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# Activity of MMP1 and MMP13 and Amino Acid Metabolism in Patients with Alcoholic Liver Cirrhosis

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**Background:** Alcoholic liver disease remains one of the most common causes of chronic liver disease worldwide. The aim of this study was to assess the usefulness of metalloproteinases (MMP1 and MMP13) as diagnostic markers of alcoholic liver disease and to determine the changes in free amino acid profile in the patients with alcoholic liver cirrhosis.





**Material/Methods:** Sixty patients with alcoholic liver cirrhosis treated in various hospitals of the Lublin region were randomly enrolled. The control group consisted of 10 healthy individuals without liver disease, who did not drink alcohol. Additionally, a group of alcoholics (22 persons) without liver cirrhosis was included in the study. The activity of MMP-1 and MMP-13 in blood plasma of patients and controls was measured using the sandwich enzyme immunoassay technique with commercially available quantitative ELISA test kits. Amino acids were determined by automated ion-exchange chromatography.

**Results:** No significant differences were observed in the activity of MMP-1 in alcoholics with or without liver cirrhosis or in controls. Increased serum MMP-13 was found in patients with liver cirrhosis (stage A, B, C) compared to the control group. Patients with alcoholic liver cirrhosis (stage A, B, C) demonstrated reduced concentrations of glutamic acid and glutamine compared to the control group. Plasma levels of valine, isoleucine, leucine, and tryptophan were significantly lower in patients with alcoholic liver cirrhosis (stage C) than in controls.

**Conclusions:** MMP-13 can be useful to confirm the diagnosis of alcoholic liver cirrhosis, but levels of MMP-1 are not significantly increased in patients with liver cirrhosis compared to controls. The serum branched-chain amino acid (BCAA) is markedly reduced in patients with stage C alcoholic liver cirrhosis.

**MeSH Keywords:** **Amino Acids • Liver Cirrhosis, Alcoholic • Matrix Metalloproteinase 1 • Matrix Metalloproteinase 13**

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## Background

Alcoholic liver disease remains one of the most common causes of chronic liver disease worldwide. The severity of liver damage related to alcohol abuse varies from individual to individual and even in the same individual in various periods. The clinical classification of alcoholic liver disease is based on the pathologic findings of fatty liver, alcoholic hepatitis, and alcoholic cirrhosis [1].

Liver fibrosis/cirrhosis is characterized by excessive accumulation of fibrous tissue components and is commonly observed in late or end-stage chronic hepatic diseases. Compelling evidence has documented the association of MMPs (matrix metalloproteinases) with liver fibrosis. The matrix metalloproteinases (MMPs) are a family of extracellular matrix-degrading enzymes that share several structural features, including the presence of a conserved zinc-binding catalytic domain [2]. MMPs have both inhibitory and stimulatory roles in fibrosis. Whereas some MMPs do indeed function to reduce fibrosis, others promote it. MMP-1, MMP-2, and MMP-8 play a protective role in chronic liver injury and attenuate the development of fibrosis. MMP-12 plays a modest role in generating fibrosis. MMP-19 seems to be pro-fibrotic in the early phases post-injury and in anti-fibrotic during resolution [3].

Under physiological conditions, the activity of metalloproteinases is regulated at the level of transcription and translation, mainly by tissue inhibitors of metalloproteinases (TIMPs). MMP-1 and MMP-13 are enzymes belonging to the group of collagenases. Their key feature is the ability to cleave type I, II, and III collagens, which are essential structural elements of the extracellular matrix [4]. MMP1 has a significant role in the pathogenesis of AMD (Age-Related Macular Degeneration) [5] and open-angle glaucoma [6].

Progressive fibrosis of the liver, the crucial organ for many metabolic changes, also induces protein-energy malnutrition, which manifests in amino acid deficiencies as well as other abnormalities.

To date, the role of collagenases in liver cirrhosis has been poorly elucidated. Therefore, the aim of this study was to assess the activity of MMP-1 and MMP-13 as potential markers of liver cirrhosis, and to determine free amino acid concentrations according to stage of disease in order to optimally diagnose patients and use the best nutritional therapy.

## Material and Methods

Sixty patients with alcoholic liver cirrhosis treated in various hospitals of the Lublin region were randomly enrolled.

The stages of cirrhosis were assessed according to the Child-Turcotte-Pugh criteria (Child-Pugh score) as P-Ch A, P-Ch B, and P-Ch C. The control group consisted of 10 healthy individuals without liver disease, who did not drink alcohol. Additionally, the group of alcoholics without liver cirrhosis (22 persons) was included in the study. Characteristics of the study population are given in Table 1. Cases and controls were age- and sex-matched.

The diagnosis of liver cirrhosis was based on clinical features, laboratory tests (Table 2), abdominal ultrasound, and history of heavy alcohol consumption (Table 1). The clinical manifestations of cirrhosis may include nonspecific symptoms: weight loss, weakness, or signs and symptoms of hepatic decompensation (jaundice, pruritus, signs of upper gastrointestinal bleeding, ascites, and confusion due to hepatic encephalopathy). Physical examination findings may include jaundice, spider angiomas, gynecomastia, ascites, splenomegaly, palmar erythema, digital clubbing, and asterixis. Laboratory abnormalities in the patients with liver cirrhosis are elevated serum bilirubin, abnormal aminotransferases, elevated alkaline phosphatase/gamma-glutamyl transpeptidase, a prolonged prothrombin time/elevated international normalized ratio (INR), hyponatremia, hypoalbuminemia, and thrombocytopenia. Characteristic findings of liver cirrhosis in ultrasound are nodular liver surface, round edge, and hypoechoic nodules in liver parenchyma, which represent regenerative nodules of cirrhotic liver. Detection of splenomegaly, ascites, and portosystemic collaterals is possible by ultrasound.

Samples of blood (5 mL) were collected from patients in the fasting state. Serum obtained by centrifugation was stored at  $-20^{\circ}\text{C}$  and then used for further analysis.

The activities of MMP1 and MMP13 in serum of patients and controls were measured using the sandwich enzyme immunoassay technique with commercially available quantitative ELISA test kits (Quantikine Elisa, R&D Systems Europe, Ltd.). Measurements were conducted according to the manufacturer's 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 and a negative control (a blank well without plasma) were run for each test plate.

To measure concentrations of free amino acids, 0.5 mL of serum sample was deproteinized with 0.5 mL of 6% sulphosalicylic acid in the lithium citrate buffer (pH 2.8) and centrifuged at high speed. Amino acids were determined by automated ion-exchange chromatography with 5 lithium-citrate buffers (pH 2.6, 3.0, 3.35, 4.05, and 4.65, respectively) using an Amino Acids Analyser (AAA 400) (INGOS Corp., Prague, Czech Republic). Amino acids were separated using the analytical column OSTION

**Table 1.** Characteristic of patients with alcoholics without liver cirrhosis (P-Ch 0), alcoholics with liver cirrhosis (P-Ch stage A, B, C) and healthy controls (C) taking part in the study.

	C (n=10)	P-Ch 0 (n=22)	P-Ch A (n=14)	P-Ch B (n=25)	P-Ch C (n=24)
Sex (male/female)	8/2	18/4	11/3	20/5	19/5
Age (years $\pm$ SD)	55.51 $\pm$ 8.89	54.91 $\pm$ 12.82	52.50 $\pm$ 16.11	54.00 $\pm$ 12.19	50.71 $\pm$ 10.00
Body weight (kg $\pm$ SD)	75.63 $\pm$ 9.83	64.54 $\pm$ 8.58	66.33 $\pm$ 11.93	84.84 $\pm$ 27.11	85.91 $\pm$ 21.76
Height (cm $\pm$ SD)	173.54 $\pm$ 10.31	169.91 $\pm$ 6.95	171.33 $\pm$ 9.86	177.36 $\pm$ 11.40	175.45 $\pm$ 6.69
Drinking period (years $\pm$ SD)	–	7.5 $\pm$ 2.89	11.16 $\pm$ 7.403	13.86 $\pm$ 7.06	18.17 $\pm$ 10.73
Daily dose of alcohol (g/day)	–	30–75	30–75	30–75	30–75
Existing medical symptoms					
Ascites	0	0	0	14	22
Encephalopathy	0	0	1	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 $\pm$ 0.22	2.66 $\pm$ 0.82	2.7 $\pm$ 0.95	5.58 $\pm$ 0.82	9.71 $\pm$ 0.98
Albumin (g/dl)	5.23 $\pm$ 0.54	4.20 $\pm$ 0.74	4.00 $\pm$ 0.67	3.80 $\pm$ 0.84	2.42 $\pm$ 0.48
ALT (U/l)	19.24 $\pm$ 8.56	34.10 $\pm$ 8.21	56.63 $\pm$ 15.51	63.19 $\pm$ 10.38	70.31 $\pm$ 18.22
AST (IU/l)	17.81 $\pm$ 5.030	42.51 $\pm$ 26.45	53.50 $\pm$ 27.36	152.9 $\pm$ 114.3	190.2 $\pm$ 255.1
ASP/ALT ratio	0.96 $\pm$ 0.21	1.96 $\pm$ 1.07	2.67 $\pm$ 2.22	2.83 $\pm$ 1.35	3.39 $\pm$ 1.73
GGTP (IU/l)	20.40 $\pm$ 8.96	234.81 $\pm$ 46.95	313.75 $\pm$ 27.96	642.24 $\pm$ 70.04	749.48 $\pm$ 72.55
Urea (mg/dl)	24.40 $\pm$ 10.07	35.45 $\pm$ 8.62	38.77 $\pm$ 6.98	44.81 $\pm$ 8.54	51.25 $\pm$ 5.39
Blood platelets count (K/uL)	340.2 $\pm$ 7.96	320.95 $\pm$ 6.46	166.75 $\pm$ 11.96	135.46 $\pm$ 12.28	105.33 $\pm$ 7.02
INR	1.26 $\pm$ 0.16	1.24 $\pm$ 0.16	1.30 $\pm$ 0.21	1.39 $\pm$ 0.23	2.01 $\pm$ 0.90
MCV (fl)	86.00 $\pm$ 7.26	97.31 $\pm$ 7.24	95.97 $\pm$ 9.36	97.09 $\pm$ 6.27	103.07 $\pm$ 6.09
Na (mmol/l)	139.50 $\pm$ 3.44	133.56 $\pm$ 4.77	129.75 $\pm$ 10.50	134.05 $\pm$ 4.78	131.85 $\pm$ 8.41
K (mmol/l)	4.17 $\pm$ 0.32	4.02 $\pm$ 0.70	3.59 $\pm$ 0.42	4.07 $\pm$ 0.77	3.86 $\pm$ 0.60

Data are expressed as mean  $\pm$ 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 count (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).

LG FA and identified in comparison to the standards provided by INGOS Corp. The original software MIKRO version 1.8.0 (INGOS) was used for amino acid determinations.

The local Ethics Committee (Medical University of Lublin) accepted the protocol of the study (agreement No. KE-0254/190/2011).

**Table 3.** Activities of Pro-MMP-1 and MMP-13 obtained in the study.

	C	P-Ch 0	P-Ch A	P-Ch B	P-Ch C
Pro-MMP-1	0.352±0.276	0.351±0.320	0.433±0.506	0.659±0.648	0.457±0.473
Pro-MMP-13	92.09±48.23	95.96±58.01	169.20±33.43*	174.90±86.41*	195.50±25.66* <sup>A</sup>

p <0.05; \*\* p<0.01; \*\*\* p<0.001 vs. control group; <sup>A</sup> p<0.05; <sup>B</sup> p<0.01; <sup>C</sup> p<0.001 vs. group P-Ch 0.

**Table 4.** Amino-acids concentration in the serum patients with alcoholics without liver cirrhosis (0), alcoholics with liver cirrhosis (stage A, B, C) and healthy controls (C) taking in the study.

	Contol	0	A	B	C
GLU	0.044±0.016	0.394±0.173*	0.440±0.043*	0.648±0.111**	0.651±0.414**
GLN	0.466±0.150	0.130±0.171**	0.128±0.077**	0.012±0.019**	0.031±0.039**
ALA	0.323±0.056	0.257±0.067	0.287±0.065	0.291±0.077	0.279±0.062
VAL	0.203±0.033	0.174±0.052	0.187±0.072	0.138±0.029	0.106±0.072*
ILE	0.064±0.012	0.043±0.013	0.039±0.018	0.036±0.012*	0.027±0.018**
LEU	0.121±0.025	0.095±0.032	0.098±0.030	0.088±0.024	0.058±0.035*
TYR	0.078±0.019	0.063±0.016	0.081±0.027	0.078±0.026	0.066±0.025
PHE	0.067±0.010	0.052±0.009	0.068±0.030	0.065±0.019	0.063±0.018
TRP	0.035±0.019	0.022±0.021	0.014±0.008	0.014±0.018	0.006±0.008*
ASN	0.007±0.003	0.005±0.004	0.010±0.009	0.005±0.005	0.012±0.010
ORN	0.065±0.016	0.076±0.021	0.074±0.032	0.056±0.018	0.047±0.013
CIT	0.027±0.012	0.022±0.007	0.031±0.004	0.026±0.009	0.032±0.009
ARG	0.063±0.020	0.040±0.028	0.039±0.018	0.050±0.014	0.041±0.021
BCAA	0.129±0.064	0.104±0.065	0.108±0.076	0.088±0.048	0.064±0.055*
AAA	0.060±0.024	0.046±0.023	0.054±0.037	0.052±0.035	0.045±0.033

\* p<0.05; \*\* p<0.01; \*\*\* p<0.001 vs. control group. GLU – glutamic acid; GLN – glutamine; ALA – alanine; VAL – valine; ILE – isoleucine; LEU – leucine; TYR – tyrosine; PHE – phenylalanine; TRP – tryptophan; ASN – asparagine; ORN – ornithine; CIT – citrulline; ARG – arginine; BCAA – branched-chain amino acid; AAA – aromatic amino acid.

### Statistical analysis

Data are expressed as mean ± standard deviation (SD) and were statistically analyzed using the one-way analysis of variance (ANOVA) (GraphPad Prism 4.0, GraphPad Software Inc.). Post hoc comparison of means was carried out with the Tukey test for multiple comparisons. P<0.05 was considered as statistically significant.

### Results

No significant differences were found in the activity of MMP-1 in alcoholics with or without liver cirrhosis or in the control group. The activity of MMP-13 in serum of patients with liver

cirrhosis (stage A, B, C) was significantly higher than that in controls (Table 3).

The study findings demonstrated significant changes in plasma amino acid concentrations in patients with alcoholic liver cirrhosis compared to the control group. Patients with alcoholic liver cirrhosis (stage A, B, C) had lower concentrations of glutamic acid and glutamine than controls. The serum levels of valine, isoleucine, leucine (branched-chain amino acid -BCAA), and tryptophan were significantly reduced in patients with alcoholic liver cirrhosis (stage C) compared to the control group (Table 4).

No statistically significant differences were observed in serum extraction of aromatic amino acids (AAAs) between the groups of patients with alcoholic liver cirrhosis and controls.

## Discussion

Hepatic fibrosis is characterized by excessive deposition of type I collagen fibrils in Disse's space. In humans, the fibrils are degraded by matrix metalloproteinase. MMP-1 possesses proteolytic activity against interstitial collagens, the most abundant classes of extracellular matrix (ECM) proteins in fibrotic livers [7].

Activity of MMP-1 is useful for diagnosing liver cirrhosis in chronic hepatitis C patients. Attallah et al. observed changes in serum MMP-1 concentrations in 129 chronic hepatitis C (CHC) patients (78 non-cirrhotic and 51 with cirrhotic liver) and 50 healthy controls. MMP-1 concentrations increased in patients with CHC according to the stage of liver fibrosis. The diagnostic potential of MMP-1 for discriminating cirrhotic patients from healthy individuals was very high, with 98% sensitivity and 97% efficiency. Moreover, the sensitivity and efficiency of MMP-1 for detecting cirrhosis in CHC patients was 71% and 73%, respectively [7].

In the present study, attempts were made to assess the clinical usefulness of measurements of serum MMP-1 and MMP-13 activities as potential markers of alcoholic liver disease severity for the management of patients with alcoholic liver disease. No significant increases in serum MMP-1 were found in patients with liver cirrhosis compared to controls and alcoholics without liver cirrhosis. The study findings did not confirm the role of MMP-1 for discriminating alcoholic cirrhosis patients from healthy individuals.

Activation of proenzyme or inactivation of MMP-1 can be inhibited by increased expression of TIMP-1 via formation of TIMP-MMP-1 complex [8]. TIMP-1 (tissue inhibitor of metalloproteinases – 1) specifically inhibits matrix MMP-1. According to Murawaki et al., hepatic levels of TIMP-1 increased with the development of liver disease, correlating closely with the degree of necroinflammation and liver fibrosis [8].

In our study the activity of MMP-13 was significantly increased in serum of patients with liver cirrhosis (stage A, B, and C) compared to the control group; therefore, MMP-13 may be considered a good marker for discriminating alcoholic cirrhosis patients from healthy individuals. Nowadays, some other metalloproteinases are considered as markers of pathological states. Peker et al. indicate that MMP-7 may be beneficial as markers of gastrointestinal stromal tumors [9].

MMP-13, the interstitial collagenase of rodents, is a highly specific protease capable of degrading insoluble fibrillar collagens, especially type I collagen, which suggests its important role in liver fibrogenesis [10]. MMP-13 mRNA expression is induced in hepatic scar-associated macrophages during spontaneous

regression of liver fibrosis [10]. MMP-13 was detected to evaluate the fibrosis in rat liver [11].

The liver is responsible for metabolism of hormones that affect protein, carbohydrate, and lipid metabolism. Chronic and acute liver disease can profoundly alter the nutritional status and amino acid metabolism [12]. A cirrhotic patient is characterized by protein catabolism and a hypermetabolic state.

Most changes in plasma amino acid concentrations in patients with chronic liver disease may be explained on the basis of impaired hepatic function, portal-systemic shunting of blood, hyperinsulinemia and hyperglucagonemia.

Morgan et al. showed that plasma concentrations of glycine, alanine, phenylalanine, and branched-chain amino acids were significantly reduced in alcoholics with liver cirrhosis, while concentrations of methionine were significantly increased [13].

BCAAs (branched-chain amino acids) are the essential amino acids that could have a stimulatory effect on hepatic protein synthesis and an inhibitory effect on proteolysis; moreover, they are the major nitrogen source for glutamine synthesis in muscles.

Circulating glutamine is mostly derived from the skeletal muscle and lung, and the relative contribution of liver to the pool of circulating glutamine is considered minor [14].

Synthesis of glutamine is activated during critical illnesses, such as cancer and trauma. However, the body demands for glutamine in certain physiologic conditions are enormous, and increased utilization of glutamine often exceeds its synthesis, resulting in its deficiency in plasma and muscles. The needs of BCAA for synthesis of glutamine are connected with the breakdown of muscle proteins, and the result is muscle-protein wasting [15].

In our study patients with alcoholic liver cirrhosis (stage A, B, C) had reduced concentrations of glutamic acid and glutamine compared to the control group.

In the liver, the glutamine synthesis pathway is involved in the detoxification of blood ammonia, a by-product of protein metabolism. Ammonia metabolism is one of the most important liver functions. Ammonia produced by protein degradation in the gut is detoxified by 2 major pathways present in the liver: the urea synthesis and glutamine synthesis pathways. Thus, the sequence of a low-affinity, but high-capacity system (urea synthesis) followed by a high-affinity system (glutamine synthesis) ensures effective and complete elimination of blood ammonia. Hepatocytes severely disrupt the glutamine synthesis pathway, which results in impaired ammonia detoxification [16].

Irimia et al. evaluated the value of oral glutamine challenge (OGC) in improving psychometric performance for the diagnosis of minimal hepatic encephalopathy. Oral glutamine challenge is a method to increase blood ammonia in patients with cirrhosis, which could lead to cognitive disturbances. Patients with hepatic encephalopathy in liver cirrhosis and healthy controls ingested a 20-g solution of glutamine dissolved in 100 ml of tap water. Arterial ammonia concentrations and psychometric tests were evaluated 60 minutes before and after a 20-g oral glutamine load. In cirrhotic patients, oral glutamine load improved the psychometric diagnostic performance for minimal hepatic encephalopathy [17].

Adequate concentrations of glutamine are crucial. Study findings demonstrate that glutamine decreases intestinal atrophy caused by total parenteral nutrition (TPN), protects the intestinal mucosa, and reduces the morbidity associated with small intestine inflammation caused by cytotoxicity of drugs [18].

Furthermore, glutamine stimulates the growth of cells, production of cytokines, and synthesis of heat shock proteins [19]. Recent studies reveal that glutamine availability is strictly connected with the induction of apoptosis, in which it acts both as a nutritional substance and a signalling molecule that directly and indirectly leads to programmed cell death [20]. Apoptosis is considered the main cause of liver damage in hepatic cholestasis.

In the present study branched-chain amino acid concentrations in serum were significantly reduced in patients with advanced liver cirrhosis (stage C) compared to the control group.

ASPEN and ESPEN guidelines recommend the use of BCAA to improve hepatic insufficiency [21]. There is a highly significant correlation between plasma BCAA and albumin levels in liver cirrhosis (i.e., patients with low plasma BCAA have low serum albumin levels and those with high BCAA have high albumin levels) [22]. BCAA increases plasma albumin, benefits quality of life, and improves survival in liver cirrhosis patients [23].

Supplementation with BCAAs increases *in vitro* synthesis and secretion of albumins by cultured rat hepatocytes without affecting the albumin mRNA expression. BCAAs recover impaired turnover kinetics of albumins both in the rat cirrhotic model and in cirrhotic patients.

Several clinical studies have suggested that administration of BCAA-enriched amino acid solution to liver cirrhosis patients dramatically and rapidly improved the consciousness of patients with portal-systemic shunt and precipitant factors [24]. High concentrations of blood ammonia in liver cirrhosis patients might be detoxified by BCAA from muscles [25]. There have been multiple reports on beneficial effects of BCAA supplementation,

including improved metabolic profiles, as measured by protein sparing and/or normalization of respiratory quotients, and clinical improvement of hepatic encephalopathy [26].

Muto et al. provide important evidence of a therapeutic benefit of BCAA supplementation in prevention of cirrhosis-associated complications. Oral supplementation with a BCAA preparation that can be administered for a long period improves event-free survival, serum albumin concentration, and QOL of patients with decompensated cirrhosis with an adequate daily food intake [27].

According to Marchesini et al., one-year nutritional BCAA supplementation of patients with advanced liver cirrhosis was useful to prevent progressive hepatic failure and to improve surrogate markers and perceived health status in these patients [28].

In our study, the highest deficit observed was that of isoleucine. Thus, the question of whether administration of BCAA mixture is more effective than supplementation of a deficient amino acid remains open.

Recent studies demonstrate that leucine shows specific ability to initiate the synthesis of proteins in muscles by modulating mRNA translation [29]. Catabolism of leucine increases 7–8 hours after BCAA administration, which, according to some researchers, evidences enhanced BCAA transamination, glutamate formation, and increased *de novo* synthesis of glutamine [30].

According to 1 study, alimentary tract hemorrhage in patients with liver cirrhosis is accompanied by decreased concentrations of isoleucine [31]. Intravenous administration of this amino acid has improved the amino acid profile and affected the synthesis of muscle and liver proteins because isoleucine is capable of modulating the translation of proteins similarly to leucine.

Murata and Moriyama [32] showed that isoleucine prevented liver metastases in a mouse colon cancer metastatic model. They found that isoleucine prevented tumor growth via a novel mechanism by inhibition of vascular endothelial growth factor (VEGF) production, partially through the mTOR pathway, independent of hypoxia-inducible factor 1- $\alpha$  (H1F1- $\alpha$ ).

## Conclusions

Our study findings demonstrate that determinations of MMP-13 can be used to confirm the diagnosis of alcoholic liver cirrhosis, but concentrations of MMP-1 are not significantly increased in patients with cirrhosis as compared to controls.

Further studies are required to explain the role of collagenases in the successive stages of liver cirrhosis. Recent studies

suggest that collagenases are not involved in the initial stages of neoplasia and that their expression increases once the basilar membrane barrier has been crossed; therefore, they can be considered as markers of tumor progression [10,33].

Thus, lack of MMP-1 expression can indicate that the cirrhotic process is not accompanied by the neoplasia process, which is essential for prognosis and evaluation of changes in amino

acid concentrations because the free amino acid profile in liver cirrhosis differs from that in liver cancer [34].

Our study findings demonstrate that serum BCAA concentrations are significantly reduced in patients with stage C alcoholic cirrhosis. In advanced alcoholic cirrhosis, BCAA supplementation seems necessary, and in stage A and B cirrhosis it is likely to improve nitrogen balance, increase glutamine availability, and improve prognosis.

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