039), HCO₃⁻ (R = −0.32, p = 0.039), TCO₂ (R = −0.33, p = 0.036), Beb (R = −0.31, p = 0.049), BEcef (R = −0.32, p = 0.043).

A statistical significance was revealed between an increase in MDA content, and therefore an increase in oxidative stress and the venous blood ABB parameters, with the occurrence of hypocapnia, partially compensated metabolic acidosis, and a decrease in the level of base excess in the blood and extracellular fluid.

We found a direct correlation between MDA and AAo2 (R = 0.31; p = 0.044), so with the MDA increase, AAo2 also increases, indicating the presence of arteriovenous pulmonary shunts, violation of ventilation-perfusion relations associated with oxidative stress.

**Discussion**

The results of this study provide further evidence that oxidative stress plays an important role in the pathogenesis of liver cirrhosis [10] and HPS in particular. In our study, cirrhotic patients with HPS demonstrated significantly higher levels of oxidative stress biomarkers such as malondialdehyde. This can be explained by the activation of lipid peroxidation processes due to an increase in the content of free radicals caused by the impaired liver function and tissue damage due to cirrhosis, tissue hypoxia that occurs in case of HPS, and therefore insufficient activation of the body’s antioxidant system to neutralize prooxidants in response to these changes due to a severity disease increase. The absence of changes in the content of catalase in cirrhotic patients with HPS can obviously be explained by the fact that the activity of catalase may not change, especially in the early stages of LC, since this enzyme is also contained in erythrocytes and its activity is compensatory maintained. So, if there is still no complete loss of liver function, the basal enzyme content can be preserved.

There are several potential mechanisms by which oxidative stress may promote the development of HPS. Oxidative stress can directly damage endothelial cells lining the pulmonary vascular system, disrupting normal barrier function, and increasing vascular permeability [11]. Reactive oxygen species can also impair nitric oxide bioavailability and signaling, leading to dysregulated vasodilation and angiogenesis [12]. The systemic inflammation in chronic liver disease further exacerbates oxidative stress and reactive oxygen species generation [13—18]. This suggests that an oxidant-antioxidant imbalance leading to excessive oxidative stress is an important contributor to the intrapulmonary vascular dilatations [19] and impaired gas exchange characteristic of HPS.

To confirm the role of oxidative stress, future studies could evaluate whether targeted antioxidant therapies improve clinical, functional, and hemodynamic parameters in HPS patients. Interventions to reduce oxidative stress such as antioxidants may represent a promising therapeutic approach [15, 16, 20, 21], so their optimal timing, dosage, and specific regimens remain to be elucidated.

In summary, the current study adds to the growing body of evidence implicating oxidative stress as a key driver of HPS development and progression in cirrhotic patients. Controlling excessive oxidative damage through lifestyle, pharmacologic, or other interventions represents a promising avenue to explore in order to prevent and treat this devastating complication of end-stage liver disease. Unraveling the precise molecular links between oxidative stress and the pulmonary vascular pathology of HPS, their determination should be a priority for future basic science and translational investigation.

**Conclusions**

In cirrhotic patients with HPS, there is an intensification of peroxidation with an increase in the cirrhosis class according to the Child–Turcotte–Pugh score, which causes the redox system imbalance in this category of patients, leading to the disorder of ventilation-perfusion relations and the occurrence of partially compensated metabolic acidosis, which requires mandatory correction of redox homeostasis and respiratory disorders.

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Accepted 25.02.2024