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NH Virstiuk
SHEI "Ivano-Frankovsk National Medical University", Ivano-Frankovsk, Ukraine Europe

IM Kobitovych
SHEI "Ivano-Frankovsk National Medical University", Ivano-Frankovsk, Ukraine Europe

Correspondence
NH Virstiuk
SHEI "Ivano-Frankovsk National Medical University", Ivano-Frankovsk, Ukraine Europe

Influence of asymmetric dimethylarginine, hypoxia of inducible factor 1 alpha and connective tissue growth factor on the course of alcoholic cirrhosis of the liver against the background of exacerbation of chronic bronchitis

NH Virstiuk and IM Kobitovych

Abstract
In patients with alcoholic liver cirrhosis a there was an increase of Asymmetric dimethylarginine, hypoxia induce factor -1α and connective tissue growth factor in the blood, especially in the exacerbation of chronic bronhitis, indicating an increase in endothelial dysfunction, hypoxia and activation of fibro- and angiogenesis in the background of increased inflammation. The positive correlations were detected between Asymmetric dimethylarginine, hypoxia induce factor -1α and the prognostic indeces ADC-MELD, CLIF-CACLF, CLIF-SOFA; they indicate their unfavourable effect on the alcoholic liver cirrhosis course and the threat of acute on chronic liver failure formation.

Keywords: Liver cirrhosis, chronic bronchitis, asymmetric dimethylarginine, hypoxia, inductive factor 1 alpha, connective tissue growth factor.

1. Introduction
In recent years hepatology has undergone significant qualitative changes, the concept of etiology and pathomorphological processes in the liver parenchyma is expanding, first of all it concerns inflammation and fibrogenesis and syndromes associated with them, in particular, syndrome of portal hypertension (PH) in liver cirrhosis (LC) of different etiology. Most of the severe complications of alcoholic liver cirrhosis (ALC) are related to PH, an increase of hepatic vascular resistance to portal blood flow is in the basis of its pathogenesis [1].
Endotoxemia in cirrhosis directly or indirectly through the cytokine cascade stimulates NOS of vascular endothelium, increasing NO production. Endothelial dysfunction – is a violation of normal endothelial functions caused by an imbalance of vasoconstriction and vasodilatation, pro- and anticoagulation, growth-stimulating and inhibiting factors. In ALC, the endothelium dysfunction is manifested by the inhibition of the endothelium of dependent vasodilation within the microcirculatory unit of the liver [2].
Dimethylarginines are formed during cellular proteolysis and methylation of nuclear proteins. Asymmetric dimethylarginine (ADMA) plays an important role as an endogenous nitric oxide synthase inhibitor (NOS) and is mainly metabolized in the liver by dimethylarginine dimethylamino hydrolase (DDAH). The role of the liver in the regulation of ADMA levels in plasma in the chronic disease of the liver is currently being actively studied [3].
Hypoxia – is an important mechanism in the formation of endothelial dysfunction, which lies at the basis of the progression of fibrosis and plays a role in the neovascularization of the cirrhotic altered liver. Pro-inflammatory mediators produced by Kupffer cells, mast cells, and leukocytes may exhibit angiogenic response by induction and increased transcriptional activity of hypoxia inducible factor 1alpha (HIF1α), which mediates the determining physiological response to hypoxia.
The connective tissue growth factor (CTGF) plays an important role in many biological processes, including cell adhesion, migration, proliferation, angiogenesis, and fibrogenesis [4]. In particular, under experimental conditions, it has been proven that CTGF mediates TGF-β-induced collagen synthesis and induces angiogenesis [5]. In the induction of immune-mediated systemic angiogenesis in PH, an important role is played by oxidative stress. A further increase in the expression level of the connective tissue growth factor (CTGF) may increase the synthesis of extracellular matrix proteins, in particular type I collagen, and a decrease in.

the level of the MMP/TIMP complex (metalloproteinase/tissue inhibitor of metalloproteinase) contributes to the decrease of the degree of degradation of extracellular matrix proteins. This leads to significant histological changes in the liver vessels. The thickness of their walls decreases in the same way as the ratio of the thickness of the medial layer to the lumen of the vessel. Elastic fibers lose their orderly location, and well-expressed collagenous fibers become narrower and divided due to the enlargement of the cell placental matrix in the interstitial medial layer with a significant decrease in the number of smooth cells [6, 7]. Similar violations occur in the mesentery resistive arteries. This contributes to the excessive NO-mediated permeability and process of angiogenesis due to the high expression level of eNOS and vascular-endothelial factor in the microvessels located here [8].

Aim of the work: to evaluate the influence of symmetric dimethylarginine, connective tissue growth factor and hypoxia of inducible factor 1α at the course of alcoholic cirrhosis of the liver against the background of exacerbation of chronic bronchitis.

2. Materials and methods of the study

Objects of the research were 123 patients with ALC, Class B and C according to the Child-Pugh classification; 56 cases were without combination with chronic bronchitis (CB) (group I), 67 – with CB combination in the exacerbation phase, which at the outpatient stage lasted more than 2 weeks (group II). The control group involved 20 conditionally healthy persons of the same age and sex.

Diagnosis of ALC was established according to the Adapted clinical guidelines "Alcoholic liver disease" (2014) and to the protocol of medical aid on the specialty "Alcoholic hepatitis" (Order of the Ministry of Health of Ukraine № 826 dated November 6, 2014). CB in the phase of infectious exacerbation was diagnosed on the basis of anamnesis of the disease, objective examination, chest radiography, spirometry, general blood analysis, sputum analysis, pulse oximetry with the exception of chronic obstructive pulmonary disease (COPD) from the diagnosis, according to the Unified clinical protocol of the primary, secondary (specialized), tertiary (highly specialized) medical aid and medical rehabilitation "Chronic Obstructive Pulmonary Disease" (Order of the Ministry of Health of Ukraine № 555 dated June 27, 2013).

The criteria for not including into the study were: LC of the viral, toxic and metabolic genesis; chronic hepatitis, steatosis of various genesis; metabolic liver diseases; decompensated somatic pathology, oncological and lymphoproliferative diseases, bronchial asthma; COPD; the presence of chronic pulmonary heart; absence of individual consent of the patient to participate in the study.

Patients were performed a general clinical examination (analysis of complaints, anamnesis of disease and life, objective status, general blood and urine analysis, biochemical blood test), ultrasound examination of the abdominal cavity, electro- and echocardiography. The degree of severity of the LC was estimated by Child-Pugh criteria [9, 10]. To stratify the ALC's severity in a single patient, the Maddrey's discriminant function and MELD index (Mayo Endstage Liver Disease, 2001) (1989) (MDF) [11] was calculated. The diagnosis of multiple organ failure was performed in accordance with the recommendations of the CLIF Consortium (European Foundation for the study of chronic liver failure), the European and American associations of liver study [10, 12] with calculation of CLIF-C ACLF (Acute-on-Chronic Liver Failure), CLIF-AD ACLF (Acute decompensation Score) and CLIF-SOFA (score insufficiency of internal organs in case of chronic liver failure) of indices using electronic calculators.

The level of ADMA in blood serum was determined by means of the set of reagents ADMA ELISA Kit "Immundiagnostik" K7828 (Germany); level of CTGF – with ELISA Kit "ADIPO BIOSCIENCE CTGF" (31-2000), (USA); the level of HIF1α – reagents ELISA Kit for Hypoxia Inducible Factor 1 Alpha (USCN Life Science Inc.), (USA), the level of the TNFα – reagents ELISA Kit Diameb (A-8756) (France), the level of C-reactive protein (CRP) – reagents ELISA Kit "Monobind Inc.", (USA), using the immune enzyme method.

The statistical processing of the obtained results was carried out using the software – Microsoft Excel spreadsheet and application software package Statistica v. 10.0 (StatSoft, USA). The statistical significance of the differences between the mean values was estimated using Student's t-criterion.

3. Results of the study

It was determined that in patients with ALC in combination with exacerbation of CB there was a more severe course of the disease, in particular, the MELD index increased by 34.79% ($p<0.05$), MDF index – by 28.55% ($p<0.05$) compared to patients of group I ($p<0.05$), (*Table 1*).

Table 1: Stratification of the severity of alcoholic cirrhosis of the liverdepending on the presence of chronic bronchitis exacerbation

Groups of patients with ALC / Prognostic indices	I group, n=56	II group, n=67
MELD index (points)	11.68±0.47	15.34±0.75•
MDF index (points)	22.73±1.81	29.22±2.24•
CLIF-SOFA (points)	6.79±0.91	12.46±0.83•
CLIF-CACLF calculator (points)	7.76±0.84	12.09±0.84•
CLIF-ADACLF calculator (points)	42.57±2.34	49.34±2.37•

Note: • - reliability of the difference in the indices in the group II compared with the group I, $p<0.05$.

An increase of the level of CRP 19.01-fold compared with the control and 2.08-fold – compared with the rate of patients in group I ($p<0.05$) and an increase of the level of pro-inflammatory cytokine TNF-α – 4.30-fold compared with the control and 1.80-fold comparable to the rate of patients in group I ($p<0.05$) was testified by activation of inflammation in patients with ALC in the exacerbation of CB (*Table 2*).

Table 2: Indices of endothelial dysfunction in blood serum in patients with alcoholic cirrhosis of the liver on the background of exacerbation of chronic bronchitis, (M±m)

Indices (M±m)	ALC, n=56	ALC and CB, n=67	Control, n=20
CRP, mg/l	6,23±0,15*	12,93±2,07*/●	0,68±0,01
TNFα, pg/ml	37,36±1,18*	87.21±3.34*/●	20.29±1.26
ADMA,mmol/l	2.572±0.017*	3.108±0.051*/●	0.461±0.012
HIF-1α, pg/ml	8.27±0.12*	15.34±1.22*/●	4.33±0.07
CTGF, ng/ml	38.22±1.86*	49.27±2.05*/●	19.11±1.07

Notes: * – reliability of the differences in the indices in groups I and II compared with healthy ones, $p<0.05$;
● – reliability of the difference in the indices in group II compared with the group I, $p<0.05$.

According to the results of the study, it was found that in patients with ALC, the content of ADMA in blood serum was increased 5.58-fold ($p<0.05$) compared with healthy ones, which may indicate an excessive blockage of the eNOS synthesis and prevalence of vasospasm. Patients in group II with exacerbation of CB showed a deepening of counterbalance of vasoconstriction, which is shown by an increase of the concentration of ADMA 6.74-fold ($p<0.05$) compared with the control and 1.2-fold ($p<0.05$) – compared with the group I.

It was determined that in patients with ALC, the level of HIF-1α was increased 1.9-fold compared to healthy ones ($p<0.05$), which, in our opinion, corresponds to the essence of the disease, which is accompanied by hypoxia. In patients of group II, the level of HIF-1α was 3.54 times higher ($p<0.05$) than that of healthy subjects and 1.85-fold ($p<0.05$) – compared with patients in group I, indicating an increase of hypoxia degree in patients with ALC in the context of exacerbation of CB. In our view, a more pronounced increase in HIF-1α in patients of group II is due to a decrease in partial pressure and blood oxygenation in the exacerbation of CB and, as a result, more severe hypoxemia.

The serum level of CTGF in examined patients with ALC was 2.0 times higher ($p<0.05$) compared to healthy ones. In patients of group II, an increase of the level of CTGF was observed in comparison with the control group – 2.58-fold, and in comparison with the patients in group I – 1.29-fold ($p<0.05$).

On the basis of statistical analysis, correlations between markers of inflammatory process and endothelial dysfunction in patients of groups I and II, in particular, between ADMA and TNF-α ($r=+0.52$; $r=+0.69$ correspondingly; $p<0.05$), hypoxia – HIF-1α and TNF-α ($r=+0.58$; $r=+0.75$; respectively, $p<0.05$); activation of fibro- and angiogenesis – CTGF and TNF-α ($r=+0.46$; $r=+0.63$; respectively; $p<0.05$), indicating an increase in the degree of endothelial dysfunction, hypoxia, and more pronounced activation of fibro- and angiogenesis on the background of exacerbation of the inflammatory process. Also, the correlation between ADMA and HIF-1α ($r=+0.77$; $p<0.05$), ADMA and CTGF ($r=+0.62$; $p<0.05$), indicating an increase of endothelial dysfunction with increasing hypoxia, activating fibro- and angiogenesis.

The negative influence of ADMA on the ALC course was determined by the detected correlations between ADMA and the predictive indices of the ALC course – MELD, CLIF-CACLF, CLIF-SOFA indexes ($r=+0.53$; $r=+0.65$; $r=+0.61$ respectively, $p<0.05$), which may be due to the prevalence of factors of vasospasm intensification and vascular overtone against the background of systemic inflammation in the exacerbation of CB. The increase in hypoxia with an increase of HIF-1α was accompanied by a deterioration of the ALC prognosis for the detected correlations between HIF-1α and MELD, CLIF-CACLF, CLIF-SOFA indexes ($r=+0.41$; $r=+0.47$; $r=+0.53$ respectively, $p<0.05$). The correlations between CTGF and MELD, CLIF-CACLF, CLIF-SOFA indexes ($r=+0.34$; $r=+0.41$; $r=+0.38$, $p<0.05$) were less pronounced; thus, CTGF influenced the course through the growth of endothelial dysfunction.

Thus, it is the activation of the inflammatory process, the strengthening of endothelial dysfunction and hypoxia in patients with ALC in the context of exacerbation of CB can lead to pathophysiological effects that can contribute to the progression of ALC and the formation of acute on chronic liver failure (ACLF). In turn, increase of the level of HIF-1α can contribute to the progression of fibrosis and the growth of endothelial dysfunction.

4. Conclusions

1. In patients with ALC there was an increase of ADMA, HIF-1α and CTGF in the blood, especially in the exacerbation of CB, indicating an increase in endothelial dysfunction, hypoxia and activation of fibro- and angiogenesis in the background of increased inflammation. 2. The positive correlations were detected between ADMA, HIF-1α and the prognostic indeces ADC-MELD, CLIF-CACLF, CLIF-SOFA; they indicate their unfavourable effect on the ALC course and the threat of ACLF formation. 3. Correlations between the CTGF and the predictive indexes MELD, CLIF-CACLF, CLIF-SOFA were less pronounced, obviously, CTGF has the ability to influence the course of ALC due to an increase of endothelial dysfunction, the correlation between CTGF and ADMA was ($r=+0.62$; $p<0.05$).

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