Future Biochemistry and Bioscience
Yenilikçi Biyokimya ve Biyobilim

FBB 2022;4(2):41-48
http://doi.org/10.48086/ASD0041

Araştırma Makalesi/Research Article

Azalan Pentraxin-2 Seviyesi, Non-invaziv Bir Biyobelirteç Olarak Non-alkolik Steatohepatit ile İlişkilidir, Ancak Hastalığın Şiddeti ile İlişkili Değildir; Bir Ön Çalışma

Decreased Pentraxin-2 Level Is Associated with Non-alcoholic Steatohepatitis as A Non-invasive Biomarker But Not The Severity of The Disease; A Preliminary Study

Umut Karabay[1*], Durmuş Ayan[2*], Emrah Erkan Mazı[3], Ersin Vanlı[4], Müveddet Banu Yılmaz Özgüven[5], Rabia Karasu[6], Fatih Borlu[3], Sibel Söylemez[7]

[1]Gülhane Research and Training Hospital, Department of Internal Medicine, Ankara
[2]Niğde Research and Training Hospital, Department of Medical Biochemistry, Niğde
[3]Seyrantepe Hamidiye Etfal Research and Training Hospital, Department of Internal Medicine, Istanbul
[4]Seyrantepe Hamidiye Etfal Research and Training Hospital, Department of Radiology, Istanbul
[5]Başakşehir Çam and Sakura City Hospital Department of Pathology, Istanbul
[6]Baltalimanı Research and Training Hospital, Department of Radiology, Istanbul
[7]Gazi University Life Sciences Research and Application Center, Department of Medical Biochemistry, Ankara

Öz

Amaç: Alkole bağlı olmayan steatohepatit (NASH), karaciğerde aşırı yağ birikmesi, fibrozis ve karaciğer iltihabı ile karakterize bir tür karaciğer hastalığıdır. Pentraxin-2 (PTX-2), inflamasyonu ve fibrozisi engelleyen bir serum proteinidir. Bu çalışmada, NASH'da biyopsi sonuçları ile PTX-2 arasındaki olası ilişki hakkında bilgi edinirken, PTX-2'nin NASH tespitinde biyobelirteç olarak rolünü araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmaya NASH tanısı ile takip edilen toplam 65 hasta ve 15 sağlıklı gönüllü dahil edildi. Hastaların yaş, cinsiyet, kilo, boy, vücut kitle indeksi (VKİ) ve bel çevresi gibi demografik verileri kaydedildi. PTX-2 düzeyleri enzyme-linked immunosorbent assay ile ölçüldükten sonra biyokimyasal, hematolojik parametreler ve karaciğer biyopsi sonuçları ile birlikte değerlendirildi. Karaciğer fibrozunun şiddeti National Institute of Diabetes and Digestive and Kidney Diseases Nonalcoholic Steatohepatitis Clinical Research Network skorlama sistemine göre yorumlandı.

Bulgular: Hasta grubunda PTX-2 düzeyleri, sağlıklı bireylere göre istatistiksel olarak anlamlı düzeyde daha düşüktü ($p<0,001$). Hastaların PTX-2 düzeyi ile karaciğer biyopsi sonuçları arasında istatistiksel olarak anlamlı bir ilişki yoktu. Hasta grubunda PTX-2 düzeyi ile alkalen fosfataz düzeyleri arasında istatistiksel olarak negatif bir korelasyon vardı ($r=-0.294$, $p=0.017$). PTX-2 düzeyi ile diğer biyokimyasal ve hematolojik parametreler arasında istatistiksel olarak anlamlı bir ilişki yoktu.

Sonuç: Serum PTX-2 seviyelerinin NASH öngören invaziv olmayan bir biyobelirteç olabileceğini söyleyebiliriz ancak serum PTX-2 düzeyleri tek başına hastalığın şiddeti ve yağlanmanın derecesi hakkında bilgi edinmek için yetersizdir.

Anahtar kelimeler: Karaciğer yağlanması, NASH, Pentraxin-2, Fibrosis, Karaciğer biyopsi

Abstract

Objective: Non-alcoholic steatohepatitis (NASH) is a type of hepatic disease characterized by excessive fat accumulation in the liver, fibrosis and hepatic inflammation. Pentraxin-2 (PTX-2) is a serum protein that inhibits inflammation and fibrosis. In this study, we aimed to investigate the role of PTX-2 as a biomarker in detecting NASH while obtaining information about the possible relationship between biopsy results and PTX-2 in NASH.

Materials-Methods: A total of 65 patients followed up with the diagnosis of NASH and 15 healthy volunteers were included in the study. Demographic data such as age, gender, weight, height, body mass index (BMI) and waist circumference of the patients were recorded. After PTX-2 levels were measured by enzyme-linked immunosorbent assay, Biochemical, hematological parameters, and liver biopsy results were compared with PTX-2 levels. The severity of liver fibrosis was interpreted according to National Institute of Diabetes and Digestive and Kidney Diseases Nonalcoholic Steatohepatitis Clinical Research Network Scoring System by the same pathologist. Liver biopsies were also performed by the same radiologist.

Results: The PTX-2 levels were statistically lower in the patient group compared with healthy subjects ($p<0.001$). No statistically significant correlation was found between PTX-2 level and liver biopsy results of patients. PTX-2 level was negatively correlated with alkaline phosphatase in the patient group ($r=-0.294$, $p=0.017$). There was no statistically significant correlation between PTX-2 level and the other biochemical and hematological parameters.

Conclusion: In conclusion we could mention that serum PTX-2 levels may be used as a non-invasive biomarker in the prediction of NASH. However, serum PTX-2 levels are insufficient to obtain information about the severity of the disease and the degree of steatosis.

Key Words: Non-alcoholic steatohepatitis, NASH, Pentraxin-2, fibrosis, liver biopsy.

İletişim adresi / Address for correspondence:

Uzm.Dr.Durmuş Ayan https://orcid.org/0000-0003-2615-8474
Niğde Research and Training Hospital, Department of Medical Biochemistry/ Niğde
E-mail: durmusayan@hotmail.com
Tel: +90 553 633 81 85
*** These authors contributed equally**
Umut Karabay: https://orcid.org/0000-0002-9632-360X, Emrah Erkan Mazı: https://orcid.org/0000-0003-4879-7584
Ersin Vanlı: https://orcid.org/0000-0002-8923-6348, Müveddet Banu Yılmaz Özgüven: https://orcid.org/0000-0002-3540-4772
Rabia Karasu: https://orcid.org/0000-0002-5538-5425, Fatih Borlu: https://orcid.org/0000-0003-4248-3066
Sibel Söylemez: https://orcid.org/0000-0002-5005-2277
Geliş Tarihi/Received:25 Eylül 2022.Kabul Tarihi/Accepted: 25 Kasım 2022.Çevrimiçi Yayın:Published Online: 30 Aralık 2022

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is classified histologically as non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NASH is a type of hepatic disease characterized by excessive fat accumulation in the liver, fibrosis, and hepatic inflammation[1,2]. NASH merely differs from fatty liver, in which there is only an accumulation of fat without fibrosis or inflammation[3]. NASH could progress to cirrhosis in up to 20% of patients and it rarely causes hepatocellular carcinoma[4]. NASH is the second major etiology of liver disease among adults who awaiting transplantation in the United States of America[5]. The average prevalence of NASH has been reported between 1.5% and 6.45%[6]. However, since liver biopsy is not operable in the general population, we have no studies that could accurately assess the prevalence or incidence of NASH [6].

The risk factors for NASH include the increasing epidemic of obesity, dyslipidemia, and insulin resistance [7]. Diabetes is the main predictor of NASH [8]. Based on these risk factors, studies have reported that NASH may have a crucial role in the development of other metabolic diseases such as cardiovascular diseases and chronic kidney disease [9, 10].

Liver biopsy remains the gold standard for making a definitive diagnosis of NASH, which is a clinicopathological presence [4]. However, its routine use is controversial due to its invasive procedure, high costs, and related complications [11, 12]. Therefore, researchers have begun to search for new non-invasive methods that could predict the severity of NASH. Methods such as NASH fibrosis score, fibrotest, and transient elastography (fibroscan) have yielded successful results in predicting fibrosis[13].

Numerous studies have shown that Pentraxin-2 (PTX-2), a member of the Pentraxin family prevents the development of fibrosis by regulating monocyte/macrophage differentiation and the PTX-2 level is inversely correlated with the histologically demonstrated fibrosis degree[14,15]. PTX-2 is a 27-kDa protein that is produced by the liver, secreted into the blood, and circulates as stable 135-kDa pentamers[16]. PTX-2 decreases the adhesion of neutrophils to the extracellular matrix, inhibits the differentiation of monocytes to fibrocytes, reduces profibrotic macrophages, and promotes phagocytosis of cell debris[17].

We investigated the role of PTX-2 as a biomarker in detecting NASH while obtaining information about the possible relationship between biopsy results and PTX-2 in NASH.

MATERIAL and METHODS

This is a multi-center prospective study. A total of 65 (33% male, 32% female) patients aged between 20-75 years, followed-up with the diagnosis of NASH and had biopsy indication with the suspicion of NASH-fibrosis who applied to Şişli Hamidiye Etfal Research and Training Hospital between 01/03/2016 and 01/09/2016 were included in the study. Patients with persistently elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) despite losing at least 5% of their weight through appropriate diet and exercise and who had a NAFLD fibrosis score (NFS)>0.676 (high probability of fibrosis) were included in the study. NAFLD fibrosis score was determined by liver biopsy and the consent was obtained from patients before liver biopsy. The diagnosis of NAFLD was based on a liver biopsy showing steatosis in at least 5% of hepatocytes or fatty infiltration of the liver confirmed by imaging study (ultrasound, computed tomography, or magnetic resonance imaging). The staging of fibrosis was divided into fibrosis stage 0 to stage 4 using the Brunt criteria[18].

Patients who diagnosed with both viral, ischemic, autoimmune hepatitis, hemochromatosis, Wilson disease, alpha-1 antitrypsin deficiency, biliary disease, malignancy during outpatient examinations; those who were using herbal products, who were receiving hormone replacement therapy and using hepatotoxic drugs, and patients with normal AST and ALT levels during the outpatient examinations were excluded from the study. It was also done the exclusion of liver disease of other etiologies, including alcohol-induced liver disease (history of excessive alcohol consumption greater than 20 g/day).

A control group consisted of 15 healthy volunteers (20% male, 80% female) aged 19-68 who had no disease and did not take any medicine. Demographic data of patients were recorded during the anamnesis such as age, gender, weight, height, body mass index (BMI), and waist circumference. The blood samples taken from patients were stored at -80°C after centrifugation until the analysis for PTX-2.

Before the biopsy, hemogram and international normalized ratio (INR) results were evaluated in all patients, and the patients were questioned regarding anticoagulant and antiaggregant use. In all patients, the procedure was performed after six hours of fasting, and 70 ml of saline infusion per hour was administered intravenously, starting two hours before the process. Liver biopsies were performed in the interventional radiology unit using a convex transducer accompanied by ultrasonography (ACUSON S3000, Siemens Healthcare, Erlangen, Germany). All biopsies were performed from the right lobe of the liver with a 16 G fully automatic cutting needle under aseptic conditions using the free hand technique by the same radiologist.

The severity of liver biopsy results was interpreted by the same pathologist. Hematoxylin eosin, Periodic acid schiff (PAS), Diastase PAS, Masson trichrome, reticulin, and iron dyes were applied to biopsy materials, and tissue sections prepared 3-5 microns after paraffin follow-up. Fibrosis, Masson trichrome, and reticulin stains were applied. Finally, the preparations were examined under a light microscope. The presence of NAFLD, presence of fatty liver as compared with steatohepatitis, NASH activity score, and fibrosis stage were assessed with the use of the previously published NASH scoring system. Pathologist blinded to subjects' details evaluated biopsy specimens based on the presence of steatosis, inflammation, and ballooning and according to the decision of the hepatopathologist in terms of NASH presence or absence and scored each liver biopsy specimen using the National Institute of Diabetes and Digestive and Kidney Diseases Nonalcoholic Steatohepatitis Clinical Research Network scoring system. The histological NAFLD activity score (NAS) was defined as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3), and ballooning degeneration (0–2), thus ranging from 0 to 8. Fibrosis was staged as follows: stage 0, no fibrosis; stage 1, perisinusoidal or periportal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, bridging fibrosis; and stage 4, cirrhosis.

PTX-2 levels were measured by enzyme-linked immunosorbent assay (ELISA) in medical biochemistry research laboratory at Gazi University Faculty of Medicine. PTX-2 levels (ng/ml) were obtained with the Invitrogen Human ELISA kit (Catalog number: EH386RB) on the Chromate 4300 Microplate Reader (Roberlab., USA). The analytical sensitivity and assay range of ELISA kit is respectively 0.08 ng/mL and 0.08-20 ng/mL. Inter-assay CV and intra-assay CV of ELISA method is respectively <12% and <10%.

Fasting Blood Glucose (FBG), AST, ALT, gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), albumin, total bilirubin, direct bilirubin, triglyceride (TG), cholesterol, C-reactive protein (CRP), high-density lipoprotein (HDL), and total cholesterol levels were measured by spectrophotometric assay on Cobas c501biochemistry device (Roche Diagnostic, Germany). Low-density lipoprotein levels were calculated by the Friedewald formula. Ferritin levels were measured by electrochemiluminescence immunoassay (ECLIA) on Cobas e601 hormone device (Roche Diagnostic, Germany). HbA1c levels were measured by High-performance liquid chromatography (HPLC) assay on Bio-Rad D-10 analyzer. Hemoglobin and platelet (PLT) levels were analysed on the SYSMEX XN 1000 Hematologyanalyzer (Sysmex Corporation, Kobe, Japan). All analyses were measured in the medical biochemistry laboratory at Sisli Hamidiye Etfal Research and Training Hospital.

Ethics Considerations

This study was approved by the local ethics committee of Sisli Hamidiye Etfal Research and Training Hospital with the 2016 dated and 704 numbered decision. All patients were informed about the objectives of the study in detail and gave written informed consent. This study was conducted in accordance with the

ethical principles of the Declaration of Helsinki revised in 2013.

Statistical Analysis

SPSS 15.0 for Windows program was used for statistical analysis. While descriptive statistics were given as numbers and percentages for categorical variables, were given as minimum, maximum, and median for numerical variables. Comparison of two independent groups were made with Student's t-test when the numerical variables met the normal distribution condition, and with the Mann-Whitney U test when the normal distribution condition did not meet. The relationships between numerical variables were analyzed by Spearman correlation since the parametric test condition was not met. The rates in the groups were compared with Chi-Square analysis. The statistical alpha significance level was accepted as $p<0.05$.

RESULTS

A total of 65 patients diagnosed with NASH were included in the study. The control group consisted of fifteen healthy volunteers. The median value of weight, BMI, and waist circumference in the patient group were statistically significantly higher than the control group. (Table 1).

Table 1. General characteristics of the groups

	Patient Group (n=65)		Control Group (n=15)		p value
	Median	Min-Max	Median	Min-Max	
Age (year)	48	20-75	36	19-68	**0.029**
Weight (kg)	89	55-134	53	49-74	**<0.001**
Height (cm)	165	139-193	160	155-177	0.197
BMI (kg/m²)	32	20.4-46.9	20.6	19.1-24.4	**<0.001**
Waist Circumference (cm)	116	88-133	68	62-85	**<0.001**

Significant p values are given as bold

The median values of FBG, HbA1c, AST, ALT, GGT, ALP, Albumin, ferritin, total bilirubin, direct bilirubin, TG, cholesterol, and CRP in patient group were statistically significantly higher than control group. The median values of HDL and PTX-2 in the patient group were statistically significantly lower than the control group (for all, $p<0.05$). Comparison of biochemical parameters between the two groups are given in Table 2.

Table 2. Comparison of biochemical parameters between the groups.

Test/Unit	Patient Group (n=65) (NASH)		Control Group (n=15)		p value
	Median	Min-Max	Median	Min-Max	
FGB mg/dL	105	74-429	88	71-102	**<0.001**
HbA1c%	6.3	4.7-13.3	5.6	4.9-6	**<0.001**
AST U/L	44	21-238	16	13-27	**<0.001**
ALT U/L	73	43-280	16	8-41	**<0.001**
GGT U/L	40	17-386	18	7-30	**<0.001**
ALP U/L	73	43-324	64	48-78	**0.011**
Albumin g/dL	4.6	4-5.3	4.3	3.8-5.3	**0.048**
Ferritin ng/mL	100	19-1094	29	3-159	**<0.001**
Total Bilirubin mg/dL	0.5	0.2-1.6	0.4	0.2-0.5	**0.008**
Direct Bilirubin mg/dL	0.2	0.1-0.9	0.1	0.1-0.5	**0.010**
TG mg/dL	155	40-612	92	51-141	**<0.001**
LDL mg/dL	117	10-251	116	75-166	0.718
HDL mg/dL	42	23-70	52	42-78	**<0.001**
Cholesterol mg/dL	194	82-358	180	141-274	0.259
CRP mg/dL	3.4	3-23	3	2-5	**<0.001**
Hemoglobin g/dL	14	10.5-16.9	12.7	11.7-15	**0.005**
PLT (x 10^3 /L)	235	124-381	252	190-377	0.249
PTX-2 ng/mL	5.9	0.8-29.1	19.1	6-34.4	**<0.001**

Significant p values are given as bold
FBG: Fasting Blood Glucose, TG: Triglyceride, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein, CRP: C-reactive protein, PLT: Platelet, PTX-2: Pentraxin-2

PTX-2 had a 95% predictive value showing whether fibrosis develops in chronic liver diseases (Figure 1).

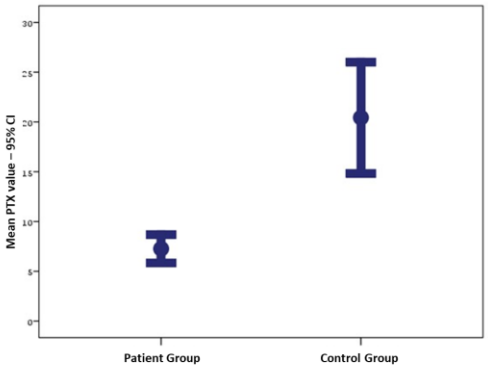

Figure 1. Box plot diagram showing PTX-2 levels between the patient and control group indicating that PTX-2 has a 95% predictive value in showing whether fibrosis develops in chronic liver diseases.

According to Spearman's correlation analysis, PTX-2 level was found to be negatively correlated with ALP in the patient group ($r=-0.294$, $p=0.017$). There was no statistically significant correlation between PTX-2 level and the other parameters. Median, minimum

and maximum of lobular activity score and biopsy results for the patient group were given in Table 3.

Table 3. Lobular activity score and biopsy results for the patient group

Test/Unit			Median (Min-Max)
Lobular activity score			5.0 (2.0-8.0)
		n	%
Grade	1	26	40.0
	2	20	30.8
	3	19	29.2
Fibrosis	1	13	20.0
	2	38	58.5
	3	14	21.5
Ballooning	0	3	4.6
	1	44	67.7
	2	18	27.7
Lobular inflammation	1	20	30.8
	2	29	44.6
	3	16	24.6
Lobular activity	None (1-2)	2	3.1
	Possible (3-4)	22	33.8
	Definitive (5-8)	41	63.1

No statistically significant correlation was found between PTX-2 level and biopsy results of patients (Table 4). In addition, there was no statistically significant difference in median of PTX-2 in lobular activity groups (p=0.593). Figure 2 shows the correlation between PTX-2 and NASH.

Table 4. The correlation results between PTX-2 level and biopsy results

	Pentraxin (PTX-2) ng/mL	
	Spearman's Rho	**p value**
Grade	0.033	0.791
Fibrosis score	0.139	0.271
Ballooning grade	-0.058	0.649
Lobular inflammation	-0.134	0.288
Lobular activity score	-0.071	0.574

PTX-2: Pentraxin-2

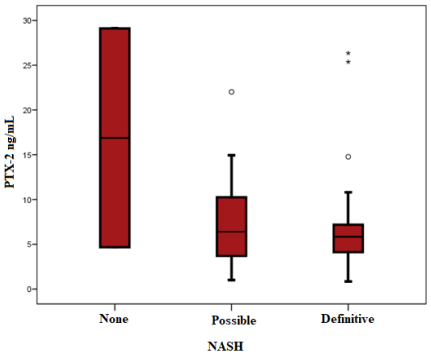

Figure 2. Decrease in serum PTX-2 levels relative to the degree of NASH

DISCUSSION

NAFLD is the most common chronic liver disease with a worldwide prevalence of 20–30%[6]. The potential of the disease for progression has been well-established and it is estimated that NAFLD will become the leading indication for liver transplantation in the future[19]. NAFLD involves a wide spectrum of diseases ranging from NAFL to NASH, cirrhosis, and HCC. NASH is characterized by the accumulation of hepatocellular fat accompanied by the ballooning of hepatocytes and lobular inflammation. Lipogenesis, inflammation, metabolic disturbances, fibrogenesis, and oxidative stress play a major role in the pathogenesis and progression of NASH [20].

The gold standard for diagnosis of NASH remains biopsy, although it is only performed in patients with a poor prognosis and progressing to cirrhosis. On the other hand, liver biopsy is an invasive method, which may result in complications[21]. Furthermore, using liver biopsy in the diagnosis of NASH is neither practical nor cost-effective. The absence of another descriptive criterion or a non-invasive marker causes insufficiency in diagnosis. Within this context, studies have focused on

several clinical and biochemical parameters and various imaging methods. Fibrosis score, fibrotest, fibroscan, HsCRP, ferritin are among these methods[13,21]. Fibrosis score and fibrotest are used in advanced form of NASH, while fibroscan is used in detecting fibrosis[13]. However, its availability is limited, making the diagnosis through fibroscan challenging. Therefore, studies have been continued on practical biochemical parameters to detect fibrosis[22].

In the present study, we demonstrated the role of PTX-2 in diagnosing NASH. PTX-2 levels were evaluated in human subjects undergoing liver biopsy for the first time. In previous years, it has been shown that inflammation markers such as CRP, a member of the pentraxin family, are increased in NAFLD, and it has been suggested that high sensitivity CRP (hs-CRP) can be used as a marker in NAFLD progression[23]. Yoneda et al. have mentioned that hsCRP significantly elevated in patients with NASH compared to those with simple non-progressive steatosis[13]. In our study, CRP was significantly higher in the NASH patient group when compared to healthy controls. However, the use of hs-CRP as a marker in the progression of NAFLD is controversial[24].

The plasma level of PTX-3 could find to be higher than normal controls in various systemic inflammatory conditions such as rheumatological diseases, asthma, coronary artery diseases, vasculitis, and sepsis[25]. While Gurel et al. have found that PTX-3 was strongly associated with endothelial dysfunction in subjects with NAFLD[26]. Another study has demonstrated markedly higher PTX3 levels in NAFLD patients compared with controls, and in biopsy-proven NASH patients compared with non-NASH ones. The authors concluded that plasma PTX3 may be a promising biomarker for the presence of NASH[27]. As a result of the study conducted in chronic hepatitis patients, it was determined that PTX-3 levels were lower than in the control group, and it was reported that a significant decrease in PTX-3 levels in patients with stage 1 fibrosis would be beneficial for the early diagnosis of fibrosis[28].

PTX-2 is known to play an important role in the regulation of fibrogenesis and wound healing, as well as inhibition of hepatic stellate cell activation, and recombinant PTX-2 protein replacement is promising[29]. Studies have shown that PTX-2 levels are decreased in idiopathic end-stage renal disease, pulmonary fibrosis, and non-alcoholic steatohepatitis[15,30,31].

Studies on animal models have shown that exogenous administration of PTX-2 reduced bleomycin TGF-induced lung fibrosis [32] and the progression of liver fibrosis[29]. Dillingh et al have compared the PTX-2 levels in healthy volunteers and patients with pulmonary fibrosis and found that PTX-2 levels were found to be lower in the group with fibrosis than in those without. They determined that there was a decrease in the percentage of circulating fibroblasts when recombinant PTX-2 was given to this group as an infusion[32]. Based on the results of these studies, the use of PTX-2 has become a current issue both for treatment purposes as it prevents fibrosis against tissue damage and as a screening marker because its level decreases as the fibrosis rate increases[34]. Besides exogenous PTX-2 application, evaluation of PTX-2 level is also recommended for screening purposes in NASH patients who developed fibrosis[15]. Verna et al. have shown that PTX-2 levels were significantly reduced in patients with NAFLD and in patients with fibrosis when compared to healthy controls. However, they could not reach any significant correlation result between the degree of steatosis and the level of PTX-2[15]. Although it is mentioned that PTX-2 has effects on wound healing, Blink et al. have stated that administration of PTX-2 (PRM-151) in patients with idiopathic lung fibrosis was not beneficial[35]. On the contrary, another study has reported that patients with idiopathic pulmonary fibrosis benefited from PTX-2 administered for 28 weeks by infusion[36].

According to our result, serum PTX-2 levels of patients with NASH were significantly lower than healthy control participants. However, there is no significant correlation between serum PTX-2 levels with the degree of steatosis and liver biopsy results.

CONCLUSION

In conclusion, we might notify that serum PTX-2 levels might be a non-invasive biomarker predicting NASH. On the other hand, we could mention that serum PTX-2 levels are insufficient to obtain information about the severity of the disease and the degree of steatosis. In order to better understand the role of PTX-2 in NASH pathology, studies with larger participants and examining changes in PTX-2 protein gene expression are needed. Finally, we believe that our research will guide prospective studies to be planned in the future.

LIMITATIONS

Although our study was well designed, there were some limitations. Firstly, we had an insufficient number of patients. Our control group number was limited and not age-matched with the patient group. Secondly, we could not evaluate according to the stage of hepatic steatosis.

Etik Onay: Bu çalışma Sağlık Bilimleri Üniversitesi Şişli Hamidiye Etfal Eğitim ve Araştırma Hastanesi Kurumsal Etik Kurulu tarafından onaylanmıştır (karar no: 704, tarih: 16.08.2016)

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemektedir.

Finansal Destek: yok

Ethics Ethics Committee Approval: This study was approved by the Institutional Ethics Review Committee of University of Health Sciences Şişli Hamidiye Etfal Training and Research Hospital (decision no: 704, date: 16.08.2016)

Conflict of Interest: The authors declare no conflict of interest to disclose

Financial Support: None

REFERENCES

1. Sheka AC, Adeyi O, Thompson J, Hameed B, Crawford PA, Ikramuddin S. Nonalcoholic Steatohepatitis: A Review. Jama, 2020, 323(12):1175-83 doi: 10.1001/jama.2020.2298.
2. Diehl AM, Day C. Cause, Pathogenesis, and Treatment of Non-alcoholic Steatohepatitis. N Engl J Med. 2017, 377(21):2063-2072 doi: 10.1056/NEJMra1503519.
3. Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA; NASH Clinical Research Network (CRN). Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology, 2011, 53(3):810-820 doi: 10.1002/hep.24127.
4. Hashimoto E, Tokushige K. Hepatocellular carcinoma in non-alcoholic steatohepatitis: Growing evidence of an epidemic?.Hepatol Res, 2012, 42(1):1-14 doi: 10.1111/j.1872-034X.2011.00872.x.
5. Cholankeril G, Wong RJ, Hu M, Perumpail RB, Yoo ER, Puri P, et al. Liver Transplantation for Non-alcoholic Steatohepatitis in the US: Temporal Trends and Outcomes. Dig Dis Sci, 2017, 62(10):2915-2922 doi: 10.1007/s10620-017-4684-x.
6. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of non-alcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology, 2016, 64(1):73-84 doi: 10.1002/hep.28431.
7. Saklayen MG. The Global Epidemic of the Metabolic Syndrome. Curr Hypertens Rep, 2018, 20(2):12 doi: 10.1007/s11906-018-0812-z.
8. Alam S, Alam M, Alam SMNE, Chowdhury ZR, Kabir J. Prevalence and Predictor of Non-alcoholic Steatohepatitis (NASH) in Nonalcoholic Fatty Liver Disease (NAFLD). J. Bangladesh Coll. Phys. 2015, 32(2):71-7 doi: 10.3329/jbcps. v32i2. 26034.
9. Labenz C, Huber Y, Michel M, Nagel M, Galle PR, Kostev K, et al. Impact of NAFLD on the Incidence of Cardiovascular Diseases in a Primary Care Population in Germany. Dig Dis Sci, 2020, 65(7):2112-2119 doi: 10.1007/s10620-019-05986-9.
10. Kaps L, Labenz C, Galle PR, Weinmann-Menke J, Kostev K, Schattenberg JM. Non-alcoholic fatty liver disease increases the risk of incident chronic kidney disease. United European Gastroenterol J, 2020, 8(8):942-948 doi: 10.1177/2050640620944098.
11. Golabi P, Sayiner M, Fazel Y, Koenig A, Henry L, Younossi ZM. Current complications and challenges in non-alcoholic steatohepatitis screening and diagnosis. Expert Rev Gastroenterol Hepatol, 2016, 10(1):63-71, doi: 10.1586/17474124.2016.1099433.
12. Pappachan JM, Babu S, Krishnan B, Ravindran NC. Non-alcoholic Fatty Liver Disease: A Clinical Update. J ClinTransl Hepatol, 2017, 5(4):384-393 doi: 10.14218/JCTH.2017.00013.
13. Yoneda M, Yoneda M, Mawatari H, Fujita K, Endo H, Lida H, et al. Non-invasive assessment of liver fibrosis by measurement of stiffness in patients with non-alcoholic fatty liver disease (NAFLD). Dig Liver Dis, 2008, 40(5):371-378 doi: 10.1016/j.dld.2007.10.019.
14. Pathik P, Ravindra S, Ajay C, Prasad B, Jatin P, Prabha S. Fibroscan versus simple non-invasive screening tools in predicting fibrosis in high-risk non-alcoholic fatty liver disease patients from Western India. Ann Gastroenterol, 2015, 28(2):281-286.

15. Verna EC, Patel J, Bettencourt R, Nguyen P, Hernandez C, Valasek MA, et al. Novel association between serum pentraxin-2 levels and advanced fibrosis in well-characterised patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther, 2015, 42(5):582-590 doi: 10.1111/apt.13292.
16. Cox N, Pilling D, Gomer RH. Serum amyloid P: a systemic regulator of the innate immune response. J Leukoc Biol, 2014, 96(5):739-743 doi: 10.1189/jlb.1MR0114-068R.
17. Pilling D, Roife D, Wang M, Ronkainen D, Crawford JR, Travis LE, et al. Reduction of bleomycin-induced pulmonary fibrosis by serum amyloid P. J Immunol, 2017, 179(6):4035-4044 doi: 10.4049/jimmunol.179.6.4035.
18. Brunt EM. Pathology of non-alcoholic steatohepatitis. Nat Rev Gastroenterol Hepatol, 2010, 7(4):195-303 doi: 10.1038/nrgastro.2010.21.
19. Pais R, Barritt AS, Calmus Y, Scatton O, Runge T, Lebray P, et al. NAFLD and liver transplantation: current burden and expected challenges. J Hepatol, 2016, 65(6):1245–57, doi: 10.1016/j.jhep.2016.07.033.
20. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. Nat Med, 2018, 24(7):908-22 doi: 10.1038/s41591-018-0104-9.
21. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, et al. Sampling variability of liver biopsy in non-alcoholic fatty liver disease. Gastroenterology, 2015, 128:1898-906 doi: 10.1053/j.gastro.2005.03.084.
22. Rossi E, Adams LA, Bulsara M, Jeffrey GP. Assessing liver fibrosis with serum marker models. Clin Biochem Rev, 2017, 28(1):3-10.
23. Yeniova AO, Küçükazman M, Ata N, Dal K, Kefeli A, Başyiğit S, et al. High-sensitivity C-reactive protein is a strong predictor of non-alcoholic fatty liver disease. Hepato gastroenterology, 2014, 61(130):422-425.
24. Hui JM, Farrell GC, Kench JG, George J. High sensitivity C-reactive protein values do not reliably predict the severity of histological changes in NAFLD. Hepatology, 2004, 39(5):1458-1459 doi: 10.1002/hep.20223
25. Ramirez GA, Rovere-Querini P, Blasi M, Sartorelli S, Di Cihio MC, Baldini M, et al. PTX3 Intercepts Vascular Inflammation in Systemic Immune-Mediated Diseases. Front Immunol, 2019, 10:1135 doi: 10.3389/fimmu.2019.01135
26. Gurel H, Genc H, Celebi G, Sertoglu E, Cicek AF, Kayadibi H, et al. Plasma pentraxin-3 is associated with endothelial dysfunction in non-alcoholic fatty liver disease. Eur Rev Med Pharmacol Sci, 2016, 20(20):4305-4312.
27. Boga S, Koksal AR, Alkim H, Yilmaz Ozguven BM, Bayram M, Ergun M, et al. Plasma Pentraxin 3 Differentiates Non-alcoholic Steatohepatitis (NASH) from Non-NASH. Metab Syndr Relat Disord, 2015, 13(9):393-399, doi: 10.1089/met.2015.0046.
28. Özer Balin Ş, Çabalak M, Sağmak Tartar A, Kazancı Ü, Telo S, Demirbağ K, et al. Pentraxin-3: A novel marker for indicating liver fibrosis in chronic hepatitis B patients?. Turk J Gastroenterol, 2021, 32(7): 581-585 doi: 10.5152/tjg.2020.19378.
29. Cong M, Jiang C, Taura K, Kodama Y, De Minicis D, Kramer MS, et al. Serum Amyloid P Attenuates Hepatic Fibrosis in Mice by Inhibiting the Activation of Fibrocytes and Hepatic Stellate Cells. Hepatology 2011, 54(4 Suppl.): 736A
30. Basturk T, Ojalvo D, Mazi EE, Hasbal NB, Ozagari AA, Ahbap E, et al. Pentraxin-2 is Associated with Renal Fibrosis in Patients Undergoing Renal Biopsy. Clinics, 2020, 75:e1809 doi: 10.6061/clinics/2020/e1809
31. Castaño AP, Lin SL, Surowy T, Nowlin BT, Turlapati SA, Patel T, et al. Serum amyloid P inhibits fibrosis through Fc gamma R-dependent monocyte-macrophage regulation in vivo. Science translational medicine, 2009, 1(5):5ra13 2doi: 10.1126/scitranslmed.3000111.
32. Murray LA, Rosada R, Moreira AP, Joshi A, Kramer MS, Hesson DP, et al. Serum amyloid P therapeutically attenuates murine bleomycin-induced pulmonary fibrosis via its effects on macrophages.2010, PLoS ONE, 5: e9683, doi: 10.1371/journal.pone.0009683.
33. Dillingh MR, van den Blink B, Moerland M, van Dongen MG, Levi M, Kleinjan A, et al. Recombinant human serum amyloid P in healthy volunteers and patients with pulmonary fibrosis. Pulm Pharmacol Ther, 2013,26(6):672-676 doi: 10.1016/j.pupt.2013.01.008.
34. Naik-Mathuria B, Pilling D, Crawford JR, Gay AN, Smith CW, Gomer RH, et al. Serum amyloid P inhibits dermal wound healing. Wound Repair Regen, 2008, 16(2):266-273, doi: 10.1111/j.1524-475X.2008.00366.x.
35. Van Den Blink B, Dillingh MR, Ginns LC, Morrison LD, Moerland M, Wijsenbeek M, et al. Recombinant human pentraxin-2 therapy in patients with idiopathic pulmonary fibrosis: Safety, pharmacokinetics and exploratory efficacy. Eur Respir J, 2016, 47(3):889-97 , doi: 10.1183/13993003.00850-2015.
36. Raghu G, Van Den Blink B, Hamblin MJ, Brown AW, Golden JA, Ho LA, et al. Effect of recombinant human pentraxin 2 vs placebo on change in forced vital capacity in patients with idiopathic pulmonary fibrosis: a randomized clinical trial. JAMA, 2018, 319(22):2299-30, doi: 10.1001/jama.2018.6129.