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The transferrin isoforms in chronic hepatitis

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ABSTRACT

Background: The aim of this study was to evaluate the effect of chronic hepatitis on the serum profile of transferrin isoforms.

Methods: Tested group consist of 160 patients with chronic hepatitis. The samples were analyzed by capillary electrophoresis on MINICAP electrophoretic system (Sebia, France).

Results: In patients with chronic hepatitis tetrasialotransferrin level was increased ($P = 0.002$) and pentasialotransferrin decreased ($P = 0.009$). Moreover, statistical analysis revealed that trisialotransferrin level was different according to the grade of portal/periportal activity ($P = 0.009$), the grade of lobular activity ($P = 0.004$) and the stage of fibrosis ($P = 0.022$). There were no differences in tetrasialotransferrin and pentasialotransferrin according to the advancement of hepatitis activity and the stage of fibrosis ($P > 0.05$ for all comparisons).

Conclusions: We conclude that chronic hepatitis affect the serum profile of transferrin isoforms, but only trisialotransferrin level could be useful in determining progression of chronic hepatitis and the stage of fibrosis.

1. Introduction

The most of serum glycoproteins are synthesized by the liver hepatocytes. Among others, there is a transferrin (TF) with molecular weight of 79.6 kDa. This glycoproteins is composed of three structural subdomains (two N-linked oligosaccharide chains and a single polypeptide chain builded of 679 amino acids) [1]. Because of structure the transferrin shows high variability (microheterogeneity) depending on the number of sialic acid residues in carbohydrate chains nine isoforms (from asialotransferrin to octasialotransferrin) [1]. The dominated isoform in healthy people is tetrasialotransferrin (64–80%) [2]. Therefore, the transferrin could be one of the best model for the analysis of the changes in proteins glycosylation. For the shift in the profile of transferrin isoforms, the changes in the activity of enzymes is responsible [3]. Also, the disorders in the transport of some intracellular proteins play a role [4]. Thus, the functional status of liver hepatocytes plays an important role in creating of profile of transferrin isoforms, which may vary in different liver diseases.

The occurrence of alterations in proteins glycosylation in liver diseases is well known, also during the course of chronic hepatitis [5]. For example, the characteristic change in the hepatitis caused by HCV viruses is the presence of an additional *N*-acetylgalactosamine (GalNAc), which rarely occurs in the normal N-glycans structure of glycoproteins [6]. The aberrations in the structure of N-glycans are also shown during the course of HBV infections, for example an extra *N*-acetylglucosamine (GlcNAc) residues linked to the mannose or an increasing number of N-glycans branches [7,8].

Taking into account above facts, we can suspect that activity of hepatitis and the stage of liver fibrosis affect the serum profile of transferrin isoforms. We undertake the assessment of these changes of using capillary electrophoresis.

2. Material and methods

2.1. Subjects

The tested group consisted of 160 patients with hepatitis (95 males and 65 females) (mean age: 39.7 years; range: 19–73) who were admitted to the Department of Infectious Diseases and Hepatology of Medical University of Bialystok. They were divided into 2 subgroups according to the clinical diagnosis of disease: chronic hepatitis B — 65 patients (40 males and 25 females) (mean age: 33.9 years; range: 19–71) and chronic hepatitis C — 95 patients (55 males and 40 females) (mean age: 43.9 years; range: 19–73).

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Table 1
The laboratory characteristics of patients with chronic hepatitis B and C, and the control group.

	AST [IU/L]	ALT [IU/L]	GGT [IU/L]	Bilirubin [mg/dL]	MCV [fL]	PLT [10^9/L]	INR
Control group	23	17.5	23.5	0.79	86.55	243	0.93
$N = 30$	14–39	6–39	8–39	0.25–1.23	73.6–94.3	135–311	0.86–1.01
HBV	25	28	17.5	0.62	87.7	191	0.97
$N = 65$	12–75	14–106	7–127	0.32–3.57	79.10–98.6	80–325	0.89–1.22
	$P = 0.107$*	$P < 0.001$*	$P = 0.089$*	$P = 0.815$*	$P = 0.415$*	$P = 0.013$*	$P = 0.005$*
HCV	44	55.5	31	0.66	88.3	196	0.97
$N = 95$	20–194	12–300	7–721	0.20–3.55	79.2–97.7	60–326	0.86–1.79
	$P < 0.001$*	$P < 0.001$*	$P = 0.045$*	$P = 0.823$*	$P = 0.149$*	$P = 0.005$*	$P = 0.007$*
	$P < 0.001$†	$P < 0.001$†	$P < 0.001$†	$P = 0.872$†	$P = 0.389$†	$P = 0.579$†	$P = 0.789$†

Data are median and range. The differences between tested groups and controls were estimated by Mann-Whitney *U* test.
AST — aspartate aminotransferase, ALT — alanine aminotransferase, GGT — gamma-glutamyl transferase, MCV — mean corpuscular volume, PLT — platelet, INR — international normalized ratio.
† In comparison between hepatitis groups.
* In comparison with the control group.

Table 2
The serum concentration of total transferrin and transferrin isoforms.

	Total transferrin [g/L]	Disialotransferrin [%]	Trisialotransferrin [%]	Tetrasialotransferrin [%]	Pentasialotransferrin [%]
Control group	2.49	0.60	3.65	78.15	17.20
$N = 30$	1.68–3.26	0.30–5.40	1.60–5.60	65–84.7	11.10–32.80
Chronic hepatitis	2.55	0.60	3.30	81.65*	14.10*
$N = 160$	1.43–3.78	0.20–5.70	0.30–8.90	66.40–89.70	6–27.4
	$P = 0.541$	$P = 0.946$	$P = 0.449$	$P = 0.002$	$P = 0.009$

Data are median and range. The differences between tested group and controls were estimated by Mann-Whitney *U* test.
* Significant differences in comparison with the control group.

The diagnosis was based on the clinical data such as: signs, symptoms, physical exams and abdominal ultrasound or abdominal CT, and laboratory tests (biochemical liver panel: PLT, MCV, INR, AST, ALT, GGT, bilirubin). Because the liver biopsy is considered a gold standard for diagnosing and assessing liver fibrosis in all patients with chronic hepatitis the liver biopsy was done. To evaluate the grade of inflammation activity and stage of fibrosis we used the Scheuer classification for grading (G0 to G4) and staging (S0 to S4) of chronic hepatitis.

2.2. Control group

The control group consisted of 30 healthy subjects (16 males and 14 females) (mean age: 29.5 years; range: 21–54). Informed consent was obtained from all individual participants (healthy and sick) included in the study. This study was in accordance with Helsinki Declaration and was approved by the Bioethical Committee at the Medical University of Bialystok.

2.3. Sample collection

Blood samples from patients with liver disease and controls were collected by peripheral vein puncture. The sera were separated by centrifugation at 1500 × *g* for 10 min at room temperature and stored at − 86 °C until analysis.

2.4. Determination of transferrin concentration

Total serum transferrin concentration (normal range: 2.0–3.6 g/L) was determined by the immunoturbidimetric method using the Transferrin reagent kit (Abbott Laboratories, USA). The Architect ci8200 analyzer (Abbott Laboratories, Abbott Park, IL, USA) measures sample turbidity created by insoluble complexes of transferrin with specific antibodies.

2.5. Determination of transferrin isoforms

The analysis of transferrin isoforms was performed on a MINICAP electrophoretic system used the MINICAP CDT reagent kit (Sebia, Evry, France). The MINICAP system uses the principle of capillary electrophoresis (CE) in free solution. System performs all sequences automatically to obtain a complete transferrin isoforms profile for quantitative analysis of each fraction. The human serum transferrin isoforms were separated in alkaline buffer (pH 8.8) into five major fractions according to their sialylation level: asialotransferrin, disialotransferrin, trisialotransferrin, tetrasialotransferrin and pentasialotransferrin.

2.6. Statistical analysis

Results were expressed as median and range. The differences between tested and control groups were evaluated by Mann-Whitney *U* test. The ANOVA rank Kruskal-Wallis test was performed to analyze data according to grading and staging of chronic hepatitis. The correlation between transferrin isoforms and laboratory results was analyzed by Spearman's correlation. We considered P values < 0.05 as statistically significant. The specificity and positive predictive values (PPV) for trisialotransferrin, terasialotransferrin and pentasialotransferrin for patients with chronic hepatitis was calculated.

3. Results

The laboratory characteristics of chronic hepatitis patients and control group is presented in Table 1. In patients with hepatitis C all tests, with exception of bilirubin and MCV, were found to be significantly different compared with controls, while in patients with hepatitis B, only the mean levels of ALT, PLT and INR. Comparing the hepatitis B with hepatitis C, the mean levels of AST, ALT and GGT were found to be significantly different (Table 1).

The total serum transferrin concentration and the distribution of