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Advanced Glycation Endproducts in Serum of Patients with Chronic Hepatitis and Liver Cirrhosis

Późne produkty glikacji w surowicy krwi chorych na przewlekłe wirusowe zapalenie i marskość wątroby

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Abstract

Background. There is a strong epidemiologic relationship between chronic viral hepatitis and the development of cirrhosis, although the cellular and molecular mechanisms of cirrhosis formation remain to be firmly established.
Objectives. Determination of serum levels of advanced glycation end-products (AGEs) in patients with chronic viral hepatitis or liver cirrhosis and examination of their relationship to neopterin, a marker of activation of the cellular immune system and haptoglobin, a marker of fibrosis.
Material and Methods. Concentration of advanced glycation endproducts, haptoglobin and neopterin were determined in patients with chronic viral hepatitis or postinflammatory liver cirrhosis. 69 patients with chronic HBV or HCV infection were divided into four groups: chronic viral hepatitis group; cirrhosis classes A, B and C (Child-Pugh classification) – groups A, B and C, respectively.
Results. Advanced glycation endproducts serum levels were increased in patients with chronic hepatitis and liver cirrhosis (groups A, B, C) as compared to normal subjects ($p < 0.001$). However, no difference was observed between chronic hepatitis and liver cirrhosis groups. Haptoglobin was significantly decreased only in sera of cirrhosis patients as compared to normal subjects or chronic hepatitis patients ($p < 0.001$). Neopterin serum levels were increased in chronic hepatitis patients and cirrhosis patients as compared to healthy subjects ($p < 0.005$ and $p < 0.001$, respectively). However, these levels were significantly higher in the liver cirrhosis group as compared to the chronic hepatitis group ($p < 0.01$). In the cirrhosis group the serum concentrations of neopterin were significantly higher in group C as compared to group B ($p < 0.001$). Interestingly, a significant relationship was observed between neopterin serum content and serum advanced glycation endproducts ($r = 0.30$; $p < 0.05$) in chronic hepatitis patients.
Conclusions. These data suggest that AGEs, neopterin and haptoglobin may be useful as additional biochemical parameters to identify patients with chronic viral hepatitis developing cirrhotic lesions (**Adv Clin Exp Med 2006, 15, 2, 259–264**).

Key words: cirrhosis, chronic hepatitis, advanced glycation endproducts, haptoglobin, neopterin.

Streszczenie

Wprowadzenie. Przewlekłe wirusowe zapalenia oraz związany z nimi rozwój zwłóknienia, a w końcu marskość wątroby są istotnym problemem epidemiologiczno-klinicznym. W związku z tym potrzebne jest znalezienie wskaźników biochemicznych użytecznych w diagnostyce różnicowej, których badanie poszerzyłoby wiedzę o patomechnizmie tych chorób.
Cel pracy. Zbadanie zależności między stężeniem późnych produktów glikacji (AGE) w surowicy chorych na przewlekłe wirusowe zapalenie lub marskość wątroby a stężeniem neopteryny (marker odporności immunologicznej typu komórkowego) i haptoglobiny (wskaźnik biochemiczny zwłóknienia).
Materiał i metody. W surowicy krwi chorych na przewlekłe wirusowe zapalenie lub pozapalną marskość wątroby oznaczono stężenie AGE, haptoglobiny oraz neopteryny. 69 chorych z przewlekłymi zakażeniami HBV lub HCV podzielono na: grupę z przewlekłym zapaleniem wątroby oraz grupy A, B, C z marskością wątroby – zgodnie z zaawansowaniem choroby według kryteriów Childa-Pugh.

Wyniki. Stężenie AGE było istotnie zwiększone w grupie chorych na przewlekłe zapalenie i marskość wątroby (grupa A, B, C) w stosunku do grupy kontrolnej ($p < 0,001$), ale bez istotnych różnic między grupami chorych. Stężenie haptoglobiny w grupie chorych na marskość było istotnie mniejsze w stosunku do grupy kontrolnej oraz do grupy z przewlekłym zapaleniem wątroby ($p < 0,001$), ale nie stwierdzonono istotnej różnicy między badanymi grupami chorych A, B i C. Różnice stężeń neopteryny między grupami B i C były istotne, a u chorych na przewlekłe zapalenie wątroby wartości tego wskaźnika są istotnie mniejsze niż u chorych na marskość wątroby ($p < 0,01$), nawet w stosunku do grupy C ($p < 0,001$). Stwierdzono poza tym dodatnią korelację między wartościami neopteryny i AGE w grupie chorych na przewlekłe zapalenie wątroby ($r = 0,30$; $p < 0,05$).
Wnioski. Wzrost stężenia AGE w surowicy chorych wskazuje na udział tych produktów w patomechanizmie zwłóknienia. Zmiany stężeń AGE, haptoglobiny i neopteryny w surowicy krwi mogą być pomocne w diagnostyce różnicowej przewlekłego wirusowego zapalenia i marskości wątroby oraz w ocenie włóknienia tego narządu, zwłaszcza łącznie z innymi wskaźnikami **(Adv Clin Exp Med 2006, 15, 2, 259–264).**

Słowa kluczowe: marskość wątroby, przewlekłe wirusowe zapalenie wątroby, późne produkty glikacji, haptoglobina, neopteryna.

Chronic lesions of the liver caused by hepatotropic viruses, mainly HBV and HCV, impair the hepatocyte function, progressing accumulation of extracellular matrix components, which both may lead to fibrosis and finally to cirrhotic changes [1]. The analysis of the histopathological biopsy of the liver is essential to differentiate between chronic hepatitis and cirrhosis as well as to determine the stage of disease. However, this is an invasive procedure, which can only be applied to a limited extent due to possible contraindication to perform liver biopsy or potential complications. Therefore, the authors tried to find new diagnostics markers useful in differential liver diagnostics and they performed a research to learn more about the pathomechanism of fibrosis.

At physiological conditions, glucose and other reducing sugars react nonenzymatically with protein amino groups to initiate a post-translational modification process known as nonenzymatic glycosylation. This reaction proceeds from reversible Schiff bases to stable, covalently bonded Amadori rearrangement products. Once formed, the Amadori products undergo further chemical rearrangement reactions to form irreversibly bound advanced glycated endproducts (AGEs) [2]. Slow metabolic turnover of proteins and higher levels of sugar accelerate the glycation process. These conditions may be observed in diabetes, during a long-term dialysotherapy, and in aging. AGEs are considered to play an important role in the pathogenesis of the chronic complications of diabetes mellitus, and atherosclerosis, Alzheimer disease, amyloidosis and nephropathy [3]. AGEs engaged in the pathogenesis of diabetes complications involve endogenic and exogenic AGEs [4].

A chronic damage of the liver affects its sugar metabolism. High levels of sugar and its metabolites can upset the balance of the body's internal chemistry, so that more AGEs are produced. The accumulation of advanced glycation endproducts both in the serum and in tissues may provoke the oxidant stress, the carbonyl stress and other cell destruction processes [1]. Sebekova et al. [5] confirmed an increased AGEs concentration, indicated by measurement of fluorescence and N(epsilon)-carboxymethyllysine (CML) in the serum of patients qualified for a liver transplantation on grounds of cirrhosis (Wilson disease, alcoholic diseases, disorders of unknown origin, primary biliary cirrhosis) and normalization of the AGE concentration after the transplantation.

During the viral hepatitis, monocytes/macrophages are stimulated as part of an immune response to the infecting virus, with neopterin (NPT) as a marker. This activation, beside the hepatocyte damage, is considered to trigger fibrosis during the chronic hepatitis [6]. Neopterin derivatives are produced by human monocyte-derived macrophages and upon stimulation with interferon (gamma). Furthermore, NPT is excreted in an unchanged form *via* the kidneys [7].

Haptoglobin (Hp) is produced mainly by hepatocytes in response to the activity of inflammatory factors (interleukin-1, interleukin-6, tumor necrosis factor – TNF-α) which are generated by macrophages in the damage place. The serum concentration of Hp in patients with hepatitis correlates with the stage of fibrosis confirmed in liver biopsy [8] and it is one of the parameters of the Fibro test developed by the French hepatogastroenterology group [9].

The objective of this study was to compare the fluorescent of AGEs (the AGEs concentrations ratio) in the serum of patients with a chronic hepatitis and patients in a different stage of postinflammatory cirrhosis with AGEs levels in the serum of healthy subjects – to see if they were different. This might point to a significant participation of the AGEs in the pathology of these disorders. The concentrations of neopterin and haptoglobin (as parameters useful in differential diagnosis of liver disease) are measured, and analysed in terms of dependence on the serum concentrations

of AGEs. As mentioned earlier, every measured biochemical parameters may reflect the advancement of liver pathology, yet in a different aspect.

Material and Methods

The authors 69 patients (22 women, 47 men): 23 with confirmed chronic viral hepatitis HBV (9 women, 14 men) aged 17–72 (median 38), 21 with chronic HCV viral hepatitis (8 women, 13 men) aged 24–69 (median 29) and 25 with liver cirrhosis (4 women, 21 men) aged 24–74 (median 30) treated at the Clinic of Infectious Diseases, Liver Diseases and Acquired Immune Deficiency in the Silesian Piasts University of Medicine in Wrocław. The diagnosis of viral chronic hepatitis and liver cirrhosis was based on generally adopted diagnostic criteria: clinical, biochemical, serological and histopathological. The HBV infection was confirmed by measurement of the following infection markers in the blood serum: HbsAg, HbeAg, HbcAb, HbeAb – MEIA methods, Abbotts IMX, HBV DNA – Digene Hybrid Capture. The HCV inflammation was confirmed by measurement of HCV Ab and HCV RNA in the serum, using the EIA methods and RT PCR – Cobas Amplicor Roche methods, respectively. The liver biopsy was performed with the Menghini method, in regional anaesthesia, in all patients (except for the group with advanced cirrhosis – the C stage, according to the Child-Pugh classification) after abdominal ultrasonography.

Patients with postinflammatory liver cirrhosis were divided into three groups: A (n = 10), B (n = 7), C (n = 8) in accordance with the Child-Pugh classification [10]. The consent of Bioethics Committee of the Silesian Piasts University of Medicine in Wrocław was obtained and all patients were informed about the character of analyses made. The control group were 20 healthy subjects (7 women, 13 men) aged 19–56 (median 23.5). Blood samples were collected in the Silesian Centre of Medical Diagnostics in Wrocław. The blood samples of patients and from registered healthy blood donors who served as the control group to examine was taken from the basilic vein. The blood samples were stored at –80°C until the moment of measurement.

AGEs levels were determined in undiluted sera by spectrofluorescent method (the results given in arbitral units) [11]. Haptoglobin serum levels were measured with the guaiacol micromethod [12], and neopterin levels were measured using the commercially available enzyme-linked immunosorbent assay kits (IBL Immuno-Biological Laboratories, Hamburg, Germany).

Data analysis was computed by the Student's *t*-test and Mann-Whitney nonparametric test. The frequency of occurrence of the characteristics in the group was compared by means of the χ^2 test. The Spearman rank correlation test was used to assess the correlation between the parameters examined. The 0.05 level of probability was taken as significant.

Results

Figure 1 presents the mean concentrations of biochemical parameters in the serum, i.e. advanced glycation endproducts, haptoglobin and neopterin in control group and the groups of patients with chronic hepatitis (CH) and liver cirrhosis (LC), as well as statistic analysis. Figure 2 presents the mean values of serum AGEs, Hp and NPT concentrations presented with the variability bands in the groups of studied patients with chronic viral hepatitis and liver cirrhosis.

The serum concentrations of advanced glycation endproducts as measured by spectrofluorometry, were significantly higher in patients with chronic hepatitis and liver cirrhosis as compared to control group ($p < 0.001$; Fig. 2). However, no difference was observed between the LC and CH groups. Mean AGEs serum levels were markedly increased in cirrhotic patients as compared to grade of severity of cirrhosis while no difference was observed between groups A, B and C.

In chronic hepatitis patients mean Hp serum concentrations were normal. In contrast, in the cirrhotic group mean Hp serum concentrations were significantly lower than those measured in control group or chronic hepatitis patients ($p < 0.001$; Fig. 1). Moreover, the Hp serum content was significantly higher in CH group as compared to group: A ($p < 0.005$), B ($p < 0.05$), C ($p < 0.005$), while no difference was observed between groups A, B and C.

Significant increase in neopterin ($p < 0.01$) was evidenced in the serum of 18 patients with chronic hepatitis (41%) and 21 patients with liver cirrhosis (37%). The mean values of NPT increased in patients with cirrhosis according to the stage of the disease (A, B, C) (Fig. 2). The significant difference was observed between the CH and C groups or B and C groups ($p < 0.001$ and $p < 0.05$ respectively).

Substantially higher levels of the NPT were found in the patients with HCV than in those with HBV, but only within the group with chronic hepatitis B ($p < 0.001$).

As presented in Figure 2, the values of the studied parameters varied to a great extent, espe-

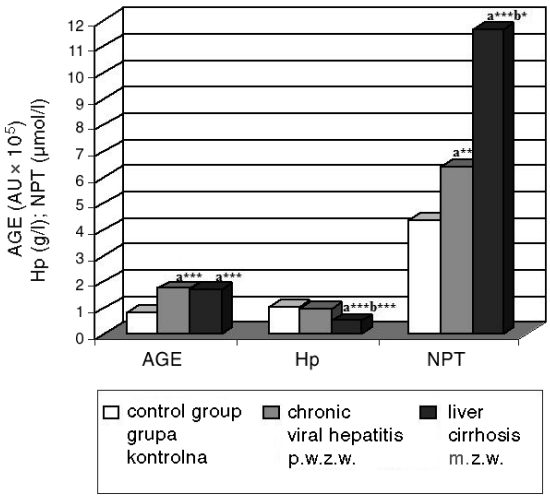

Fig. 1. Mean values of serum advanced glycation endproducts (AGE), haptoglobin (Hp) and neopterin (NPT) concentration in patients with chronic hepatitis and liver cirrhosis as compared to normal subjects. Significance levels for overall among groups; ** $p < 0.005$; *** $p < 0.001$ *vs* normal subjects [a]; *$p < 0.01$; *** $p < 0.001$ *vs* liver cirrhosis[b]

Ryc. 1. Średnie stężenia późnych produktów glikacji (AGE), haptoglobiny (Hp) oraz neopteryny (NPT) w surowicy krwi osób z grupy kontrolnej i u chorych na przewlekłe wirusowe zapalenie wątroby (p.w.z.w.) oraz na marskość wątroby (m.w.). Istotność różnic: a – względem osób zdrowych, b – względem chorych na marskość wątroby; * $p < 0,0l$; ** $p < 0,005$; *** $p < 0,001$

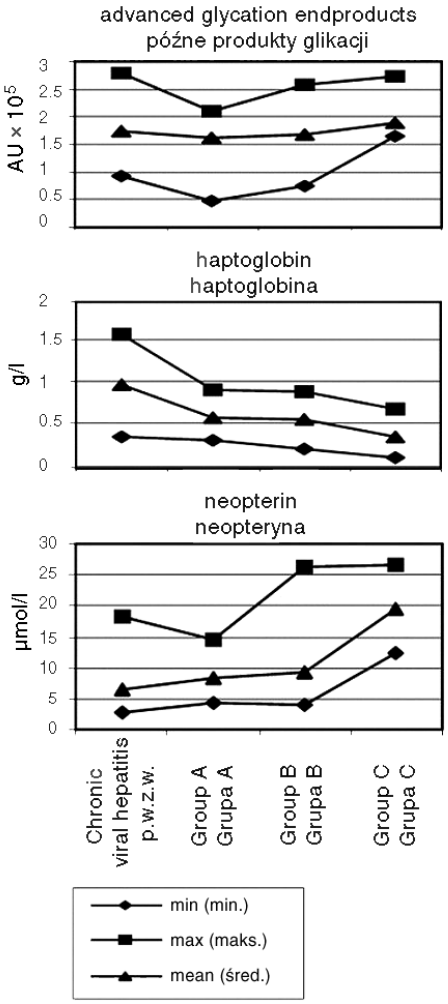

Fig. 2. Mean values of serum advanced glycation endproducts, haptoglobin and neopterin concentration presented with the variability bands (minimal and maximal values) in the groups of studied patients with chronic hepatitis (group CH) and liver cirrhosis (groups A, B and C)

Ryc. 2. Średnie stężenia późnych produktów glikacji, haptoglobiny i neopteryny w surowicy krwi, przedstawione wraz z pasmami zmienności (wartości minimalne i maksymalne) w poszczególnych grupach badanych chorych na przewlekłe wirusowe zapalenie wątroby (p.w.z.w.) oraz na marskość wątroby (grupa A, B i C)

cially for the group of patients in advanced stages of liver cirrhosis (B and C).

In patients with chronic hepatitis a significant correlation was evidenced between the NPT serum content and AGE fluorescence ($r = 0.3$; $p < 0.005$).

Discussion

For a long time, the advanced glycation endproducts were considered to be the expression of nonenzymatic protein glycation in patients with diabetes. Analysing the results of examination of patients who suffer from the liver disease and diabetes at the same time, the authors observed that most of the patients with liver cirrhosis had the liver diseases diagnosed earlier than the carbohydrate metabolism disorders [13]. For that reason the authors became interested in the role of AGE in the liver diseases.

Described research showed the increased levels of AGE in the serum of patients with chronic viral hepatitis and postinflammatory liver cirrhosis as compared to healthy subjects (up to 2-fold). The values of AGE increased in patients with cirrhosis according to the stage of the disease, but differences were not significant. The examined groups were not too numerous and the observed variability bands were wide. Sebekova et al. [5] confirmed a 3-fold increase in AGE fluorescence in the serum of patients with advanced liver cirrhosis without diabetes, who were qualified for the liver transplantation. Authors also observed a significant correlation ($r = 0.708$) between the severity of liver cirrhosis and plasma AGEs levels (fluorescent AGEs and N(epsilon)-carboxymethyllysine – CML). An increased level of AGEs in circulation may reflect both the reduction of effective liver mass and disrupted carbohydrate metabolism in

the liver, as well as the diminished degradation of AGEs due to endothelium damage caused by capillaryzation of blood vessels [5, 14]. Moreover, compared to non-diabetic patients with chronic hepatitis C infection, HCV patients with diabetes had increased transforming growth factor (TGF), a liver marker of increased fibrosis [13]. Thus, given the correlation between the activity of the liver disease and AGEs accumulation, a link between fibrosis, TGF and diabetes is an attractive hypothesis.

Kidneys and liver are engaged in removing AGEs from the serum. AGEs interact with specific receptors. p60 (OST-48, AGE-R1), p90 (80K-H, AGE-R2), galectin-3 (AGE-R3), the macrophage scavenger receptor type II (ScR-II), and CD36 regulate the uptake and clearance of AGEs in the liver. The best-characterised is receptor for advanced glycation endproducts (RAGE) [15–17]. At *in vivo* conditions, the proteins glycation was determined in portal, hepatic and peripheral venous blood plasma of cirrhotic patients and hepatic and peripheral venous blood plasma (as a surrogate of portal venous blood) of control subjects. There was no evidence for hepatic extraction of glycated proteins in subjects with normal liver function and limited extraction of methylglyoxal-modified protein in cirrhotic subjects [14].

Inflammation and fibrosis are usually observed at the same time in the course of a liver disease [1, 18] and, therefore, it is difficult to draw conclusions with respect to each of this disorders separately, based on the concentration of AGEs. That is why the fluorometric method for measuring the AGEs levels in the blood serum of patients with postinflammatory liver cirrhosis and chronic viral hepatitis still has to be confirmed as a marker of liver fibrosis.

The authors did not observe the decreased levels of Hp in the serum of patients in early stages of chronic hepatitis, but at the onset of cirrhosis the Hp concentrations in the serum give additional information about the advancement of liver cirrhosis [19]. According to the results of presented research, the mean value of Hp in patients with chronic viral hepatitis was normal, and differences between levels of Hp in patients with chronic viral hepatitis and liver cirrhosis were considerable. It confirms the information from other authors that this parameter is useful in differential diagnostic of liver diseases [8, 19]. A decrease concentration of Hp in the serum is an evidence of lower synthesis which corresponds to the process of cirrhosis and week secretion which can result from liver cell damage.

Described research also confirmed that there was a correlation between neopterin levels and the severity of liver diseases [20]. The present data evidenced the best correlation between NPT and the grade of severity of liver disease, compared to other parameters, i.e. AGE and Hp. Neopterin concentrations measured in blood reflect activation of cellular immunity. The release of NPT (as a mediator and/or modulator in the course of inflammatory process) enhances the cytotoxic potential of activated macrophages [6, 7]. Finally, the poor correlation was evidenced between NPT serum content and AGE fluorescence in patients with chronic viral hepatitis ($r = 0.30$).

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