Serum Levels of Carboxy-Terminal Propeptide of Type 1 Procollagen Do Not Reflect the Severity of Nonalcoholic Steatohepatitis

SERUM TİP 1 PROKOLLAJEN KARBOKSİ-TERMİNAL PROPEPTİD DÜZEYLERİ NON-ALKOLİK STEATOHEPATİT ŞİDDETİNİ YANSITMAZ

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Abstract

Objective: High serum levels of carboxy-terminal propeptide of Type 1 procollagen (PICP) were reported in patients with chronic viral hepatitis, alcoholic liver disease and cirrhosis as a marker of liver fibrosis. However, its status in nonalcoholic steatohepatitis (NASH) is uncertain. The aim of this study was to determine serum PICP levels in NASH patients comparing with cirrhotic patients and healthy individuals, and its association with the degree of necroinflammatory activity and fibrosis.

Material and Methods: PICP levels were measured by a specific enzyme immunoassay in 29 patients with biopsy-proven NASH, 18 patients with cirrhosis and 18 healthy controls. Histological grading and staging for NASH group were evaluated using Brunt's classification and the scores were compared with their PICP levels.

Results: Compared to controls (mean ± SD: 57.4 ± 17 ng/mL), PICP levels were significantly higher in cirrhotics (120.67 ± 102 ng/mL) and NASH patients (92.78 ± 33.6 ng/mL) [p= 0.004 and p< 0.001, respectively]; however, the concentrations did not differ between NASH and cirrhotic patients (p> 0.05). PICP levels did not correlate with necroinflammatory activity and fibrosis scores in NASH patients.

Conclusion: Serum PICP levels are elevated in NASH patients, similar to those with cirrhosis; however they do not reflect the degree of histological grade and stage.

Key Words: Procollagen Type 1 carboxy terminal peptide, liver cirrhosis

Özet

Amaç: Tip 1 prokollajen karboksi-terminal propeptid (PICP) yüksek serum düzeylerinin kronik viral hepatitli, alkolik karaciğer hastalığı ve sirozu olan hastalarda karaciğer fibrozisinin bir belirteci olabileceği bildirilmiştir. Ancak, bu belirtecin non-alkolik steatohepatit (NASH)'li hastalardaki düzeyleri bilinmemektedir. Bu çalışmanın amacı, NASH'lı hastalardaki serum PICP düzeylerini sirotik hastalar ve sağlıklı bireylerle karşılaştırarak tayin etmek ve nekroinflamatuar aktivite ve fibrozis derecesiyle ilişkisini belirlemekti.

Gereç ve Yöntemler: PICP düzeyleri biyopsiyle kanıtlanmış 29 NASH'li hastada, 18 sirozlu hastada ve18 sağlıklı kontrolde EIA'le ölçüldü. NASH grubunda histolojik derecelendirme ve evrelendirme Brunt sınıflaması kullanılarak yapıldı ve skorlar PICP düzeyleriyle karşılaştırıldı.

Bulgular: Kontrollerle karşılaştırıldığında (Ort. ± SD: 57.4 ± 17 ng/mL) PICP düzeyleri sirotiklerde (120.67 ± 102 ng/mL) ve NASH hastalarında (92.78 ± 33.6 ng/mL) anlamlı bir şekilde daha yüksekti [sırasıyla, p= 0.004 ve p< 0.001]; ancak konsantrasyonlar NASH ve siroz hastalarında farklılık göstermiyordu (p> 0.05). PICP düzeyleri NASH hastalarında nekroinflamatuar aktivite ve fibrozis skorlarıyla korele değildi.

Sonuç: Serum PICP düzeyleri sirozlu hastalardakine benzer şekilde NASH'lı hastalarda yükselmiştir, ancak bu yükselme histolojik derece ve evre şiddetini yansıtmamaktadır.

Anahtar Kelimeler: Tip 1 prokollajen karboksi-terminal propeptid, karaciğer fibrozisi

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NASH, which is the most severe form of nonalcoholic fatty liver disease, may lead to end stage liver disease showing a progressive course.[1] Thus, it is important to identify patients who are at risk of progression to cirrhosis. Patients with high levels of liver enzymes and high grade of inflammation and stage of fibro-

sis are considered to have a higher risk of progressive liver disease. Although qualitative or quantitative measurements of extracellular matrix components are the most reliable methods in determining the degree of hepatic fibrosis, the use of histological methods, especially in follow-up, is not a practical approach due to the invasiveness of liver biopsy procedure. Recently, several studies have focused on serological fibrosis markers, which have no risk for patients, allow reproducible measurements and are well correlated with the degree of fibrosis determined by histological examinations. Among these, carboxy-terminal propeptide of Type 1 PICP assay reflects synthesis of Type 1 collagen in the body and high serum levels of this peptide were reported in patients with chronic viral hepatitis, alcoholic liver disease and cirrhosis as a marker of liver fibrosis.[2-5] However, its status in NASH is uncertain. The aim of this study was to determine serum PICP levels in NASH patients comparing with cirrhotic patients and healthy individuals, and its association with the degree of necroinflammatory activity and fibrosis.

Material and Methods

Patients: Twenty-nine patients with biopsy proven NASH (24 males, 5 females, mean age: 41.7 ± 11; range: 20-65), 18 cirrhotic patients (11 males, 7 females; mean age: 60.5 ± 9.9; range: 41-76) and 18 healthy subjects (13 males, 5 females; mean age: 45.2 ± 11; range: 25-65) as a control group were included in the study. All NASH patients had persistently elevated liver enzymes and fatty liver detected by ultrasonography. Definitive diagnosis of NASH was histologically established according to the liver biopsy findings showing steatosis, ballooning and lobular and portal inflammation with fibrosis. Histological necroinflammatory activity (grading) and severity of fibrosis (staging) were evaluated using Brunt's classification.[6] According to this classification, separate scores of steatosis, ballooning and inflammation (lobular and portal) were combined (global grading) and the results were evaluated as mild (Grade-1), moderate (Grade-2) and severe (Grade-3). Fibrosis scores were classified as Stage-1 (zone 3 perisinusoidal/pericellular fibrosis), Stage-2 (as in Stage-1 with additional portal fibrosis), Stage-3 (bridging fibrosis), and Stage-4 cirrhosis. Laboratory tests for HBsAg, anti-HBcIgG, antiHCV, autoimmune markers (ANA, AMA, ASMA, anti-LKM1) were negative and iron and copper profile and α1-antitrypsin levels were normal in all NASH patients. There was no history of alcohol consumption (none or less than 20 grams of ethanol per week), total parenteral nutrition, rapid weight loss, extensive small bowel resection, gastroplasty for morbid obesity and use of the drugs known to increase hepatic steatosis (corticosteroids, methotrexate, estrogen etc.). Of 29 NASH patients, 24 were associated with obesity and/or hyperlipidemia; three patients had Type 2 Diabetes mellitus with additional obesity and hyperlipidemia. No risk factor (s) for NASH were identified in 2 patients.

Diagnosis of cirrhosis was established by liver biopsy in 7 out of 18 patients and by clinical, laboratory, abdominal ultrasound, Doppler ultrasound and endoscopic findings in the remaining 11 patients. Of 18 cirrhotic patients, 14 were Child-B class and 4 were Child-C class according to Child-Pugh classification. Etiology of cirrhosis was HBV infection in 6 (with alcohol in one) cases, alcohol in 5, HCV infection in 4 (with alcohol in 2), Type 1 autoimmune hepatitis in 2, and cryptogenic in one.

In all control subjects, liver function tests (AST and ALT) were normal; there were also no serological evidence for HBV and HCV, and fatty liver detected by ultrasound and history of underlying disease.

PICP measurement: In order to determine PICP levels in serum samples a sandwich enzyme immunoassay was used (Metra Biosystems, Inc., Mountain View, CA94043, USA). The antibodies demonstrated 100% cross reactivity with PICP in human serum. Since the number of cases enrolled was limited, we could not establish our own analytical performance characteristics. Therefore, we used analytical performance data suggested by the manufacturer. The intraassay coefficients of variation were

5.5% and 6.8% at levels of 98.1 and 80.8 ng/ml, respectively. The interassay coefficients of variation were 5% and 7.2% at 98.1 and 296.7 ng/mL, respectively. Recovery determined by adding known quantities of purified PICP to serum samples were 96% and 102% at levels of 32.7 and 31.7 ng/mL, respectively. Analytical sensitivity was 0.2 ng/mL.

Statistical analysis

Statistical analysis were performed using the chi square test for non-numerical values and Kruskal Wallis test for numerical values. Because the numerical values in the groups did not show a normal distribution, we used Kruskal Wallis test as a nonparametric test. If median values were found to be different in Kruskal Wallis test, comparisons among groups were done with the Mann Whitney U test; in this case, a p value of less than 0.016 (0.05/3) was considered significant because a total of three comparisons were made. Correlations among ordinal, numerical or ordinal and numerical variables were evaluated by Pearson and Spearman coefficients of correlation test. A p value of <0.05 was considered significant except for the analysis of parametric values noted above.

The study was approved by the Dokuz Eylül Research Foundation Ethical Committee and informed consent was taken from all subjects participating in the study.

Results

General characteristics of the study groups are shown in Table 1. All study groups were similar with regard to sex and body mass index (BMI). While control and NASH group were similar with regard to age, mean age of the cirrhotic group was significantly higher than the age of both the control and NASH groups ($p< 0.001$). There was no correlation between age and PICP levels in all groups. Compared to controls (mean ± SD: 57.4 ± 17 ng/mL), PICP levels were significantly higher in cirrhotics (120.67 ± 102 ng/mL) and NASH patients (92.78 ± 33.6 ng/mL) [$p= 0.004$ and $p< 0.001$, respectively]; however, the concentrations did not differ between NASH and cirrhotic patients ($p> 0.05$) [Figure 1]. A total of 13 women in all study groups (4 in control group, 3 in NASH group and 6 in cirrhotic group) were in the postmenopausal period. Since low estrogen production or estrogen deficiency in post menopausal women may lead to additional increases in PICP levels, comparisons of the PICP levels were repeated after exclusion of all women in the study groups from the statistical analysis,.[7,8] Compared to controls

Table 1. Characteristics of the study groups.

	Control	Cirrhosis	NASH	p
Age (years)	45.3 ± 11	60.5 ± 9.9	41.7 ± 11	< 0.001*
Sex (male/female)	13/5	11/7	24/5	NS
BMI (kg/m^2), mean ± SD	25.2 ± 2.9	25.3 ± 3.5	28.3 ± 3.8	NS
ALT (IU/L), mean ± SD	15.9 ± 8.7	27.3 ± 20.8	99.9 ± 68	< 0.001**
Cholesterol (mg/dL), mean ± SD	-	143.8 ± 65.1	212.7 ± 47.2	< 0.001
Triglyceride (mg/dL), mean ± SD	-	77.4 ± 39.7	92.7 ± 33.6	< 0.001
PICP (mg/dL), mean ± SD	57.4 ± 17	120.7 ± 101.9	92.7 ± 33.6	= 0.001***
Histological grading (global grading)	-	-		-
Mild			15	
Moderate			11	
Severe			3	
Histological staging	-	-		-
Stage-1			17	
Stage-2			8	
Stage-3			4	
Stage-4			-	

Statistically significant differences were detected *Between the cirrhotic group and both the control and NASH groups, **Between NASH group and both the cirrhotic and control groups, and ***Between the control group and both the cirrhotic and NASH groups. NS: Not significant.

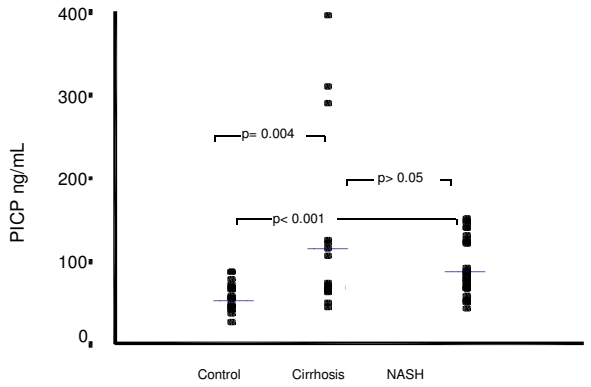

Figure 1. Scatter-plot graphics of serum PICP levels for all groups with horizontal lines indicating mean values.

(mean ± SD: 61.3 ± 13.2 ng/mL), once again, PICP levels were significantly higher in NASH patients (mean ± SD: 95.4 ± 35 ng/mL) [p= 002]. Although PICP levels of cirrhotics (mean ± SD: 126.3 ± 112.4 ng/mL) were higher than that of controls, there was a borderline significance (p= 0.055). PICP levels did not differ between NASH and cirrhotic patients (p> 0.05). Because exclusion of women had no effect on the general results of the first comparisons of PICP levels between the groups, other statistical analyses were performed using the original sample size. In this context, PICP levels did not correlate with age, BMI, ALT, cholesterol, triglyceride, steatosis, ballooning, inflammation (lobular and portal), global grading and fibrosis scores in NASH patients. In NASH group, analyses of correlation among AST, ALT, cholesterol and triglyceride levels and histological scores demonstrated that there was a weak-moderate correlation between triglyceride levels and fibrosis scores (r_s= .43, p= 0.03) and between global grading and fibrosis scores (r_s= .42, p= 0.03). Among components of global grading, only portal inflammation scores showed moderate correlation with fibrosis scores (r_s= .53, p= 0.04).

Discussion

The increase in PICP levels was similar in cirrhotic and NASH patients in this study. Because PICP may closely be associated with bone metabolism and women in all groups were mostly in the postmenopausal period, estrogen deficiency in these subjects may have led to additional increases in PICP levels. However, after excluding all women from the study, the statistical analyses of the repeated comparisons of PICP levels did not differ from the first results. Therefore, we may suggest that a probable estrogen deficiency in postmenopausal women had no effect on PICP levels in this study. In this context, the finding that the mean age of cirrhotic patients was higher than those of both NASH and control group does not seem to have any effect on PICP levels again, because we did not find any correlation between age and PICP levels in all groups. Moreover, although PICP levels were increased in NASH patients, no positive correlations were present between PICP levels and both the degree of necroinflammatory activity and fibrosis scores. Besides, there were considerable overlapping values of PICP among the study groups. Thus, we suggest that PICP is not a sensitive marker for the measurement of the degree of fibrosis and hepatic inflammation. However, we interestingly found a weak-moderate positive correlation between triglyceride levels and fibrosis scores.

There are only a few studies examining PICP levels in chronic liver diseases. In two separate studies including patients with various etiologies of chronic hepatitis (HBV-related, autoimmune, drug induced, unknown etiology) and alcoholic liver disease, Trinchet et al found a positive correlation between fibrosis scores and Type 1 collagen and suggested this peptide as a fibrosis marker for initial assesment and follow-up in these patients.[9,10] Savolainen et al reported that PICP values were less increased in patients with fatty liver and those with fatty liver and early fibrosis when compared to patients with alcoholic hepatitis and cirrhosis.[3] They also showed that there was a positive correlation between PICP levels and both hepatic inflammation and fibrosis. However, the patients in this study were mostly alcoholic. Lin et al reported increased PICP levels in patients with chronic viral hepatitis.[5] PICP levels before and after progressing to cirrhosis were similar, as in the comparison of PICP between our NASH and cirrhotic patients.

However, the authors did not perform correlation analysis between PICP levels and fibrosis scores at the chronic hepatitis stage. In another study by Fabris et al, although PICP levels decreased in both responders and non-responders of chronic hepatitis C patients treated with interferon, fibrosis scores remained unaltered in both groups.[11]

In conclusion, we found that serum PICP levels increased in NASH patients, similar to values found in cirrhotic patients. We also did not find any correlation between these increased values and histological degree of fibrosis. Therefore, we consider that PICP is not a sensitive marker of liver fibrosis in NASH patients and may not be used either for the initial assesment or for follow-up in such patients to monitor disease progression. However, the reason for the increased PICP levels in NASH similar to those in cirrhosis should be elucidated. One possible explanation may be the dysfunction of sinusoidal endothelial liver cells and their receptors; in normal conditions, circulating PICP is mainly cleared via the mannose receptors in these cells. This function may be disturbed in NASH, as in other chronic and fibrosing liver diseases.[12,13] Thus, other extracellular matrix components in liver tissue with their concomitant serum levels and their clinical significance should be investigated in NASH patients. Further studies also seem to be worth evaluating triglyceride as a marker of hepatic fibrosis.

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