ORIGINAL ARTICLE

Tumour necrosis factor-α and interleukin-6 gene expression in liver tissue and blood leukocytes in patients with liver damage – a preliminary study

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Abstract. *Background: Pathophysiologic mechanisms participating in liver fibrosis and cirrhosis development from chronic liver diseases have not been understood yet. Recently there have been many studies of the role of cytokines in this process. The aim of this study was to assess TNF-α and IL-6 gene expression and subsequent cytokine synthesis in human liver tissue and blood leukocytes.*

Patiens and methods: After institutional approval 13 patients (8 male and 5 female) indicated for liver biopsy were examined. The remnant parts of liver tissue obtained primarily for histopathological examination as well blood leukocytes were used for TNF-α and IL-6 gene expression estimation by Real-time PCR, LightCycler Instrument (Roche Diagnostics, Mannheim, Germany). Defined sections of liver tissue from liver biopsy were cultivated with William's medium and then cytokine concentrations (median, pg/ml) were estimated in culture media supernatant and in homogenated liver tissue (Quantikine, R&D Systems, USA). According to histopathological results patients were divided into three groups: group I (n=4), patients with liver cirrhosis, group II (n=5), patients with liver steatosis or other chronic liver disease and group III (n=4), patients with mild or acute hepatic damage.

Results: TNF-α gene expression was detected in one patient with liver cirrhosis. IL-6 gene expression was detected in all patients in group I (with liver cirrhosis). IL-6 and TNF-α gene expression was not demonstrated in leukocytes of venous blood in all groups. There were not significant differences in TNF-α concentration [group I (68.9), II (59.8) and III (48.2)] as well as IL-6 concentration [group I (745), II (572) and III (599)] in supernatants of cultivated liver between groups. TNF-α concentrations in homogenized liver tissue were higher in group I (37.2)

and II (53.8) in comparison with group III (15.6). Similarly IL-6 concentrations in homogenized liver tissue were higher in group I (257) and II (315) in comparison with group III (31.0).

Conclusion: Liver cirrhosis is accompanied by TNF-α and IL-6 gene expression in liver tissue. TNF-α and IL-6 gene expression in human liver tissue is accompanied by not significantly higher TNF-α and IL-6 synthesis in cell culture supernatants. The absence of IL-6 and TNF-α gene expression in leukocytes demonstrated that these processes (liver cirrhosis) influenced cytokine metabolism especially locally.

Key words: *liver, fibrosis, cirrhosis, steatosis, cytokine*

Živný P, Živná H, Bureš J, Bártová J, Hrochová K, Šimáková E, Cyrany J, Palička V. Exprese genu pro tumour necrosis factor-α a IL-6 v jaterní tkáni a v leukocytech periferní krve u pacientů s jaterním onemocněním – předběžné výsledky. Folia Gastroenterol Hepatol 2005; 3 (4): 135 – 143.

Souhrn. *Patofyziologické mechanismy, které se podílejí na vzniku jaterní fibrózy a cirhózy, nejsou dosud zcela objasněny. Recentně se objevila řada studií, zabývajících se rolí cytokinů v těchto procesech. Cílem studie bylo zhodnotit expresi genu pro TNF-α and IL-6 v jaterní tkáni a v leukocytech periferní krve a zhodnotit následnou syntézu těchto cytokinů.*

Pacienti a metody: Po schválení lokální etickou komisí bylo 13 pacientů (8 mužů a 5 žen), indikovaných k jaterní biopsii, zařazeno do studie. Zbytek jaterní tkáně, která byla primárně získána pro histopatologické vyšetření, a leukocyty periferní krve, získané venepunkcí, byly použity pro stanovení exprese genu pro TNF-α and IL-6 pomocí Real-time PCR, LightCycler Instrument (Roche Diagnostics, Mannheim, Germany). Definované vzorky jaterní tkáně z jaterní biopsie byly kultivovány ve Williamsově médiu a následně byly stanoveny koncentrace cytokinů (TNF-α a IL-6, udáno jako medián v pg/ml) v supernatantu kultivačního média a ve vzorku homogenizované jaterní tkáně pomocí setu (Quantikine, R&D Systems, USA). Podle výsledků histopatologických vyšetření byli pacienti rozděleni do tří skupin: skupina I (n=4), pacienti s jaterní cirhózou, skupina II (n=5), pacienti s jaterní steatózou nebo jiným chronickým jaterním onemocněním a skupina III (n=4), pacienti s lehkým (event. lehkým akutním) jaterním poškozením.

Výsledky: V jaterní tkáni byla exprese genu pro TNF-α prokázána u jednoho pacienta s jaterní cirhózou, exprese genu pro IL-6 byla prokázána u všech pacientů skupiny I (s jaterní fibrózou/cirhózou). Exprese genu pro IL-6 and TNF-α nebyly prokázány v leukocytech periferní krve v žádné skupině. Dále nebyly nalezeny signifikantní rozdíly v koncentraci TNF-α [skupina I (68.9), II (59.8) a III (48.2)], podobně jako u IL-6 [skupina I (745), II (572) a III (599)] v supernatantech kultivovaných jater mezi skupinami. Koncentrace TNF-α v homogenizované jaterní tkáni byla vyšší u skupiny I (37.2) a II (53.8) ve srovnání se skupinou III (15.6). Podobně koncentrace IL-6 v homogenizované jaterní tkáni byly vyšší u skupiny I (257) a II (315), ve srovnání se skupinou III (31).

Závěr: U pacientů s jaterní cirhózou byla prokázána exprese genu pro TNF-α and IL-6 v jaterní tkáni. Exprese genu pro TNF-α a IL-6 v lidské jaterní tkáni není doprovázena statisticky významně zvýšenou syntézou těchto cytokinů in vitro. Chybění exprese genu pro IL-6 a TNF-α v leukocytech periferní krve ukazuje, že procesy, které se odehrávají v játrech, nemusí mít žádnou odezvu v celém organismu.

Klíčová slova: *jaterní fibróza, cirhóza, cytokiny*

Liver diseases are relatively very frequent for men. The main aetiological causes are an excessive consumption of alcohol, viral infections or the effect of toxic substances but recently non-alcoholic steatohepatitis/non-alcoholic fatty liver disease (NASH/NAFLD) has often been discussed. Despite the fact that alcohol intake is a common problem throughout the population, NASH/NAFLD is a serious health problem, too.

The aim of this pilot study was to contribute to understanding the role of selected cytokines in variety of liver diseases. Pathophysiologic mechanisms participating in liver fibrosis and cirrhosis development from chronic liver diseases have not been understood yet. Recently there have been many studies of the role of cytokines in this process.

Although NASH obviously represents a special form of lipotoxicity (6), its pathogenesis remains poorly understood. Patients with NASH have increased lipid peroxidation, increased tumour necrosis factor-α concentrations (TNF-α) and increased hepatic mito-

chondrial beta-oxidation rates (22). Hong (12) stated that no standard protocol for treating fatty liver exists at this time and therefore he examined the effect of 10 days interleukin-6 (IL-6) injection in 3 murine models of fatty liver: leptin deficient ob/ob mice, ethanol-fed mice, and mice fed a high fat diet. In all three models, IL-6 injection has decreased steatosis and normalized serum aminotransferase.

Cytokines (TNF-α, IL-6, IL-1), leptin but also growth differentiation factor 3 (GDF-3) or stress-activated mitogen-activated protein kinase – MAPK and other seems to play an important role in the development of liver damage and subsequent reparation processes (11).

Cytokines are most distinguished for their activities associated with inflammation, immune reactivity, tissue injury or repair and organ dysfunction. Evidence for the crucial role of cytokines in the evolution of the inflammatory response has come from the demonstration that certain viruses encode the genetic information of soluble receptors for some cytokines, which are expressed by an infected cell inactivates a particular cytokine and allow the virus to evade the immune system.

However, many cytokines also play important roles in the modulation of normal physiological functions of cells. The functions of cytokines are diversed and clearly include a role in normal physiologic functions and homeostasis. The normal metabolic functions of the liver, including gluconeogenesis, lipid and protein metabolism are affected by cytokines. TNF-α and IL-1β inhibit the activity of bile acid transporters on the biliary canalicular membrane (19).

It is a known fact that the cellular source and biological targets of cytokines are not restricted to cells of the immune system, but endothelial cells, stellate cells, hepatocytes and other cells are capable of producing and responding to a number of different cytokines. Especially the stellate cell plays a central role in the development of hepatic fibrosis and cirrhosis.

The liver is an important organ in the metabolism of cytokines, with the both capacity to produce and to remove cytokines. Hepatic uptake of circulating cytokines is inhibited by alcohol (4) and this may contribute to the elevated levels of TNF-α and IL-6 that are observed in such patients. Moreover ethanol induced IL-8 production in primary hepatocyte cultures (17).

Cytokines TNF-α and IL-6 are reported as main mediators of initial liver fibrosis response (9) and liver cirrhosis development (15).

PATIENTS AND METHODS

Study design

The Local Ethical Committee has approved the protocol of the study. All patients provided a written informed consent. We examined 13 patients (8 men and 5 women) indicated for liver biopsy at the 2nd Department of Internal Medicine, Gastroenterological Unit, University Hospital, Hradec Králové due to biochemical or clinical signs of liver disease (elevated transaminase, γ-glutamyl-transferase and/or bilirubin). We used remnant part of liver tissue obtained primarily for histopathological examination by needle biopsy for:

1) TNF-α and IL-6 gene expression estimation in liver tissue
2) Parts of liver tissue from liver biopsy were cultivated with culture media (William's medium) and then TNF-α and IL-6 concentrations were estimated in culture media supernatant and in homogenated liver tissue.

At the time of liver biopsy blood samples for TNF-α and IL-6 mRNA expression measurement were collected into EDTA Vacutainer (Becton Dickinson, Le Pont de Claix, France).

The results of routine clinical chemistry examination were assessed and all obtained results were confronted with the results of routine histopathological examination.

According to histopathological results irrespective of other results patients were divided into three groups:

Group I (n=4), patients with liver fibrosis or cirrhosis (2 men and 2 women)

Group II (n=5), patients with liver steatosis (4 men and 1 woman)

Group III (n=4), patients with mild acute or subacute hepatic damage with no important changes in liver parenchyma (2 men and 2 women)

Methods

Liver biopsies were done under ultrasonographic guidance using standard Menghini liver biopsy needle.

Liver tissue for histopathological examination was fixed in 10% buffered formalin, embedded in paraffin

and sectioned at 3 μm. The sections were stained with haematoxylin and eosin.

The next obtained samples of liver tissue from liver biopsy were weighted in special test tubes with a culture medium (William's medium). Culture media dosing was 100 μL per 1 mg of wet liver tissue. Cultivation plates were placed into a thermostat box with 5% CO_2 atmosphere. Liver tissue samples were cultivated 24 hours and then cell culture supernatants were stored at -80 °C for later analysis.

Fresh culture medium was added to cultivated liver tissue sample in above mentioned ratio (100 μL per 1 mg of wet liver tissue) and then this material was frozen at -80 °C. Liver samples were repeatedly frozen and de-frosted and then homogenized by glass stick and centrifuge in 1000 r.p.m. The supernatant obtained was frozen at -80 °C until being analysed.

Total RNA was extracted from each specimen (liver, blood leukocytes) according to protocol modification for isolation of RNA from tissue by Qiamp RNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). After that real time RT-PCR was performed with control PCR primers for the housekeeping gene (human beta actin). The expression levels of h-IL-6 and h-TNF-α

Table 1
Patients' demographic data, clinic data and histological description of liver

Group	Sex Age	Clinical diagnosis	Degree of liver damage
I. **Liver cirrhosis**	M 52 year	Ethylic liver cirrhosis Child-Pugh B MELD 15	**Micronodular cirrhosis**
	F 48 year	Acute alcoholic hepatitis on the basis of ethylic liver cirrhosis Child-Pugh C MELD 19	**Severe fibrosis alcoholic steatohepatitis** **Progress to cirrhosis**
	M 20 year	Primary sclerosing cholangitis ulcerative colitis Child-Pugh A MELD 8	**Susp. sclerosing cholangitis** **Severe fibrosis** **Progress to cirrhosis**
	F 47 year	Hepatitis C Positive AMF Positive anti CMV IgM	**Chronic hepatitis** **Severe fibrosis** **Progress to cirrhosis**
II. **Liver steatosis or mild fibrosis**	M 29 year	Primary sclerosing cholangitis Indeterminate colitis	**Mild fibrosis** **Not very expressed acute hepatitis with hepatocyte necrosis**
	M 41 year	Obesity Elevation of functional liver tests	**Mild high-drop steatosis (30 % of liver parenchyma)** **Mallory bodies**
	F 50 year	Febrile status Elevation of functional liver tests	**Steatosis (10-15 % of liver parenchyma)** **Solitary glycogen nuclei**
	M 44 year	Dysfunction of Oddi sphincter Elevation of functional liver tests Analgesics abuse (acetaminophen)	**Mild high-drop steatosis (10 % of liver parenchyma)** **Minimal chronic nonactive inflammatory infiltration**
	M 45 year	Elevation of functional liver tests	**Mild high-drop steatosis** **Normal parenchyma**
III. **Normal liver parenchyma or acute hepatic damage**	M 37 year	Elevation of functional liver tests Positive anti CMV IgM Positive anti EBV IgM	**Normal parenchyma** **Rare high-drop steatosis (< 1 %)**
	F 46 year	Elevation of functional liver tests Hormonal replacement therapy	**Normal parenchyma** **Rare necrosis (drug-induced)**
	M 40 year	Elevation of functional liver tests	**Normal parenchyma** **Mild intracelular cholestasis** **Minimal extracelular cholestasis**
	F 58 year	Elevation of functional liver tests Susp. primary sclerosing cholangitis Positive AMF	**Normal parenchyma** **Small granuloma = susp. incipient primary biliary cirrhosis**

MELD – the Model for End-Stage Liver Disease scoring system
M – male, F – female

genes in liver tissue were determined by quantitative real time-PCR performed on LightCycler instrument (Roche Diagnostics, Mannheim, Germany). The amplicon was detected by fluorescence using the double strain DNA binding dye SybrGreen.

Concentrations of TNF-α and IL-6 were estimated in cell culture supernatants and in homogenized liver tissue resuspended in culture medium after 24 hours cultivation (Quantikine, R&D Systems immunoassay kits, Minneapolis, MN, USA) test sensitivity for TNF-α > 15.6 pg/mL and for IL-6 > 31.2 pg/mL).

Serum alanine-transaminase (ALT), aspartate-transaminase (AST), γ-glutamyl-transferase (GGT) and alcaline phosphatase (ALP) activities (μkat/L), total bilirubin concentration (μmol/L), total protein concentration (g/L), urea concentration (mmol/L) and C-reactive protein (mg/L) concentration were determined in serum using routine kits (Modular Roche Analyser, Mannheim, Germany). Electrophoresis of serum proteins was performed on Paragon analyser (Beckman Coulter Inc, Fullerton, CA, USA).

Statistical evaluations

SigmaStat software (Jandel Scientific Corporation, San Rafael, CA, USA) was used. The tests used were: unpaired t-test, one way ANOVA. The data are presented as median and quartiles (cytokines) and the mean ± S.E.M. (standard error of the mean). Statistical significance was expressed by using the number of signs: one sign = $p<0.05$, two signs = $p<0.01$, three signs = $p<0.001$.

Results

Results of the study are given in Tables 1 – 5. TNF-α gene expression was detected in the only patient with completely developed liver cirrhosis (Group I, M 52 years). IL-6 gene expression was detected in all patients in group I (with histologically proven fibrosis or cirrhosis). IL-6 and TNF-α gene expression was not proved in leukocytes of venous blood in all groups. There were not significant differences in TNF-α as well as IL-6 concentrations in supernatants of cultivated liver among groups (Table 2). TNF-α concentrations in homogenized liver tissue were higher in group I and II in comparison with group III. Similarly IL-6 concentrations in homogenized liver tissue were higher in group I and II in comparison with group III (Table 2).

Serum albumin concentrations were significantly lower in groups I and II in comparison with group III. Relative proportion of γ-globulin in protein electrophoresis was significantly higher in fibrosis/cirrhosis group (group I) in comparison with group II and III (Table 3).

Serum C-reactive protein concentrations were significantly lower in group I in comparison with group II. ALT, AST and GMT activities were lower in group

Table 2
Expression of genes (mRNA quantity in ng/μL) estimation for TNF-α and IL-6 and TNF-α and IL-6 protein concentrations in cultivated liver tissue and in leukocytes of venous blood

	Unit	Group I	Group II	Group III
TNF-α gene expression (mRNA quantity)	**ng/μL**	1.3	0	0
TNF-α concentration in supernatant	**pg/mL**	68.9 53.2-94.0	59.8 51.1-83.4	48.2 35.9-73.3
TNF-α concentration in homogenized liver tissue	**pg/mL**	37.2 37.0-65.2	53.8 44.5-58.6 *	15.6 15.6-26.5 * p< 0.019
TNF-α gene expression (mRNA quantity) in blood leukocyte	**ng/μL**	0	0	0
IL-6 gene expression (mRNA quantity)	**ng/μL**	12.3±1.9	0	0
IL-6 concentration in supernatant	**pg/mL**	745 382-958	572 215-851	599 301-738
IL-6 concentration in homogenized liver tissue	**pg/mL**	257 88-582	315 236-472 **	31 30-32 **p< 0.005
IL-6 gene expression (mRNA quantity) in blood leukocyte	**ng/μL**	0	0	0

Table 3
Selected indicators of liver proteosynthesis (serum proteins and their electrophoresis)

	Unit	Group I	Group II	Group III
S-total protein	**g/L**	77.73±6.59	72.46±2.96	86.65±2.95
S-albumin	**g/L**	36.18±3,43 **	38.78±3,07 +	47.95±0,55 **p < 0.002 +p < 0,026
Protein electrophoresis albumin		0.526±0.015 *	0.551±0.038	0.602±0.016 *(p< 0.012)
α1 globulin		0.024±0.002	0.044±0.012	0.018±0.0004
α2 globulin		0.101±0.009 ***	0.126±0.019 *** p< 0.001	0.109±0.010
β globulin		0.111±0.003 ***	0.128±0.009 *** p< 0.001	0.116±0.010
γ globulin		0.238±0.024 ** ++	0.150±0.014 ++ p < 0.019	0.155±0.011 ** p< 0.024
A/G quotient		1.11±0.07	1.28±0.20	1.51±0.09

Table 4
Other direct and indirect biochemical markers of liver damage (assessed at the time of liver biopsy)

	Unit	Group I	Group II	Group III
S- CRP	**mg/L**	16.0±5,8	55.5±25,2	N/A
S- total bilirubin	**μmol/L**	37.5±26.74	14.67±2.94	12.5±3.5
S – ALT	**μkat/L**	0.66±0.13	1.89±0.68	0.79±0.04
S – AST	**μkat/L**	0.97±0.16	1.02±0.17	0.69±0.18
S - GMT	**μkat/L**	4.97±2.04	8.39±3.38	0.76±0.21
S - ALP	**μkat/L**	2.36±0.73	3.53±1.45	2.50±0.83
S -glucose	**mmol/L**	6.39±0.34	6.48±0.61	5.43±0.37
S - urea	**mmol/L**	3.60±1.12	3.36±0.74	4.75±0.65

Table 5
Biochemical markers of liver damage in the period of maximum activity of the disease

	Unit	Group I	Group II	Group III
Max S - ALT	**μkat/L**	0.89±0.22 *	3.42±1.17 *p < 0.05	1.01±0.18
Max S - AST	**μkat/L**	1.52±0.36	3.31±1.07 *	0.80±0.07 *p < 0.05
Max S - GMT	**μkat/L**	7.76±3.52	14.43±6.24	0.80±0.21
Max S- ALP	**μkat/L**	3.33±0.97	6.35±2.97	2.59±0.74

I comparing to group II (Table 4). The situation was similar in these markers assessed in the period of maximum activity of the disease (assessed by means of biochemical activity of liver damage).

Discussion

This pilot study is only a contribution for understanding of possible development mechanisms of fibrosis and cirrhosis from chronic liver diseases. The high regeneration ability of liver after acute liver diseases in clinical medicine or after partial hepatectomy and simple providing of hepatotoxic substances during the experiment is well known and it has been described many times in literature (10).

The exact causes participating in liver fibrosis and cirrhosis development from chronic liver diseases

have not been understood yet. Recently there have been many studies dealing with the possible role of different signal molecules, especially cytokines in initiating and maintaining of these processes. We used these studies for selecting cytokines where we determined gene expression and their concentration in culture media supernatant and in homogenated liver tissue.

The biological effect of cytokine, such as TNF-α, is dependent on the effective concentration: low concentrations are involved in homeostasis, while an increasing local concentration of TNF-α is associated with local inflammatory response and focal hepatic necrosis, and massive release of TNF-α into the circulation results in systemic activation with adult respiratory distress syndrome and multiple organ failure.

TNFR p55-mediated signals may regulate activation of Kupffer cells and stellate cells and eventually enhance fibrotic processes (13). Kupffer cells, neutrophils, and lymphocytes have the potential to influence stellate cells, on the contrary stellate cells can promote leukocyte chemotaxis and adherence, and they may also influence leukocyte activation by producing other regulatory cytokines (16).

TNF-α participates in liver repair and regeneration and increase of local production of the neutrophil chemoattractant (ENA-78) in the liver (3), directly inducing the expression of TGF-α (8), induce the expression of adhesion molecules on the endothelial surface and on circulating inflammatory cells. The next cause of cytokine elevation is its production within liver. It often depends on the initial induction of early-response cytokines released from tissue-resident macrophages (Kupffer cells). These early-response of proinflammmatory cytokines activate macrophages via an autocrine effect and recruit the stromal cells of the liver (endothelial cells, stellate cells, hepatocytes) to principate in the inflammatory response (paracrine effect) by inducing expression of cytokines and chemokines by these cells (16, 25).

Reactive oxygen intermediates are commonly produced by hepatocytes during the metabolism of free fatty acids (FFA), ethanol, paracetamol and a variety of other drugs. Free radicals can activate the transcription factor, nuclear factor-kappa B (NF-kB), which can induce the transcription of a variety of cytokines and chemokines (24). Platelet-activating factor (PAF) and LPS activate nuclear factor-kappa B in hepatocytes, Kupffer cells, and neutrophils, a key transcription factor for TNF-α and cytokine-induced neutrophil chemoattractant (CINC) (23).

Elevated circulating TNF-α or IL-1, has been observed in patients with alcoholic liver disease, especially those who are malnourished, and correlate with survival rate (18). Epidermal growth factor (EGF), transforming growth factor alfa (TGF-α) and hepatocyte growth factor (HGF) are completed hepatocyte mitogens, but recent studies have suggested a central role of TNF-α in hepatic regeneration following both toxic damage and 70% hepatectomy (1).

The role of IL-6 in liver fibrosis and cirrhosis development has not been made clear yet. Natsume (21) demonstrated participation of IL-6 in the onset of liver fibrosis, IL-6 gene expression was enhanced in liver and immunoreactive IL-6 was detected in infiltrating inflammatory cells.

The synthesis of positive acute phase proteins is induced by IL-6, while the production of negative acute phase proteins is inhibited (20). IL-1β, IL-4 and IL-6 can also modulate stellate cell collagen and other cytokine synthesis. Recent reports have correlated the degree of mast cells infiltration with the severity of hepatic fibrosis (5).

Several negative immune modulators exist, including anti-inflammatory cytokines (e.g. IL-10 and IL-4), soluble cytokine receptors (soluble TNF receptors) and cytokine antagonists (IL-1 receptor antagonist). IL-10, for example, down-regulates endotoxin-mediated IL-6 release from Kupffer and sinusoidal endothelial cells (14). However, not all soluble cytokine receptors are inhibitory: soluble IL-6R can induce IL-6 effects on cells (7).

Non-parenchymal cells play an important role in the production of inhibitory factors for liver regeneration after resection of the liver. The termination of liver regeneration is regulated by anti-inflammatory cytokine IL-10, which is released by the spleen and liver and might downregulate a TNF-α production, thereby inhibiting the liver regeneration after partial hepatectomy in rats (26).

The examined patients were divided into 3 groups according to histological results of severity (grade) of liver damage. The patients with mild hepatic damage were placed in the 3rd group. This concerned a simple acute liver damage of different aetiology followed by very good reparation. Proteosynthesis was not

decreased in the liver, the ALT, AST and GMT activities were returning to normal concentrations. The expression level of TNF-α – even the IL-6 genes was not proved in this group. The cytokines concentration gained after 24 hours cultivation of the liver tissue samples was the lowest one. Both cytokines were releasing in higher concentrations into the culture media while very low concentrations were found in the intracellular way. We might assume that the both cytokines in these concentrations were essential for fulfilling their physiological functions. They may have been used as regulators of reparation and finishing of regeneration of liver tissue damage.

In the second group – the patients with liver steatosis – there was one case attached with mild fibrosis, the ALT, AST and GMT elevation had chronic character, inflammatory indicator was increased – C-reactive protein (the connection with liver diseases is however questionable). The TNF-α and the IL-6 concentrations were increased as in homogenized liver tissue as in culture media supernatant. We may speculatively conclude that enhanced regeneration effort was processing in the liver.

In the first group – patients with proved heavy fibrosis or cirrhosis, the ALT, AST and GMT activities were lower than with patients from the second group (with liver steatosis). The TNF-α and the IL-6 concentrations were increased as in homogenized liver tissue as in culture media supernatant. The expression of the gene for IL-6 in liver tissue was demonstrated in all patients in this group and the expression of the gene for TNF-α was demonstrated in the case of one patient. However, the expression levels of IL-6 and TNF-α genes were not demonstrated in leukocytes of venous blood in all groups. This finding confirms that it concerns the processes, which are proceeding in liver but not in entire organism.

Our results related to many authors' studies that also dealt with the role of cytokines in initiating and maintaining of fibrosis reaction in chronic liver tissue damage. Most authors put the TNF-α in direct connection with initiating of fibrotic processes. For example, Cao et al. (2) found increased concentrations of the IL-6 and the TNF-α in T-cells of rats to which the ethanol was served in a chronical way. Lin (15) indicates that the activation of the whole TNF-α system, together with the leptin increase, leads to malnutrition in the patients with liver cirrhosis. The interferon-γ plays a crucial role in the protection of the patients infected with *Schistosoma mansoni* against periportal fibrosis, while the TNF-α makes this process worse (9). The TNFR p55-mediated signal leading to activation of Kupffer cells, stellate cells and to direction of fibrotic processes (13). Wang et al. (27) mention in their studies that total concentrations of collagen, hyaluronate and laminin as well as a number of the TNF-α positive cells are gradually on the increase from the patients with mild chronic hepatitis ranging to the patients with severe chronic hepatitis and there is the highest level in patients with liver cirrhosis. These cells are localized mostly at periportal areas.

IL-6 is mentioned, in connection with the inflammatory and regenerate processes, in literature as well as with processes immediately related to initiation of fibrosis. The expression level of IL-6 gene was observed in the liver of mice and immunoreactive IL-6 was detected in infiltrating inflammatory cells at chronic intermittent administration of carbon tetrachloride. The fibrotic changes were also lower in IL-6 deficient mice during tetrachlormethane administration. The authors summarize that IL-6 can participate in fibrotic changes but at the same time in maintaining serum concentrations of albumin (21). The DNA synthesis, after partial hepatectomy, appeared to be lower in IL-6 deficient mice in comparison with the inspections. The IL-6 seems to take share in recruiting of hepatocytes into the process of cell mitosis and their synchronization during the cell division.

We found that the expression level of IL-6 gene happened in patients with histologically proved fibrosis and cirrhosis. It is also undisputable that IL-6 is included into the process of regulation of DNA synthesis and regeneration of the remaining hepatocytes in such damaged liver.

Limitations of the study

One essential limitation of this study is the low number of patients and relatively heterogeneous groups and following unreliable statistical analyses. Taking this fact into account the basic information in this study had a methodical character. Further studies will be needed.

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