ORIGINAL ARTICLE

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Activity of MMP-2, MMP-8 and MMP-9 in serum as a marker of progression of alcoholic liver disease in people from Lublin Region, eastern Poland

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Abstract

In alcoholic liver cirrhosis, normal liver cells are replaced by scar tissue (fibrosis). Liver fibrosis is a dynamic process in which activated hepatic stellate cells are involved in the synthesis of matrix proteins and the regulation of matrix degeneration. The aim of the presented study was to assess the usefulness of MMP-2, MMP-8 and MMP-9 as diagnostic markers of alcoholic liver disease. Sixty patients with alcoholic liver cirrhosis were randomly enrolled during hospitalization in departments of hospitals from the Lublin Region in eastern Poland. The stage of cirrhosis was estimated according to Child-Turcotte-Pugh criteria (Child-Pugh score) as P- Ch A, P-Ch B, P-Ch C. The control group consisted of 10 healthy persons without liver disease, who did not drink alcohol. Additionally, a group of alcoholics without liver cirrhosis was included in the study. Blood sample were obtained, and after centrifuge, serum was collected for further analysis. The activity of MMP-2, MMP-8 and MMP-9 in the blood plasma of the patients and the control group were measured by using the sandwich enzyme immunoassay technique with commercially available quantitative ELISA test kits. Activity of MMP-2, MMP-8 and MMP-9 in patients with liver cirrhosis were increased gradually according to Child-Pugh stages. The activity of MMP-2, MMP-8, MMP-9 were the highest in patients with liver cirrhosis stage C. MMP-2, MMP-8, MMP-9 concentrations in the people with liver cirrhosis (stage C) were significantly increased compared to controls. A significant difference were observed between activity MMP-2 in control group, alcoholics without liver cirrhosis, and those with liver cirrhosis (stages A, B, C according Child-Pugh score). MMP-2, MMP-8 and MMP-9 may be markers of alcoholic liver cirrhosis in the alcoholics. Elevated levels of MMP-2, MMP-8 and MMP-9 in the alcoholic patients indicated that cirrhosis has developed. The most sensitive is MMP-2, because the activity of this parameter is increased in all liver cirrhosis stages. MMP-8 and MMP-9 activity were significantly elevated only in serum patients with advanced liver cirrhosis, compared to controls.

Key words

alcoholic liver disease, liver cirrhosis, matrix metalloproteinases, MMP-2, MMP-8, MMP-9

INTRODUCTION

Alcohol is the most frequent cause of liver disease in developed countries. The level of alcohol consumption necessary for the development of these advanced forms of alcoholic liver disease is probably 80 g of alcohol per day, the equivalent of 6 – 8 drinks daily for several years [1]. Women have a significantly higher risk of developing alcoholic liver disease than men for any given level of alcohol intake [2].

Alcoholic liver disease may take the form of chronic illness (steatosis, steato-hepatitis, fibrosis and cirrhosis) or acute involvement (alcoholic hepatitis). Patients with steato-hepatitis may develop progressive fibrosis [3]. In alcoholic liver disease, the fibrotic tissue is typically located in the pericentral and perisinu-soidal areas. In advanced stages, collagen bands are evident and bridging fibrosis develops. This condition precedes the development of regeneration nodules and liver cirrhosis. Liver cirrhosis is a dynamic process of deposit and removal of extracellural matrix [4]. Alcohol metabolites, such as acetaldehyde, can directly activate hepatic stellate cells (HSC), the main collagen-producing cells [5]. Hepatic stellate cells (HSC) and liver myofibroblasts (LM) are sources of extracellular matrix, matrix metalloproteinases (MMP): MMP-1, MMP-13, MMP-2 and tissue inhibitors of matrix metalloproteinases (TIMPs 1 and 2) [6].

Kupffer cells participate in the modulation on the extracellular matrix. Kupffer cells express MMP-9 [7], and sinusoidal endothelial cells are sources of MMP-2 and MMP-9.

The presented study evaluates the activity of MMP-2, MMP-8 and MMP-9 in serum, patients with different stages of alcoholic liver cirrhosis, alcoholics without liver cirrhosis, and a control group.

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OBJECTIVE

The aim of the present study was to assess the usefulness of MMP-2, MMP-8 and MMP-9 as diagnostic markers of alcoholic liver disease.

MATERIALS AND METHOD

Sixty patients with alcoholic liver cirrhosis were randomly enrolled during hospitalization in departments of hospitals from the Lublin Region of eastern Poland. The stage of cirrhosis was estimated according to Child-Turcotte-Pugh criteria (Child-Pugh score) as P- Ch A, P-Ch B, P-Ch C. The control group consisted of 10 healthy persons without liver disease, who did not drink alcohol. Additionally, a group of alcoholics without liver cirrhosis was included in the study. Characteristics of study participants are given in Table 1. The age and gender distributions of cases and controls were similar, due to matching. The diagnosis of liver cirrhosis was based on clinical features, laboratory tests (Tab. 2), ultrasonography of the abdominal cavity, and history of heavy alcohol consumption (Tab. 1). Blood sample were obtained, and after the centrifuge, the serum was collected for further analysis.

The activity of MMP-2, MMP-8 and MMP-9 in the blood plasma of the patients and the control group were measured by using the sandwich enzyme immunoassay technique with commercially available quantitative ELISA test kits (Quantikine Elisa, R&D Systems Europe, Ltd.). The measurements were conducted according to the manufactures guidelines on a microplate reader (EPOCH; BioTek Instruments, Inc.) at 450 nm. All samples were measured as duplicates and the mean was calculated for data analysis. A calibration curve, as well as a negative control (a blank well without plasma probes), were run for each test plate.

Statistical analyses. The data were expressed as the means ± standard deviation (SD). The statistical analyses were performed by the MANOVA. The hypothesis regarding differences between the dependent variables MMP-2, MMP-8, MMP-9 and the independent variable 'group' divided into healthy individuals, alcoholics without liver cirrhosis (O), alcoholics with liver cirrhosis (stages A, B and C) was verified using MANOVA. The analysed data fulfilled the assumptions of MANOVA (normality of distribution and equality of covariance matrices in subgroups).

The Wilks` lambda test (Λ=3.8359, p=0.00008) demonstrated statistically significant differences in vectors of 5 means. The above tests were followed by one-dimensional tests, which were significant for MMP-2, MMP-8 and MMP-9. Intergroup differences in means were assessed using the Tukey *post-hoc* test. Means and 95% confidence intervals are presented in figures.

Table 1. Characteristic of alcoholic patients without liver cirrhosis (P-Ch 0), alcoholics with liver cirrhosis (P-Ch stage A, B, C) and healthy controls (C)

	C (n = 10)	P-Ch 0 (n = 22)	P-Ch A (n = 14)	P-Ch B (n = 25)	P-Ch C (n=24)
gender (male/female)	8/2	18/4	11/3	20/5	19/5
Age (years ± SD)	55.51 ± 8.89	54.91 ± 12.82	52.50 ± 16.11	54.00 ± 12.19	50.71 ± 10.00
Body weight (kg ± SD)	75.63 ± 9.83	64.54 ± 8.58	66.33 ± 11.93	84.84 ± 27.11	85.91 ± 21.76
Height (cm ± SD)	173.54 ± 10.31	169.91 ± 6.95	171.33 ± 9.86	177.36 ± 11.40	175.45 ± 6.69
Drinking period (years ± SD)	-	7.5 ± 2.89	11.16 ± 7.403	13.86 ± 7.06	18.17 ± 10.73
Existing medical symptoms					
Ascites	0	0	0	14	22
Encephalophaty	0	1	5	8	17
Oesophageal *varices*	0	0	0	9	16

Table 2. Biochemical data of the study participants

	C (n = 10)	P-Ch 0 (n = 22)	P-Ch A (n = 14)	P-Ch B (n = 25)	P-Ch C (n=24)
Bilirubin (mg/dl)	0.64 ± 0.22	2.66 ± 0.82	2.7 ± 0.95	5.58 ± 0.82	9.71 ± 0.98
Albumin (g/dl)	5.23 ± 0.54	4.20 ± 0.74	4.00 ± 0.67	3.80 ± 0.84	2.42 ± 0.48
ALT (U/l)	19.24 ± 8.56	34.10 ± 8.21	56.63 ± 15.51	63.19 ± 10.38	70.31 ± 18.22
AST (IU/l)	17.81 ± 5.030	42.51 ± 26.45	53.50 ± 27.36	152.9 ± 114.3	190.2 ± 255.1
ASP/ALT ratio	0.96 ± 0.21	1.96 ± 1.07	2.67 ± 2.22	2.83 ± 1.35	3.39 ± 1.73
GGTP (IU/l)	20.40 ± 8.96	234.81 ± 46.95	313.75 ± 27.96	642.24 ± 70.04	749.48 ± 72.55
Urea (mg/dl)	24.40 ± 10.07	35.45 ± 8.62	38.77 ± 6.98	44.81 ± 8.54	51.25 ± 5.39
Blood platelets (K/uL)	340.2 ± 7.96	320.95 ± 6.46	166.75 ± 11.96	135.46 ± 12.28	105.33 ± 7.02
INR	1.26 ± 0.16	1.24 ± 0.16	1.30 ± 0.21	1.39 ± 0.23	2.01 ± 0.90
MCV (fl)	86.00 ± 7.26	97.31 ± 7.24	95.97 ± 9.36	97.09 ± 6.27	103.07 ± 6.09
Na (mmol/l)	139.50 ± 3.44	133.56 ± 4.77	129.75 ± 10.50	134.05 ± 4.78	131.85 ± 8.41
K (mmol/l)	4.17 ± 0.32	4.02 ± 0.70	3.59 ± 0.42	4.07 ± 0.77	3.86 ± 0.60

Data are expressed as mean±SD. Normal range: bilirubin (0.-1.2 mg/dl), albumin (3.5–5.20 g/dl); ALT – alanine aminotransferase – (5–40 U/l); AST – aspartate aminotransferase (5–40 IU/l); GGTP – Gamma-glutamyl transpeptidase (11–50 IU/l); Urea (21–43 mg/dl), blood platelets (120–400 K/uL); INR (0,86–1,30); MCV (80–94 fl); K – potassium (3.5–5.1 mmol/l); Na-sodium (136–145 mmol/l); K – potassium (3.5–5.1 mmol/l)

Table 3. Activities of MMP-2, MMP-8 and MMP-9

	C	P-Ch 0	P-Ch A	P-Ch B	P-Ch C
MMP-2	282.1 ± 100.0	231.1 ± 75.11	424.8 ± 156.3^{*A}	422.0 ± 96.15$^{** B}$	506.3 ± 138.9$^{*** B}$
MMP-8	9.569 ± 3.982	12.04 ± 5.228	13.15 ± 1.804	16.49 ± 6.748	17.88 ± 7.474*
MMP-9	67.11 ± 31.94	125.7 ± 127.0	165.6 ± 96.49	179.0 ± 132.9	208.3 ± 120.9*

*p<0.05; ** p<0.01; *** p<0.001 vs. control group
^{A} p<0.05; ^{B} p<0.001 vs. group P-Ch 0

RESULTS

The mean activities of MMP differed between patients with healthy liver, alcoholics without liver cirrhosis and patients with liver cirrhosis. Activity of MMP-2 [F=12.687, p=0.000], MMP-8 [F=2.89, p=0.034] and MMP-9 [F=2,761, p=0.039] in patients with liver cirrhosis were increased gradually according to Child-Pugh stages. The activity of MMP-2, MMP-8, MMP-9 was the highest in stage C of liver cirrhosis.

Activities of MMP-2, MMP-8, MMP-9 in patients with liver cirrhosis (stage C) were increased in comparison with the control group. A significant difference was observed between MMP-2 activity in the control group, alcoholics without liver cirrhosis and patients with liver cirrhosis (A, B, C stage acc. Child-Pugh score). MMP-8 and MMP-9 activity in patients with stage C liver cirrhosis (p<0.05) were significantly higher than that in controls. No significant differences in MMP-2, MMP-8, MMP-9 activities were observed among Child's groups in patients with alcoholic liver cirrhosis.

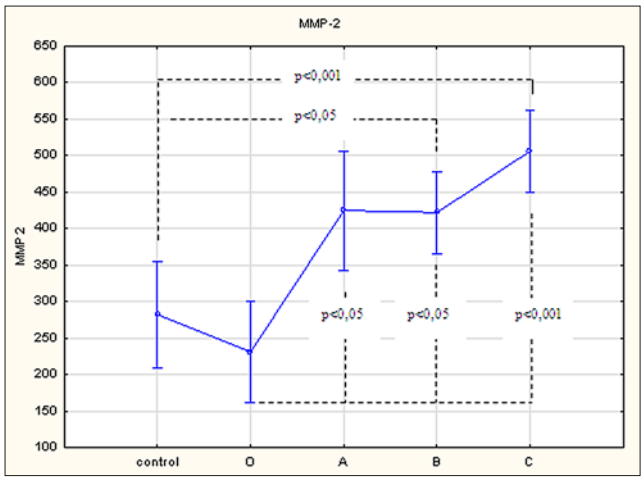

Figure 1. Activity of MMP-2 in the serum of alcoholics without liver cirrhosis (P-Ch 0), alcoholics with liver cirrhosis (P-Ch stage A, B, C) and healthy controls (C). MMP-2 activity in patients with stage A, B (p<0.05) and C (p<0.001) liver cirrhosis was significantly higher compared to the control group. Moreover, MMP-2 activity in patients with stage A, B (p<0.05) and C (p<0.001) liver cirrhosis was significantly higher compared alcoholics without liver cirrhosis

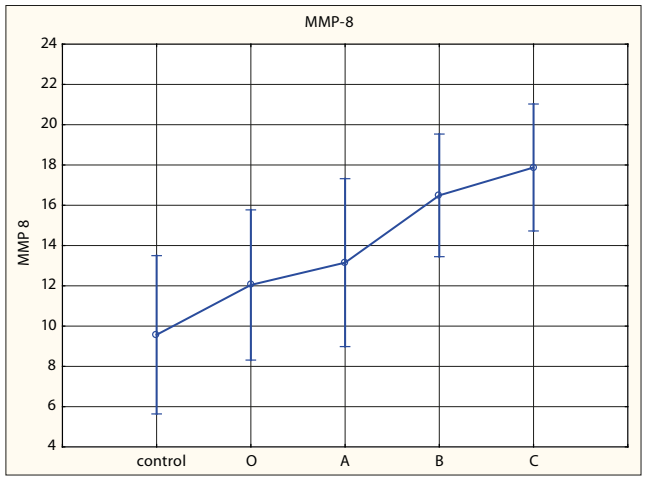

Figure 2. Activity of MMP-8 in the serum alcoholics without liver cirrhosis (P-Ch 0), alcoholics with liver cirrhosis (P-Ch stage A, B, C) and healthy controls (C). MMP-8 activity in patients with stage C liver cirrhosis (p<0.05) was significantly higher than that in controls

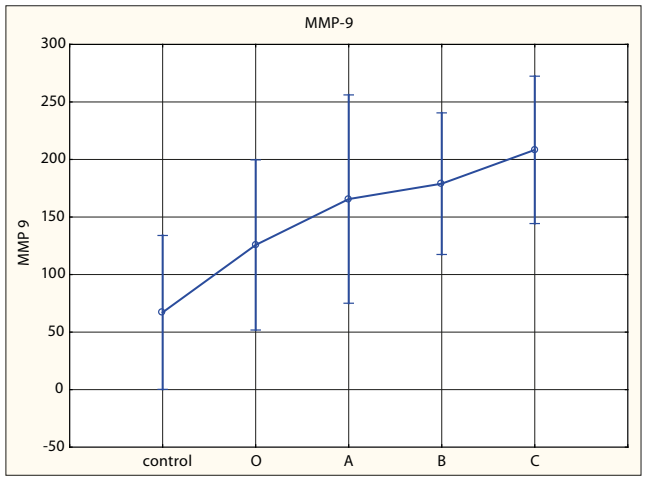

Figure 3. Activity of MMP-9 in the serum alcoholics without liver cirrhosis (P-Ch 0), alcoholics with liver cirrhosis (P-Ch stage A, B, C) and healthy controls (C). MMP-9 activity in patients with stage C liver cirrhosis (p<0.05) was significantly higher than that in controls

DISCUSSION

Matrix metalloproteinases (MMPs) are the main degrading enzymes of the extracellular matrix proteins, and they play an important role in the process of tissue remodeling and repair in physiological and pathological states [8]. In liver cirrhosis, normal liver cells are replaced by scar tissue (fibrosis), and consequently the liver is unable to perform many of its usual functions [9]. Liver fibrosis is a dynamic process in which activated hepatic stellate cells are involved in the synthesis of matrix proteins and the regulation of matrix degeneration. The value of MMPs determination as a diagnostic tool for the assessment of disease progression has not been established.

The presented study evaluated the clinical usefulness of serum measurements of MMP-2, MMP-8 and MMP-9 activities as potential markers of alcoholic liver disease severity in the management of patients with alcoholic liver disease. A significant increase was demonstrated in MMP-2, MMP-8 and MMP-9 in serum of patients with liver cirrhosis (stage C), compared to control group. MMPs detectable in the liver and in the serum may derive from a number of cells and tissues, of which the liver is most likely not the predominant one.

Firstly, the activity of MMP-2 in the serum of patients with alcoholic liver cirrhosis was determined. MMP-2 degrades native collagen types IV, V, VII, X and XI, denatures interstitial collagens, elastin, fibronectin and vitronectin [10]. In the current study, MMP-2 activity in the serum of patients with alcoholic liver cirrhosis (stages A, B and C) was significantly increased compared to the control group. There was also a significant difference between MMP-2 activity in the serum of patients with alcoholic liver cirrhosis (stages A, B and C) and alcoholics without liver cirrhosis.

The elevated serum MMP-2 level in cirrhotic patients may be explained by its over-production in the cirrhotic liver. Expression of MMP-2 has been found to be strong in hepatic tissue from patients with liver disease [11]. Increased expression of MMP-2 and MT1-MMP mRNA in hepatic stellate cells has been reported in patients with chronic hepatitis and cirrhosis [12]. In accordance with increased production of MMPs in liver tissue samples, serum MMP-2 concentrations were markedly increased in patients with liver cirrhosis, and showed a good correlation with the degree of liver fibrosis [13]. The presented finding that serum MMP-2 levels increased with the progression of alcoholic liver disease, as indicated by Child-Pugh classification, is in agreement with the findings of Murawake et al. Also, as in the current study, increased MMP-2 was reported in patients with liver cirrhosis in comparison to healthy controls.

MMP-8 is a member of the zinc-dependent interstitial collagenase subgroup of the MMP family of neutral proteinases [14]. Polymorphonuclear neutrophils (PMNs) are the main source of MMP-8 in humans and mice. MMP-8 is stored in the granules of PMNs and released upon degranulation [15]. In the rat, MMP-8 and MMP-13 are the primary MMPs capable of digesting type I collagen, the predominant component of scar tissue in fibrotic liver [16]. Neutrophils, which infiltrate the liver and accumulate in the periportal/fibrotic regions, produce and store MMP-8 in specific granules as a normal part of their maturation [17]. Harty et al. reported that polymorphonuclear cell derived MMP-8 plays an important role for liver repair in their reversible biliary obstruction model [18].

In the presented study, the activity of MMP-8 was significantly elevated in serum patients with alcoholic liver cirrhosis (stage C), compared to control group.

MMP-9 might play an important role for predicting the status of liver damage and inflammation. MMP-2 and MMP-9 have been implicated in liver injury and remodeling. The knockout of MMP-9 and other MMP attenuates liver injury [19]. MMP-9 in plasma is comparably increased in NASH (non-alcoholic liver disease) and hepatitis C infected patients [20], while hepatic MMP-9 mRNA is significantly higher in NASH compared to a cohort of patients suffering from hepatitis B or C virus infection [21]. Plasma MMP-9 was found to be elevated in patients with hepatocellular carcinoma, compared to normal controls and patients with liver cirrhosis [22]. In the current study, serum MMP-9 levels were higher in the patients with alcoholic liver cirrhosis (stage C), compared to controls.

In conclusion, this study shows that the activity of MMP-2, MMP-8 and MMP-9 might be markers of alcoholic liver cirrhosis in alcoholics. Elevated levels of MMP-2, MMP-8 and MMP-9 in the alcoholic patients indicated that cirrhosis had developed. Additionally, determinations of MMP-2 can be useful to confirm the diagnosis of alcoholic liver cirrhosis. MMP-2 has been found to be the most sensitive as its activity was increased in all stages of liver cirrhosis. MMP-8 and MMP-9 activity were significantly elevated only in the serum of patients with advanced liver cirrhosis vs. healthy controls.

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