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Original paper

Analysis of some immune system indicators in patients with liver cirrhosis

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Abstract Nowadays, a significant health problem across the globe is represented by chronic liver diseases including liver cirrhosis. Cirrhosis is characterized by the development of chronic liver injury with necrosis of liver cells followed by fibrosis and nodule formation. TNF-α is an inflammatory cytokine involved in liver inflammation and sustained liver inflammation leads to liver fibrosis. Its activity is increased in such cases and is generally believed to be associated with cirrhosis. According to our results, levels of cytokines such as IL-4 and TNF-α may indicate a more aggressive development of liver cirrhosis in patients from older age groups._The most favorable course of the disease was observed in viral and non-alcoholic fatty liver cirrhoses._ The prognosis for patients with autoimmune liver cirrhosis was the worst. The data shown here may open the prospect for the possible use of TNF-α and IL-4 as predictors of the course of viral hepatitis.

Keywords Liver disease, inflammation, cirrhosis.

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Introduction

The frequency of cirrhotic lesions of the liver is significantly increased every decade therefore the development of liver fibrosis and cirrhosis is a significant problem of modern medicine (MANOJ [1]). This is associated with a rise of the incidence of hepatitis triggered by infection with hepatitis B, C and D viruses (HBV, HCV, HDV). According to statistics, chronic viral hepatitis is diagnosed in 5% of the world's population leading to cirrhosis in 30% of the cases(BABAK & al [2]).

The second leading etiological factor of liver fibrosis and cirrhosis development is alcohol consumption (VERHELST & al [3], EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER [4]). According to statistics, 13,000 people of 1000000 of the human population is diagnosed with alcoholic liver damage, which in 40% of cases lead to the formation of liver cirrhosis. In some patients, cirrhosis is formed as a result of non-alcoholic steatohepatitis and autoimmune hepatitis (EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER [4]). Fundamental research conducted in recent years has found that fibrogenesis processes are crucial for the progression of chronic diffuse hepatic disease. The pathogenesis of the liver cirrhosis formation and its progression include several factors such as hepatocytes necrosis and progressive fibrosis wherein the liver cells are damaged due to the direct effects of etiological factors on them as well as products of cellular necrosis and inflammation.

An important link in fibrogenesis is the cytokine activation. Nowadays the role of the cytokine system in hepatology has been studied profoundly. The importance of cytokines as regulators of the inflammatory and fibrogenic response of the human body to damaging factors, to the condition of hepatocytes and their living cycle has been proven (EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER [4], HERBEIN [5]). Herewith the greatest significance is given to the duration of maintaining the increased level of pro-inflammatory cytokines and their relationship with the anti-inflammatory ones (HERBEIN [5]). The main representative of the proinflammatory cytokines is TNF-α, which stipulate the fibrogenesis by releasing active oxygen from the mitochondria, cytochrome oxidase and inducing apoptosis (EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER [4], HERBEIN [5]].

The process of excessive activation of macrophages is inhibited by anti-inflammatory cytokines, in particular IL-4. They control the regulation of proinflammatory cytokine products (EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER [4]).

Therefore, the purpose of the research was studying the cytokine profile in patients with cirrhosis of the liver of different etiologies.

Materials and Methods

The study included 101 patients with liver cirrhosis and 20 healthy individuals (control group) aged (56.0±1.1) years. Among them, there were 61 male patients and 40 females. A clinical and laboratory examination was conducted. Alcoholic etiology of cirrhosis was observed in the majority of patients (51.5%), while viral – in 32.7%. Among patients with viral cirrhosis, the hepatitis C virus was the most commonly reported infection and detected in 25 people (81.8%). Non-alcoholic fatty liver disease was noted in 12.8% of patients, and cirrhosis with autoimmune etiology was observed in 3.0% of them.

Selected patients were without comorbid pathology, HIV infection; and have been suffering with cirrhosis for not more than 2 years after the diagnosis was taken. Patients with viral cirrhosis were included in the study in the absence of markers of viral infection.

The diagnosis of liver cirrhosis was proved by the Los Angeles Classification (1994). The verification of the diagnosis was performed by anamnestic and clinical features, the results of biochemical blood analyses, and ultrasonic examination of the liver, by the presence of serum markers of hepatitis B and C according to unified methods approved by the Ministry of Health of Ukraine. The concentration of TNF-α and IL-4 cytokines were determined by ELISA using StatFax303Plus analyzer.

Results and Discussions

An imbalance in the cytokine system was detected in patients with liver cirrhosis. The level of pro-inflammatory cytokine TNF-α in the blood serum had increased in all patients (100%) and compiled (39,05±4.79) pg/ml, however, a relative insufficiency of anti-inflammatory cytokine IL-4 had been noted. It had decreased in most patients (98.0%) to (2.61 ±0.58) pg / ml in comparison with control group ($p < 0.05$). The inverse correlation between pro-and anti-inflammatory cytokines levels is TNF-α and IL-4 had been detected ($r = -0.475$; $p < 0.05$).

Data on analyses of cytokines concentration in the patients' blood sera of genders had shown more profound changes in women with liver cirrhosis than in males. The probable differences were recorded in TNF-α or IL-4, which, apparently, indicates a more conductive course of liver cirrhosis in female (Table 1).

Table 1. An evaluation of serum level of TNF-α and IL-4 in patients with the liver cirrhosis according to their gender, pg/ml

Cytokine	**Patients**			
	control group, n=20	**with liver cirrhosis, n=101**	**male, n=61**	**female, n=40**
TNF-α	1,5±0,09	38,18±4,52*	43,62±7,27*	32,65±0,17*
IL-4	17,10±1,12	2,61 ±0,58*	2,03±0,65*	3,42±0,14*

1. * – p<0,05

In the analysis of indicators in patients of different age groups, a significant increase in the concentration of TNF-α with age was revealed (Table 2). In patients aged 45-59 and over 60 years, it had exceeded that in patients under 45 years 1.5 times (p <0.05). IL-4 concentration tended to increase in patients in the younger group. Such levels of pro- and anti-inflammatory cytokines may indicate a more aggressive development of liver cirrhosis in patients of older age groups.

Table 2. Cytokines in patients of different age groups, pg/ml

Cytokine	Control group, n=20	With liver cirrhosis, n=101	25-44-year-old, n=13	45-59-year-old, n=49	Over 60 years, n=38
TNF-α	1,5±0,09	38,18±4,52*	26,72±1,58*	41,39±4,58*	40,55±5,04*
IL-4	17,10±1,12	2,61±0,58*	2,84±0,19*	2,44±0,43*	2,75±0,37*

* –p<0,05

Patients with liver cirrhosis of different etiology experienced a significant increase in TNF-α level, along with a decrease in IL-4 compared to the control group. Comparative analysis showed a significantly lower level of TNF-α in non-alcoholic fatty liver cirrhosis (p <0.05), with no significant differences in the other etiological groups (p> 0.05). The level of anti-inflammatory IL-4 was significantly reduced in autoimmune cirrhosis relative to the remaining etiologic groups (p <0.05) (Table 3).

Table 3. The level of cytokines in patients with liver cirrhosis of different etiology, pg/ml

Cytokine	**Control group, n=20**	**Alcoholic liver cirrhosis, n=52**	**Viral liver cirrhosis, n=33**	**Non-alcoholic fatty liver cirrhosis, n=13**	**Autoimmune liver cirrhosis, n=3**
TNF-α	1,5±0,09	45,29±7,95*	31,83±3,98*	27,94±4,99*/**	45,30±5,12*/#
IL-4	17,10±1,12	2,26 ±0,71*	3,23±0,21*	2,40±0,41*	0,80±0,12*/**/***/#

* - the difference is likely compared with the indicator in the control group (p <0.05);
** - the difference is likely compared with the indicator in patients with alcoholic liver cirrhosis (p <0,05);
*** - the difference is likely compared with the indicator in patients with viral liver cirrhosis (p <0,05);
# - the difference is likely compared with the rate in patients with non-alcoholic fatty liver cirrhosis (p <0.05).

The concentration of pro- and anti-inflammatory cytokines in different patient groups were quantified separately. The following values were set: at alcoholic liver cirrhosis – 20.0; viral liver cirrhosis – 9.9; non-alcoholic fatty liver cirrhosis – 11.6; autoimmune liver cirrhosis – 56.6.

By obtained data, the conclusion was done that the most favorable course of the disease in viral cirrhosis (given that the study included patients with minimal activity of disease) and non-alcoholic fatty liver cirrhoses. The prognosis for patients with autoimmune liver cirrhosis had been the worst.

Nowadays the significant health problem across the globe is chronic liver diseases, includes liver cirrhosis. Cirrhosis is characterized by the development of chronic liver injury with necrosis of liver cells followed by fibrosis and nodule formation. TNF-α is an inflammatory cytokine involved in liver inflammation and sustained liver inflammation leads to liver fibrosis. Its activity is increased in such cases and is generally believed to be combined with some complications related to cirrhosis (HERBEIN & al [5], MOURTZIKOU & al [6]).

Thus, an increase of TNF-α was detected in liver cirrhosis similar to other studies (WANG & al [7], ODEH & al [8], ATASEVEN [9]). In the analysis of the TNF-α and IL-4 concentration in representatives of different sex, the probable differences are recorded in the level of these cytokines, which may indicate a milder process of the liver cirrhosis development in women. The increased concentration of TNF-α had associated with the activation of an immune response, on the one hand, due to the development of inflammation, and on another hand, stimulation of fibrogenesis, which corresponded to the pathogenesis of liver cirrhosis (HERBEIN & al [5]).

Analysis of the level of cytokines in patients with sub- and decompensated liver cirrhosis revealed only a tendency to the TNF-α level increasing and IL-4 decreasing with decompensation developing ($p > 0.05$). The ratio of TNF-α level to IL-4 was 14.6 at decompensated liver cirrhosis, and 15.7 in at subcompensated one. These identified differences have opened the prospect for the possible use of these cytokines as predictors of the course of viral hepatitis.

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