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HIGH SENSITIVITY C-REACTIVE PROTEIN AS PREDICTION FACTOR OF DISEASE PROGRESSION IN PATIENTS WITH CHRONIC HEPATITIS C AND MILD LIVER STEATOSIS

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Chronic hepatitis C (CHC) is a major cause of liver cirrhosis and hepatocellular carcinoma. Steatosis occurs in almost 50% of patients with CHC, who make a faster progression to cirrhosis. The "atypical " patients are registred too, with normal values of blood sugar, cholesterol, triglycerides, BMI, body weight having non-alcoholic steatohepatitis (NASH) and CHC. The hsCRP levels rise before and simultaneously with the chronic hepatitis and cirrhosis progression, therefore, it is a useful prognostic parameter. According to our knowledge, there are no sufficient data concerning hsCRP concentrations in CHC patients, although it predicts or detects different grades of cirrhosis. For that reason, the aim of our research was to assess the hsCRP in patients with CHC.

The investigation involved 45 patients (28 males and 17 females), mean age 41±15 years, with CHC, without any accompanying disease. The control group consisted of 45 healthy volunteers (22 males, 23 females), mean age 34±10 years. The CHC patients' group was divided into two subgroups, the first, which consisted of 23 patients with evidenced histological signs of mild steatosis, and the second one, comprising 22 patients without the mentioned signs; hsCRP concentrations were measured in each patients' (sub)group.

The findings indicate that the hsCRP value had a statistically significant increase in the CHC patients' group compared to the control group ($p<0.05$). In the CHC and mild liver steatosis patients subgroup, even more statistically significant hsCRP increase occured compared to the other subgroup ($p<0.001$).

It can be concluded, based on the acquired results, that hsCRP should be considered as a CHC progression prognostic factor, in order to make a well-timed and special therapeutic approach to the CHC individuals even more prone to the disease progression. *Acta Medica Medianae 2010;49(3):14-18.*

***Key words**: chronic hepatitis C, high sensitivity C-reactive protein, liver steatosis*

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Introduction

Hepatitis C virus (HCV) is a leader in inducing chronic liver disease worldwide. Chronic hepatitis C (CHC) is a major cause of liver cirrhosis and hepatocellular carcinoma. The HCV infection characterizes by its predisposition to chronicity. Because of its high genetic variability, HCV has the ability of escaping the host's immune response. This virus is not directly cytopathic and the liver damage is mostly a consequence of immune-mediated mechanisms, predominantly of the T helper cells. Co-factors influencing the disease outcome include age, gender, alcohol consumption. Recent studies have shown that the combined peginterpheron and ribavirin therapy leads to a sustained virologic response in half of the CHC patients, on an average (1,2).

C-reactive protein (CRP) is a non-specific marker of inflammation and a predictor of a coronary heart disease, cardiovascular disorders, subclinical vascular diseases. Available cognizances presume that moderately increased CRP directs attention to elevated risk of myocardial infarction. The CRP concentration is affected by: body mass index (BMI), diabetes, hypertension, cigarette smoking, estrogen replacement therapy, as well as the presence of rheumatoid arthritis, chronic lung diseases, acute viral infections. High sensitivity CRP (hsCRP) has been even more often proposed for heart diseases risk evaluation, for the purpose of preventive procedures (3).

Although not being a specific biomarker, its concentrations rise fast as a response to tissue damage, infections and other inflammatory phenomena, such as autoimmune diseases and malignances (4).

Higher sensitivity of hsCRP unlike the "usual" CRP is reflected through lower minimal normal value as well as lower range of reference values (from 0 to 1 mg/l, matched with the "usual" hsCRP, with 0 to 5 mg/l), so its predictive ability is much more accurate concerning future cardiovascular pathologic events in basically healthy individuals (5). Its use is particularly frequent in the pediatric population (6-8).

Levels of CRP raise parallelly with the chronic liver disease progression, such as chronic hepatitis and liver cirrhosis, as well as before the progression beginning, therefore it is a useful prognostic parameter (9). It is interesting that hsCRP concentrations decrease linearly with the serum bilirubin increase. Also, there was noticed a coherence of increased hsCRP concentrations with the non-alcoholic steatohepatitis (NASH), frequent CHC accompanist (10). Patients with the active form of the NASH (grade 2-3) have even more increased hsCRP concentrations compared to those with the stagnant form (grade 1 or the simple steatosis), so hsCRP could be a promising biomarker for screening the NASH (11).

According to our knowledge, there are not sufficient data concerning hsCRP concentration in CHC patients, although it predicts emergence or the existence of NASH. From that reason, measuring hsCRP concentration in CHC patients is the aim of our study.

Patients and methodology

The study was carried out on 45 patients (28 males and 17 females), mean age 41±15 years, with chronic HCV infection, selected among the patients anamnestically and clinically examined in the Infectious Disease Clinic of Clinical Centre Niš. Diagnosis of the CHC was established on the basis of elevated amino-transferases serum activity, positive anti-HCV antibodies (the third generation ELISA, Bioelisa ELx800 Biokit, Spain) and presence of serum HCV-RNA (PCR testing), and histological proof of chronic hepatitis. Patients with the additional diseases (acute, chronic or autoimmune, neither of the liver nor of the other organ systems) were excluded of the reserch (the attention was particularly addressed to potential hsCRP increase including disorders), after the detailed clinical and laboratory examinations, apart from the CHC patients subgroup with the mild steatosis, patho-histologically evaluated. Only the patients with no pathohistologically diagnosed signs of liver fibrosis nor cirrhosis were enrolled into the study. The control group consisted of 45 healthy volunteers (22 males, 23 females), mean age 34±10 years, selected after the general systematic examination, who were seronegative of HCV, HBV, HIV, HbsAg, total anti-HBc antibodies and anti-HCV testing, without the previous history of hepatitis or the chronic alcoholism, with no laboratory results abnormalities including liver function testing. All the patients and the control group individuals signed the inform consent. The aspartat aminotransferase (AST) and the alanin aminotransferase activity were determined by a ready-made test of Ellitech Company, on the biochemical analyzer BTS-370 (BioSystems). The values of enzyme activity were expressed in U/l. The measurment of hsCRP was performed by a commercial test of the Dade Behring company, on the analyzer Dimension Expand. The values were expressed in mg/l. In further investigation, the CHC patients' group was divided into the two subgroups, the first, which consisted of 23 patients with the presence of mild steatosis histological signs, ant the second, which included 22 patients without the above mentioned signs; so hsCRP concentrations were determined on both subgroups.

The statistical analysis was performed by SPSS (Statistical Package for the Social Sciences Program) for Windows, version 11,0, whereby the results were presented in tables. The Students' t-test was applied on account of comparing the mean values of the laboratory parameters of the CHC patients and the control group, as well as the two CHC patients subgroup. A value of $p<0.05$ was considered statistically significant.

Results

The obtained results demonstrated that hsCRP values were significantly increased in the CHC patients' group compared to the control group ($p<0.05$) (Table 1). As it was expected, the enzymes AST and ALT activity values had a statistically significant increase in the CHC patients compared to the controls. The CHC and liver steatosis including subgroup had an even more statistically significant hsCRP rise compared to no histologically proven liver steatosis subgroup ($p<0.001$) (Table 2).

Table 1. Concentrations of hsCRP and plasma AST and ALT activity in the CHC patients and control group

	CHC patients (N=45)	Control group (N=45)
hsCRP (mg/l)	3.44±0.52 *	0.11±0.09
AST (U/l)	79.43±16.32 ***	20.34±6.03
ALT (U/l)	96.97±23.57 ***	19.49±5.92

Data are shown as mean±SD. *p<0.05; ***p<0.001

Table 2. Concentrations of hsCRP in plasma of CHC patients with and without presence of mild liver steatosis

	CHC patients with mild steatosis (N=23)	CHC patients without mild steatosis (N=22)
hsCRP (mg/l)	6.26±1.09 ***	0.57±0.13

Data are shown as mean±SD. ***p<0. 001

Discussion

Not enough of adequate data concerning hsCRP in CHC patients could be found in literature recently. Our research confirmed the presumption that hsCRP, as an inflammatory marker, is also increased in CHC, being an inflammatory liver disease. The result of increased hsCRP concentration in pathohistologically proven mild steatosis without any accompanying clinical and laboratory indicators of itself, was expected, too. The mentioned results pointed out the eventual significance of measuring hsCRP in CHC patients, in the sense of being a prediction factor, and well-timed detecting of "atypical" patients with potentially increased risk of disease progression.

Although a mild steatosis per se is usually benign, patients with histologically proven NASH can make a progression towards liver cirrhosis. Steatosis occurs in almost 50% of HCV infected patients. Liver steatosis in HCV infection facilitates the mentioned disease progression. A significant fibrosis was noticed in HCV infected patients, who were confirmed a liver steatosis or NASH by liver biopsy (12). The presence of liver steatosis correlates with the fibrosis grade in CHC patients, which emphasizes the importance of the phenomena (13).

After the complex multivariate analyses, there was noticed an occurrence of normal blood levels of glucose, triglycerides, and other indicators of NASH, in patients with NASH and CHC (14). Also, even the patients without signs of insulin resistance, with low cholesterol and triglycerides levels, and having BMI and body weight in frames of normal values, could develop NASH (15, 16).

From the above mentioned studies, one could have concluded that even the "atypical" patients (with unnoticeable clinical and laboratory finding) may develop liver steatosis, which leads CHC patients to the further disease progression. Therefore, a diagnostic parameter for anticipating liver steatosis emergence is needed, in order to have a well-timed and timely initiated therapy, especially for not considering liver biopsy anymore as inevitable diagnostic procedure (17).

Hepatitis C is still a serious progressive chronic liver disease, despite advancements in therapy methods. Viral afctors and host characteristics are related to progression towards fibrosis,cirrhosis and therapy response. Steatosis is for a long time regarded as a frequent histological finding in hepatitis C. Recent evidence propose steatosis in hepatitis C as a non-accidental finding, which could be a consequence of various cytopathic effects, synergistic mechanisms of viral proteins with the host metabolism of the lipids and factors related to steatosis or steatohepatitis in NASH: visceral obesity, diabetes type 2, insulin resistence. Steatosis increases the incidence of fibrosis towards cirrhosis progression (18).

In CHC, the prevalence of steatosis is two and a half times increased than expected, compared to non-infected individuals, which confirms that HCV participates in its development. In some studies it has been reported (although there are some who stand against) that NASH occurs more often in HCV patients with genotype 1 and increased BMI, but also with genotype 3 and increased viral load. Anyway, patients with increased progression grade of NASH have less chances to have a positive interferon therapy response (19). In patients with CHC, NASH and normal BMI, there is a frequent confusion in the interpretation of the examination results and the proper diagnosis establishing; in the meanwhile, patient's health condition is getting worse (20).

C-reactive protein plays an important role in inflammatory processes connected with metabolic syndrome, which is more often associated with individuals with CHC than HCV negative population (21).

High sensitivity CRP could be used in the beginning as a detector of the liver inflammatory response in patients with the metabolic syndrome. Limitation of the sensitivity and the specificity of such testing could be annulled by identifying, i.e. defining various grades of hepatic inflammation (22).

In our case, circumstances of CHC presence in patients without other accompanying disorders are facilitating easier reclining on hsCRP concentrations measuring. Some researches give information of significant steatosis diminishing in patients with the sustained virologic response to the therapy. On the other hand, the recurrence of steatosis in some patients is registered after the HCV infection relapse. The presence of fatty infiltrations in CHC patients is a usual finding during the ultra-sound examination, although the definite diagnosis is ungrateful, because the displayed signs of the "bright liver" are often present in hepatic fibrosis and inflammation. On the other hand, laboratory analysis in patients with a chronic course did not always correlate with the evolution of the disease, when the clinical course is mostly mild (23). Pathohistological finding is the most accurate, but the liver biopsy is not infallibly indicated in all the patients, neither all of them give their consents for the procedure (24-27).

Conclusion

High sensitivity CRP should be considered as a CHC progression factor of prediction, having in mind statistically significant increase of its concentrations in CHC patients compared to the healthy population, and in patients with the liver steatosis in comparison with the ones without the steatosis, within the CHC population.

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VISOKO SENZITIVNI C-REAKTIVNI PROTEIN KAO PREDIKTORNI FAKTOR PROGRESIJE BOLESTI KOD BOLESNIKA SA HRONIČNIM HEPATITISOM C I BLAGOM STEATOZOM JETRE

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Hronični hepatitis C (HHC) je glavni uzrok ciroze jetre i hepatocelularnog karcinoma. Steatoza je prisutna kod skoro 50% bolesnika sa HHC i oni brže progrediraju ka cirozi. Registrovani su i "atipični" bolesnici, normalnih vrednosti glukoze, holesterola i triglicerida u krvi, BMI, telesne mase sa nealkoholnim steatohepatitisom (NASH) i HHC. Nivoi hsCRP rastu pre i paralelno sa progresijom hroničnog hepatitisa i ciroze jetre, te je koristan prognostički parameter. Prema našim saznanjima, nema dovoljno podataka o koncentraciji hsCRP kod belesnika sa HHC, iako predviđa ili detektuje različite stepene steatoze. Iz tog razloga je određivanje koncentracije hsCRP kod bolesnika sa HHC cilj našeg rada.

U istraživanje je uključeno 45 bolesnika (28 muškaraca i 17 žena), prosečne starosti 41±15 godina, sa HHC, bez pridruženih oboljenja. Kontrolnu grupu činilo je 45 zdravih dobrovoljaca (22 muškarca i 23 žene), prosečne starosti 34±10 godina. Grupa bolesnika sa HHC podeljena je u dve podgrupe, prvu, koja se sastojala od 23 bolesnika sa prisutnim histološkim znacima blage steatoze, i drugu, koju su činila 22 bolesnika bez pomenutih znakova; te je kod svih (pod)grupa merena koncentracija hsCRP.

Dobijeni rezultati pokazuju da je vrednost hsCRP statistički značajno povišena u grupi bolesnika sa CHC u poređenju sa kontrolnom grupom ($p<0.05$). Kod podgrupe sa HHC i blagom steatozom jetre došlo je do statistički značajnijeg porasta hsCRP u odnosu na drugu podgrupu ($p<0.001$).

Iz priloženih rezultata može se zaključiti da bi hsCRP trebalo uzeti u obzir kao prognostički faktor progresije HHC, radi posebnog i blagovremenog terapijskog pristupa osobama sa HHC sklonijim progresiji bolesti. *Acta Medica Medianae 2010;49(3):14-18.*

***Ključne reči**: hronični hepatitis C, visoko senzitivni C-reaktivni protein, steatoza jetre*