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Original Article

Serum prolidase, urotensin-2 and nesfatin levels in patients with compensated or uncompensated cirrhosis

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Abstract

Aim: To assess the feasibility of serum prolidase, urotensin-2, and nesfatin measurements as diagnostic and follow-up markers of compensated or uncompensated cirrhosis.

Material and Methods: The study included 97 cirrhosis patients and 51 healthy individuals who were admitted to the Internal Medicine and Gastroenterology Clinics of Harran University Research and Application Hospital between May 2014 and June 2015. Patients with esophageal variceal bleeding, ascites, hepatic encephalopathy were considered as having decompensated cirrhosis. Blood samples obtained from patient and control groups were stored at -85 C. ELISA methodology was used for measurements.

Results: A total of 97 cirrhotic patients (43 with compensated and 54 with decompensated cirrhosis) with a median age of 53.98±15.67 years (range: 19-89) were included in the study. Serum prolidase, urotensin-2, and nesfatin in patients with cirrhosis compared to 51 healthy controls. Serum prolidase levels were significantly lower in the overall group of patients with cirrhosis as well as in the decompensated cirrhotic patients as compared to controls ($p<0.001$). Urotensin-2 was significantly lower in the compensated cirrhosis group than in controls ($p<0.001$), while the difference between controls and the overall cirrhotic group and decompensated cirrhotic group was not significant ($p>0.05$). Nesfatin was significantly higher in the overall and decompensated cirrhotic than in controls ($p<0.001$), while there were no significant differences between controls and compensated cirrhosis patients ($p>0.05$).

Conclusion: Our results suggest that serum prolidase levels can be used as a marker for diagnosis and follow-up in patients with decompensated cirrhosis and that urotensin-2 may play a role in the pathogenesis of cirrhosis. Again, a higher nesfatin level in the overall and decompensated cirrhosis patients was suggestive of its potential role in the development of loss of appetite and cachexia characteristic of advanced cirrhosis as well as a role as a potential marker.

Keywords: Cirrhosis; prolidase; urotensin-2; nesfatin.

INTRODUCTION

Hepatic cirrhosis is a chronic condition characterized by diffuse hepatocellular necrosis, regenerative changes, formation of nodules, impaired intra-hepatic circulation, and increased fibrosis (1). Although fibrosis was considered irreversible until recently, new findings indicate that it may actually be reversible (2).

Regardless of the etiology, the clinical manifestations of hepatic cirrhosis mainly depend on two derangements in the liver, namely the hepatocellular failure and portal hypertension (PHT). The two clinical stages of cirrhosis include the compensated and decompensated forms, which vary in terms of the clinical signs. While nearly half of the patients are diagnosed after occurrence of ascites and icterus (decompensated stage), the remaining cases are identified either after presenting with non-specific complaints or coincidentally after a routine medical examination (3).

Since proline and hydroxyproline comprise approximately 25% of the amino acids in the collagen tissue, prolidase plays an important role in collagen breakdown (4). In one previous study, significantly lower serum prolidase levels were reported in cirrhotic patients as compared to controls, indicating an altered collagen turnover in human liver as a result of cirrhosis and suggesting a potential role for prolidase activity to reflect the disorders of the collagen in this degenerative liver disease (5).

Urotensin-2 is the most potent vasoconstrictor peptide

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ever discovered, with its mRNA having been isolated in vessels, endothelium, heart, leukocytes, brain, spinal cord, kidneys, liver, adrenal glands, pituitary gland, spleen, small intestines, colon, placenta, and other tissues. Elevated plasma urotensin levels have been reported in renal failure, congestive heart failure, diabetes, hypertension, and portal hypertension (6).

Nesfatin has been shown to play a regulatory role on the functions of the pituitary-ovarian axis (7), and it is not only found in the brain tissue, but also in peripheral tissues such as adipose tissue, stomach, pancreatic islets, liver, and testicles (8). In a study involving patients with non-alcoholic steatohepatitis, reduced serum nesfatin levels have been reported (9).

This study was undertaken to the feasibility of using serum prolidase, urotensin-2 and nesfatin levels as a marker for diagnosis and follow-up in patients with compensated or decompensated cirrhosis.

MATERIAL and METHODS

A total of 97 cirrhotic patients were included in this study that treated on an outpatient or inpatient basis at the Departments of Internal Medicine and Gastroenterology, Research and Training Hospital of Harran University between May 2014 and June 2015. Also, 51 age and sex-matched healthy individuals were included in this study.

All patients provided written informed consent and the study was conducted in accordance with the 2nd Declaration of Helsinki, as approved by the local ethics committee. The diagnosis of cirrhosis was based on liver biopsy and/or clinical, laboratory, and imaging studies. Decompensated cirrhosis patients consisted of those who had esophageal variceal bleeding, ascites, or hepatic encephalitis.

In all study subjects, 5 cc of blood sample from the forearm veins were placed in biochemistry tubes. Sera were separated for prolidase, urotensin-2, and nesfatin assays by centrifuging the blood at 1500 rpm for 10 minutes. All serum samples were labelled and kept at -85 °C until the time of analysis.

Serum prolidase levels were determined using Cusabio ELISA kits, while Blue Gene ELISA kits and Boster ELISA kits were used for urotensin-2 and nesfatin levels, respectively.

Statistical Analysis

For statistical analyses, SPSS 20.0 (SPSS Inc, Chicago, IL, USA) software pack was used. Descriptive data were expressed as arithmetical mean ± standard deviation for parametric data and minimum, maximum, and median for non-parametric data. The normal distribution of the data was tested using Kolmogorov-Smirnov test. Data with parametric distribution were analyzed with one-way ANOVA, and Bonferoni test was used for post hoc analyses. Kruskal-Wallis test was used for non-parametric data and the groups responsible for the difference were determined using Mann-Whitney U test. A p level of less than 0.05 was considered statistically significant.

RESULTS

Of the 97 cirrhotic patients 43 (44.3%) and 54 (55.7%) of them had compensated and decompensated cirrhosis, respectively.Overall, there were 57 male (58.7%) and 40 (34%) female subjects in the overall group of cirrhotic subjects with a mean age of 53.98±15.67 years (19-89 y). Among patients with compensated cirrhosis, 29 (67.4%) were male and 14 (32.6%) were female, with a mean age of 50.74±15.94 years (range: 19-85 y), while there were 28 male (51.8%) and 26 female (48.2%) female patients with a mean age of 56.56±15.10 years (19-89) in decompensated cirrhosis. Of the control subjects, 13 (25.4%) were male, 33 (%64.7) were female, and the mean age was 44.75±11.4 years (18-72 y).

Prolidase, urotensin-2, and nesfatin levels in patient and control groups are shown in Table 1.

Table 1. Laboratory Results in Patient and Control Groups

	Compensated Cirrhosis	Decompensated cirrhosis	Overall cirrhosis	Controls	P1	P2	P3	P4
PROLIDASE (ıu/l)	5418.50±1522.24	3534.93±1157.13	4369.91±1624.08	5977.40±1029.70	0.091	<0.001	<0.001	<0.001
UROTENSİN-2 (ng/ml)	16.37±4.96	23.24±7.21	20.24±7.16	23.48±6.15	<0.001	1	<0.001	0.121
NESFATİN (ng/ml)	163.04±49.91	271.91±88.97	224.27±91.85	175.27±47.19	1	<0.001	<0.001	<0.001

P values= P1: Compensated cirrhosis vs. controls; P2: decompensated cirrhosis vs controls; P3: compensated vs. decompensated cirrhosis; P4: overall cirrhosis vs controls. P<0.05 is considered significant

The mean prolidase in the overall cirrhotics, compensated cirrhotic, decompensated cirrhotic, and control subjects were 4369.9±1624.0 ıu/l, 5418.5±1522.2 ıu/l, 3534.9±1157.1 ıu/l, and 5977.3±1029.7 ıu/l, respectively. Accordingly, prolidase was significantly lower in the overall cirrhosis and decompensated cirrhosis groups as compared to controls (p<0.001), while the difference between controls and compensated cirrhosis patients was insignificant (p>0.05). Figure 1 shows the mean, standard deviation, and distribution of serum prolidase in study groups.

The mean urotensin-2 levels in the overall cirrhosis, compensated cirrhosis, decompensated cirrhosis, and

control groups were 20.2±7.1 ng/ml, 16.3±4.9 ng/ml, 23.2±7.2 ng/ml, and 23.4±6.1 ng/l respectively. Patients with compensated cirrhosis had significantly lower urotensin-2 levels as compared to controls (p<0.001). On the other hand, there were no significant differences between controls and decompensated and overall cirrhotic (p>0.05). Figure 2 shows the mean, standard deviation, and distribution of serum urotensin-2 levels in study groups.

The mean nesfatin levels in the overall cirrhosis, compensated cirrhosis, decompensated cirrhosis, and control groups were 224.2±91.8 ng/ml, 163.0±49.9 ng/ml, 271.9±88.9 ng/ml, and 175.2±47.1 ng/ml, respectively.

Nesfatin was significantly higher in the overall and decompensated cirrhotic than in controls (p<0.001), while there were no significant differences between controls and compensated cirrhosis patients (p>0.05). Figure 3 shows the mean, standard deviation, and distribution of serum nesfatin levels in study groups.

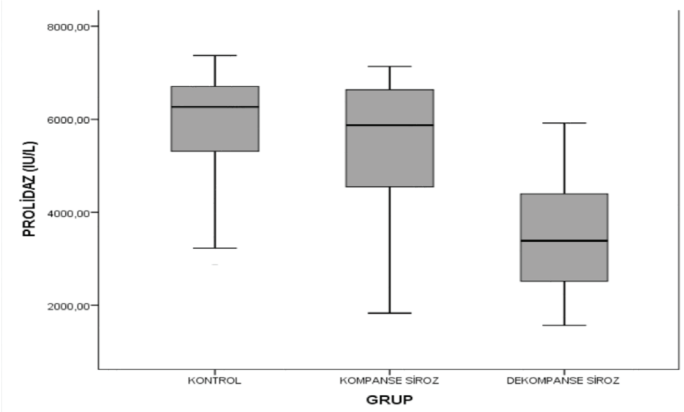

Figure 1. Mean, standard deviation and distribution of serum prolidase levels (IU/L) in patients with compensated cirrhosis, decompensated cirrhosis, and controls

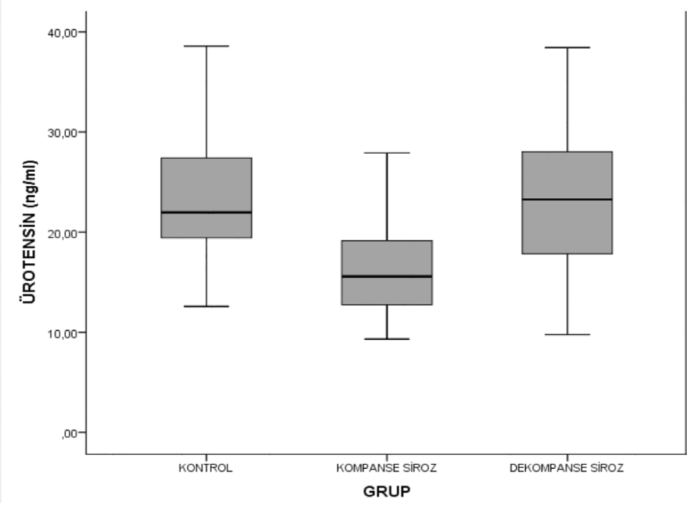

Figure 2. Mean, standard deviation and distribution of serum urotensin-2 levels (ng/ml) in patients with compensated cirrhosis, decompensated cirrhosis, and controls

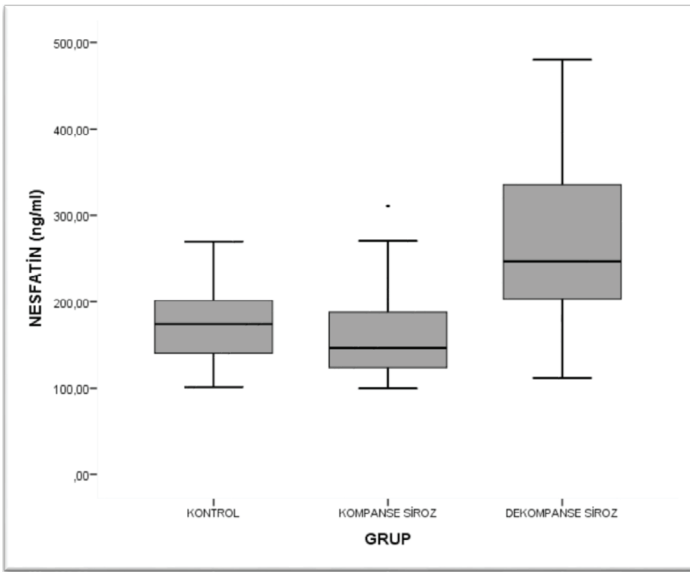

Figure 3. Mean, standard deviation and distribution of serum nesfatin levels (ng/ml) in patients with compensated cirrhosis, decompensated cirrhosis, and controls

DISCUSSION

Hepatic cirrhosis is characterized by hepatocellular injury and accompanying inflammatory infiltration that may arise due to a variety of causes. Prolonged inflammatory infiltration leads to the formation of excessive connective tissue deposition, i.e. fibrosis, in the liver. The pathogenesis is known to involve a number of factors such as the vascular alterations, which are thought to play a role in the inability to alleviate fibrosis resulting from repetitive injury (10). In this study we aimed to measure prolidase (an enzyme involved in collagen breakdown), urotensin-2 (a vasoconstrictor peptide), and nesfatin (involved in the regulation of nutrition) levels and to determine the feasibility of using these molecules as potential laboratory markers in cirrhotic patients.

Prolidase is actively involved in the breakdown of collagen and in the recycling of proline in collagen synthesis. Since prolidase is the only enzyme breaking down the peptide bond between proline and glycine, its activity is considered to be directly related with the rate of collagen turnover (11). In a study by Çelik et al. cirrhotic patients were found to have significantly lower serum prolidase levels than controls, and the authors suggested that prolidase activity may reflect impaired collagen metabolism in this degenerative liver disease due to the altered collagen turnover (12). Similarly, we observed significantly lower serum prolidase levels in the overall cirrhosis group as compared to controls. On the other hand, although the difference between decompensated cirrhotic patients (a more advanced disease stage) and controls was significant, no such differences could be detected between controls and compensated patients. This may be due to the fact that serum prolidase may be lowered in the more advanced stages of cirrhosis due to impaired collagen metabolism, and that this reduction in prolidase may have a role in the progression of cirrhosis. Accordingly, serum prolidase levels may potentially be used as a marker of

collagen tissue injury.

Urotensin-2 is a potent vasoconstrictor hormone primarily affecting the cardiac, renal, and vascular systems (12). In a study by Kemp et al. an increase in portal pressure and hepatic fibrosis was shown with urotensin-2 infusions (14). Again, Liu et al. examining the alterations in urotensin-2 in patients with cirrhosis and portal hypertension, elevated levels were found (14). Pawer et al. again found elevated urotensin-2 in children with severe portal hypertension, suggesting that urotensin-2 may correlate with the severity of the liver disease and that urotensin-2 may be used as a marker of severe portal hypertension (15). In this study, while serum urotensin-2 was significantly lower in patients with compensated cirrhosis as compared to controls, the difference between controls and the overall cirrhotics and decompensated cirrhotics was not significant. Lower urotensin-2 levels in compensated cirrhosis patients than in controls seems to suggest that urotensin-2 may play a role in the pathogenesis of cirrhosis via differential mechanisms, and further studies are warranted to test its usability as a marker in this disease.

Nesfatin is secreted from the pituitary gland in nuclei regulating the nutrition. Nesfatin is synthetized after degradation of the prohormone by a convertase, and it has been shown to reduce feeding behavior and bodyweight (16). Nesfatin is not only found in the brain tissue, but also in peripheral sites such as the adipose tissue, stomach, pancreatic islets, liver, and the testicles, although its exact function in these locations remains unknown (8). Also, the relationship between serum nesfatin and body mass index (BMI) has been examined. Stengel et al. reported a reduced food intake and bodyweight by nesfatin (17). In one study involving patients with non-alcoholic fatty liver disease, serum nesfatinlevels were found to be reduced (18). Our literature search did not reveal any studies examining serum nesfatin levels in cirrhotic patients, and therefore this study is the first of its kind in this regard. According to our results, overall group of cirrhotics and decompensated cirrhotics had significantly higher serum nesfatin levels than controls. Nesfatin may play a role in the development of appetite and cachexia in cirrhotic patients, and particularly in patients with decompensated cirrhosis, and therefore it may be used as a marker for diagnosis and treatment in this condition.

CONCLUSION

In conclusion, it seems that serum prolidase levels may have a potential role as a marker for follow-up and diagnosis in cirrhotic patients. Lower levels of urotensin-2, which is a potent vasoconstrictor, in patients with compensated cirrhotic it suggests that it may play a role in the pathogenesis of urotensin-2. Its role in pathogenesis and potential use as a marker requires further studies. Higher levels of nesfatin in the overall group of cirrhotic and decompensated cirrhotic suggests that it may play a role in the decreased appetite and cachexia, particularly in patients with decompensated cirrhosis, and it may be utilized as a marker.

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