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Thrombospondin 1 and Nuclear Factor Kappa B Signaling Pathways in Non-alcoholic Fatty Liver Disease

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ABSTRACT

Aim: We aimed to evaluate the circulating thrombospondin-1 (TSP-1) and nuclear factor kappa B (NF-κB) in nonalcoholic fatty liver disease (NAFLD) in order to integrate these signaling pathways in the inflammatory and fibrogenic processes of this liver disorder.

Methods: Ninety-five NAFLD patients were recruited in the study. The study also included 83 age-sex matched healthy controls.

Results: The number of patients with metabolic syndrome (MetS) criteria was 57 (60%). TSP-1 level was found to be statistically significantly lower in the NAFLD group compared to the control group (p=0.037). However, NF-κB level was found to be significantly higher in the NAFLD group compared to the control group (p=0.004). There was a significant negative correlation between plasma TSP-1 levels with glucose (r=-0.235, p=0.022), alanine aminotransferase (r=-0.261, p=0.011) and aspartate transaminase (r=-0.328, p=0.001) levels. In addition, a significant negative correlation was found between plasma TSP-1 and NF-κB levels (r=-0.729, p<0.001).

Conclusions: Our results suggest a close relationship between increased NF-κB and reduced TSP-1 in NAFLD. TSP-1 and NF-κB signaling pathways might have a role in the inflammatory and fibrogenic processes. Furthermore, they may be used as a noninvasive marker and could assist as a therapeutic target for NAFLD.

Key words: Non-alcoholic fatty liver disease – thrombospondin-1 – nuclear factor kappa B – inflammation – fibrogenesis.

Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CRP: C-reactive protein; HDL: high density lipoprotein; HOMA-IR: homeostatic model assessment for insulin resistance; IL-1β: interleukin-1Beta; IL6: interleukin-6; IR: insulin resistance; LDL: low density lipoprotein; MetS: metabolic syndrome; NAFLD: non-alcoholic liver disease; NF-κB: nuclear factor kappa B; T2DM: type 2 diabetes mellitus; TC: total cholesterol; TG: triglycerides; TNF- α: tumor necrosis factor-α; TSP-1: thrombospondin-1.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as histopathological lipid accumulation in ≥5% of hepatocytes in people whose secondary causes (such as high alcohol use, long-term use of drugs, hepatitis C, parenteral nutrition, Wilson's disease) are excluded [1]. Metabolic diseases such as type 2 diabetes mellitus (T2DM), prediabetes, insulin resistance (IR), obesity, dyslipidemia, metabolic syndrome (MetS), polycystic ovary syndrome are important risk factors for the development of NAFLD [2, 3].

Hepatic steatosis has adverse effects on liver function and is triggered by inflammation. Expressions of interleukin-1Beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) are up-regulated with the increase in adiposity [4]. Increased TNF-α production is associated with NAFLD and IR. TNF-α can activate apoptotic and proapoptotic signaling events by activating nuclear factor kappa B (NF-κB). TNF-α stimulates IR through NF-κB, which can result in liver inflammation and metabolic changes [5]. NF-κB plays a central role in regulating the expression of cytokines and chemokines and recent data suggest that hepatic steatosis activates NF-κB [6, 7].

Thrombospondins (TSPs) are extracellular, oligomeric, multifunctional glycoproteins that bind to Ca^{2+}. They are produced by various cell types and have diverse roles in cell signaling; their expression varies mainly in pathophysiological conditions. Thrombospondins was originally named the "thrombin-sensitive protein" due to its release by thrombin-activated platelets [8, 9]. Thrombospondins family is divided into two subgroups: A (TSP-1, TSP-2) and B (TSP-3, TSP-4, TSP-5). The thrombospondins 1 is an extracellular matrix glycoprotein that can be secreted from both normal and tumor cells [10]; it plays important roles in many biological processes, including regulation the function of many organs, angiogenesis, apoptosis, latent TGF-activation, and immune regulation. However, the role of TSP-1 in liver diseases has not been extensively addressed [10-13]. At the same time, it is unknown whether circulating TSP-1 could be an important factor in the development and progression of inflammation, fibrogenesis and NAFLD.

The aim of our study was to evaluate the circulating TSP-1 and NF-κB in NAFLD, which is an inflammatory process, and to analyze the production of inflammatory and fibrogenesis stimuli to explain the mechanism in these signaling pathways.

METHODS

This case-control study was conducted at the Department of Internal Medicine, Kanuni Sultan Suleyman Research and Training Hospital, Health Sciences University, Istanbul, Turkey. The protocol for sample collection was approved by the Kanuni Sultan Suleyman Training and Research Hospital Ethical Committee (Number: KAEK/2021.02.56). The study was performed in accordance with the Helsinki Declaration and informed consent was acquired from all patients and controls prior to their inclusion in the study.

Inclusion and exclusion criteria are presented in Table I.

Both the patient and control subjects were invited to participate in the study shortly after the ultrasonographic examination and laboratory tests.

Ultrasonographic examinations were performed by the radiology department prior to the enrollment of the patients and volunteers in the study, regardless of the purpose of the study and patient characteristics. The level of steatosis on ultrasound was defined according to the criteria previously defined in the literature (grade 1 steatosis: a slight and diffuse increase of liver echogenicity with normal visualization of the diaphragm and of the portal vein wall; grade 2 steatosis: a moderate increase of liver echogenicity with slightly impaired appearance of the portal vein wall and the diaphragm; grade 3 steatosis: marked increase of liver echogenicity with poor or no visualization of portal vein wall, diaphragm, and posterior part of the right liver lobe) [14].

Prior to the enrollment, the body mass index (BMI) and the following values of the biochemical parameters were obtained from hospital records: glucose, insulin, creatinine, alanine transaminase (ALT), aspartate transaminase (AST), low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides (TG), total cholesterol (TC) and C-reactive protein (CRP). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated with the following formula: HOMA-IR= (glucose*insulin)/405. For the hepatic steatosis index (HSI) the following formula was used: HSI= 8*ALT/AST + BMI (+2 if type-2 DM yes, +2 if female). Hepatic steatosis index values below 30 indicate that NAFLD can be ruled out with a sensitivity of 93.1%; while HSI values of 36 and above indicate that NAFLD positive diagnosis with a specificity of 92.4% [15].

The number of patients identified as potentially eligible patients was 213 but 43 of them could not be reached. The remaining 170 patients were examined for study eligibility and 58 of them were excluded for the following reasons: hepatic steatosis index score ≤36 (n=9), alcohol consumption (n=22), pregnancy (n=2), active infection (n=7), chronic kidney disease (n=4), stage 3-4 heart failure (n=3), chronic lung disease (n=5), hepatitis B infection (n=3), hepatitis C infection (n=2) and heavy exercise (n=1). Seventeen patients did not consent to the study and finally 95 patients were included in the study. Among

Table I. Inclusion and exclusion criteria for the study groups

Inclusion criteria	Exclusion criteria
For the patient group: • ≥18 years age, • Liver steatosis detected on ultrasonography, • Hepatic steatosis index score: ≥36 For the control group: • ≥18 years age, • No history of any comorbid disease, • Absence of liver steatosis on ultrasonography	For both the patient and control group: • <18 years age, • Pregnant and/or breastfeeding women, • The patients with: o a history of alcohol consumption, o any active infection, o chronic kidney disease, o receiving peritoneal dialysis treatment, o severe malnutrition, o stage 3-4 heart failure, o chronic inflammatory diseases, o chronic lung disease (such as chronic obstructive pulmonary disease, bronchiectasis, asthma, pulmonary hypertension), o hepatitis B and C infections, • Individuals who heavy exercise in the last week (exercises that cause excessive sweating and/or severe fluid loss and / or obvious tachycardia (for example, brisk walking - 6.5-8 km, running 10 km/hour, hiking uphill, cycling >10 mil/hour or steeply uphill, lap swimmming, roller blading 2-3 hours, court sports, strength training and weightlifting, heavy gardening with digging).

the potential 173 eligible healthy subjects (consisting of hospital personnel subjected to annual routine control examinations), 31 were excluded [alcohol consumption (n=12) and active infection (n=19)] and 59 did not consent to the study. The remaining 83 subjects were included and completed the study as a control group.

Finally, we included 95 NAFLD patients older than 18 years with liver steatosis detected on ultrasonography, hepatic steatosis index score ≥36, and 83 age and gender matched healthy controls.

Age, gender, BMI and smoking status were recorded in all subjects. Subjects were grouped in terms of obesity according to their BMI values (normal: 18.5-24.9; overweight: 25-29.9; class 1 obesity: 30-34.9; class 2 obesity: 35-39.9; class 3 obesity: ≥40). Waist circumference was measured horizontally at the level of the umbilicus with a non-elastic tape measure when the participants were standing with the arms relaxed. Hip circumference was measured around the buttocks, below the iliac crest where the circumference appeared to be largest. Waist-hip ratio was calculated by dividing waist circumference to hip circumference. Comorbid diseases, especially T2DM, hypertension and hyperlipidemia were questioned in the patient group. Rate and degree of liver steatosis detected in ultrasonography in the patient group was recorded as grade 1, grade 2 and grade 3, and it was noted whether hepatomegaly accompanied the picture in ultrasonography. The patient group was evaluated through MetS criteria, and it was noted whether each patient had MetS.

After an overnight fast, venous blood was drawn from all subjects included in the study before administration of any contrast agent or medications. Procession of blood samples was according to methods of Novelli et al. [16]. In summary, to minimize the confounding effect of platelet activation and degranulation ex vivo, possibly leading to artifactual elevation of TSP-1 levels, the use of a tourniquet for arm occlusion was avoided or minimized whenever possible. All blood samples were collected in 3.8% trisodium citrate tubes and immediately transferred to laboratory facilities adjacent to the collection sites at 4°C. The tubes were inverted 8–10 times and then subjected to double centrifugation at 1,500 g at 4°C to obtain platelet poor plasma. The supernatant was aliquoted into cryotubes and were stored at -80 C until assayed for determination of TSP-1 and NF-κB concentrations. All icteric or hemolytic blood samples were discarded. All parameters were analyzed in all samples together in a single batch; after we had finished our protocol (control and patient samples were analyzed in the same batch).

Biochemical parameters were analyzed by a COBAS 8000 (ROCHE-2007, Tokyo, Japan) device in the morning after an overnight fast.

Plasma TSP-1 concentrations were in duplicates analyzed by a commercially available competitive enzyme linked immunoassay kit (R&D Systems, Minneapolis, MN, USA). The coefficients of intra and inter assay variation were 4.7% (n=25) and 5.9% (n=25), respectively.

Plasma NF-κB concentrations were in duplicates analyzed by a commercially available competitive enzyme linked immunoassay kit (R&D Systems, Minneapolis, MN, USA). The coefficients of intra and inter assay variation were 4.4% (n=25) and 6.0% (n=25), respectively.

Statistical analysis was performed using SPSS for Windows 26.0 (IBM Corporation, Chicago, IL, USA). In the power analysis made through the GPower 3.1.9.4 program, it has been determined that the achieved power value (1-β err prob) of the study is 0.94. Mean, standard deviation, median, lowest, highest, frequency, and ratio values were used in the descriptive statistics of the data. Distribution of the variables was measured by the Kolmogorov–Smirnov and Shapiro-Wilk tests. For comparison of numeric variables between two groups, student's t-test was applied when data were normally distributed and Mann-Whitney U-test when non-normally distributed. For more than two numeric variables, ANOVA was used when the data were normally distributed, and Kruskal-Wallis was used when non-normally distributed. Pearson correlation test and Spearman's correlation test were used to detect the association between parameters for the normally and non-normally distributed parameters, respectively. To evaluate the relationship between plasma TSP-1 and NF-κB levels and development of NAFLD, cut-off values for plasma TSP-1 and NF-κB levels were determined by the receiver operating characteristic (ROC) analysis. A univariate regression analysis performed to observe the effects of parameters on the cut-off values of plasma TSP-1 and NF-κB. A p-value <0.05 was regarded as statistically significant.

RESULTS

One hundred seventy-eight subjects (116 female; mean age: 43.65±9.7 years) were included in the study (54 were active smokers and there were no participants who consumed alcohol).

From the 95 NAFLD patients 40 (42.1%) had T2DM, 25 (26.3%) hypertension and 17 (17.9%) hyperlipidemia. The number of patients with MetS criteria was 57 (60%). The number of patients with grade-1, grade-2 and grade-3 liver steatosis was 31 (32.6%), 55 (57.9%) and 9 (9.5%), respectively. Forty (42.1%) of the NAFLD patients had hepatomegaly. Demographic data and laboratory parameters for NAFLD patients and healthy controls are presented in Table II.

Plasma TSP-1 and NF-κB levels in NAFLD patients subdivided in groups according to gender, smoking, comorbid diseases, liver steatosis grade, presence of hepatomegaly and obesity classification are displayed in Table III.

Thrombospondin 1 level was found to be statistically significantly lower in the NAFLD group compared to the control group (p=0.037). However, NF-κB level was found to be significantly higher in the NAFLD group compared to the control group (p=0.004).

There was a significant negative correlation between plasma TSP-1 levels, glucose (r=-0.235, p=0.022), ALT (r=-0.261, p=0.011) and AST (r=-0.328, p=0.001) levels. Moreover, a significant negative correlation was found between plasma TSP-1 level and NF-κB levels (r=-0.729, p<0.001).

For the analysis of the association between TSP-1 and NF-κB levels with NAFLD development, cut-off values were determined by ROC analysis. As shown in Fig. 1 and Table IV, TSP-1 level of 0.6975 and below (63.9% sensitivity, 55.8% specificity, area under the curve: 0,412, p:0.042) and NF-κB level of 0.543 and above (56.8% sensitivity, 55.4% specificity, area under the curve: 0,627, p: 0.042)) were determined as cut-off values for the diagnosis of NAFLD.

Table II. Comparison of demographic, routine biochemistry parameters, thrombospondin-1 and nuclear factor-kappa B levels of the study groups

	NAFLD group	Control group	p
	n = 95	n = 83	
Age, years	44.95±9.31	42.15±9.97	0.056
Gender			
Male. n	38	24	0.156
Female. n	57	59	
Weight, kg	93.33±18.9	65.86±11.17	<0.001
BMI, kg/m^2	35.1±6.47	23.51±3.44	<0.001
Hypertension; n (%)	25 (26.3)	-	-
BMI			
Normal (<25 kg/m^2), n	0	58	<0.001
Overweight (25-29,9 kg/m^2), n	20	22	
Class-1 obesity (30-34,9 kg/m^2), n	39	3	
Class-2 obesity (35-39,9 kg/m^2), n	21	0	
Class-3 obesity (≥40 kg/m^2), n	15	0	
Waist circumference, cm	113.06±12.2	79.53±9.37	<0.001
Hip circumference, cm	116.46±11.74	96.83±7.31	<0.001
Waist circumference/hip circumference ratio	0.97±0.07	0.82±0.07	<0.001
Glucose, mg/dL	114.51±42.79	90.09±8.35	<0.001
HOMA-IR	7.56±7.95	2.48±1.76	<0.001
Creatinine, mg/dL	0.73±0.16	0.67±0.14	0.037
Platelet, 10^{3}µ/L	281.28±68.94	250.62±53.43	<0.001
ALT, U/L	37.15±25.92	15.42±8.98	<0.001
AST, U/L	26.56±14.7	16.32±4.78	<0.001
LDL cholesterol, mg/dL	117.06±35.18	100.5±29.71	0.001
HDL cholesterol, mg/dL	43.31±9.27	55.93±14.28	<0.001
Total cholesterol, mg/dL	193.88±39.12	174.84±34.72	0.001
Triglyceride, mg/dL	176.7±91.49	93.31±51.37	<0.001
C-reactive protein, mg/L	4.68±5.21	2.31±4.56	<0.001
Thrombospondin, ng/mL	0.65±0.25	0.73±0.26	0.037
Nuclear Factor-κB, ng/mL	0.59±0.15	0.51±0.09	0.004

AST: aspartate transaminase; ALT: alanine aminotransferase; BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; HDL: high-density lipoprotein; LDL: low density lipoprotein

The results of univariate regression analysis for the possible confounding parameters (T2DM, hypertension, hyperlipidemia and BMI ≥35 kg/m^2) on plasma TSP-1 and NF-κB cut-off values predicted by ROC analysis are displayed in Table V.

DISCUSSION

An *in vitro* model of NAFLD showed that free fatty acid treatment induces fat deposition in hepatocytes and TSP-1 expression in these cells, which was associated with inflammatory and fibrogenic responses [17-19]. In addition, TSP-1 and NF-κBp65 signaling pathways have been reported to be interactive in the evolution of infantile hemangioma, by a potential role in the progression of angiogenesis [20, 21]. In the current study, we revealed that NF-κB might have a general inflammatory effect under conditions of fatty liver; the deficiency of TSP-1 increased NF-κB, a well-known transcription factor, and would thus be expected to contribute to the hepatic and systemic inflammatory state in patients with NAFLD.

Our NAFLD patients had a significant decrease in HDL cholesterol and a significant increase in the fasting glucose, HOMA-IR, creatinine, AST, ALT, LDL cholesterol, total TG compared to the control group. These results suggest prominent characters of the NAFLD participants, similar to other studies [22-27]. Individuals with these metabolic changes should be investigated for NAFLD because possible complications such as cirrhosis and hepatocellular carcinoma could be prevented with early diagnosis and treatment approaches. In addition, the higher BMI and waist circumference values of the NAFLD patients compared to the controls suggest that obesity/central obesity may also contribute to the inflammatory processes. Therefore, in future studies, it will be important to compare the NAFLD group with the case group in terms of BMI, waist and hip circumferences, and also waist-hip ratio, in terms of

Table III. Demographic characteristics of NAFLD patient group

	N	Trombospondin 1	p	Nuclear Factor-κB	p
Gender					
Male	38	0.66±0.21	0.758	0.58±0.12	0.582
Female	57	0.65±0.27		0.60±0.17	
Smoking					
Yes	32	0.67±0.23	0.626	0.59±0.15	0.560
No	63	0.64±0.26		0.60±0.01	
Hypertension					
Yes	25	0.62±0.19	0.531	0.57±0.12	0.422
No	70	0.66±0.27		0.60±0.16	
Metabolic syndrome					
Yes	57	0.62±0.24	0.160	0.62±0.17	0.122
No	38	0.70±0.26		0.56±0.11	
Type 2 diabetes mellitus					
Yes	40	0.61±0.24	0.147	0.62±0.16	0.095
No	55	0.68±0.25		0.57±0.14	
Obesity					
Overweight	20	0.63±0.28	0.904	0.63±0.17	0.639
Class 1 obesity	39	0.66±0.25		0.59±0.16	
Class 2 obesity	21	0.63±0.24		0.58±0.12	
Class 3 obesity	15	0.68±0.24		0.58±0.15	
Liver steatosis					
Grade 1	9	0.52±0.21	0.191	0.66±0.2	0.560
Grade 2	55	0.68±0.25		0.58±0.14	
Grade 3	31	0.63±0.25		0.59±0.15	

obtaining stronger results. Nowadays NAFLD is considered a hepatic consequence of the MetS, a disorder characterized by obesity, hypertension, diabetes, hypertriglyceridemia and low HDL cholesterol levels. 60% of our patients had MetS. Similar results were found in another study conducted in France, hypertriglyceridemia being considered as a risk factor [28]. These results suggest that NAFLD may be a manifestation of MetS in the liver. The presence or absence of these metabolic risk factors may guide clinicians to select patients for liver biopsy.

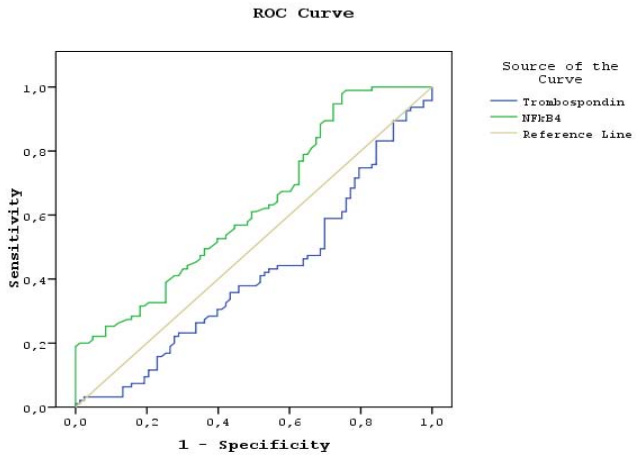

Fig. 1. ROC analysis for plasma TSP-1 and NF-κB levels for the diagnosis of NAFLD.

In our study, TSP-1 level was found to be statistically significantly lower in the NAFLD group compared to the control group. To our knowledge, no previously published study investigated the plasma TSP-1 levels together with NF-κB in NAFLD patients. Furthermore, the studies investigating the relationship between TSP-1 and fibrosis were done at the tissue level; no previous study reported the circulating plasma TSP-1 levels in this category of patients. Trombospondin 1 levels were significantly lower in those with NAFLD compared to the healthy group. Min-DeBartolo et al. [29] observed that TSP-1 knockout mice also presented with similar NASH-like manifestations; however, it was with statistically significant decreased of serum lipid levels, markers of inflammation and fibrosis. Serum AST and ALT were not different between TSP-1

Table IV. ROC analysis for plasma TSP-1 and NF-κB levels of all patients for the diagnosis of NAFLD

	Sensitivity	Specificity	Area under the curve	p
Thrombospondin level ≤0.6975	63.9	55.8	0.412	0.042
Nuclear factor kappa B ≥0.543	56.8	55.4	0.627	0.042

Table V. Univariate regression analysis for parameters predicted plasma thrombospondin-1 (TSP-1) levels ≤0.6975 and plasma nuclear factor kappa B (NF-κB) ≥0.543 in patients with NAFLD

Variable	Univariate regression analysis for plasma TSP-1 levels ≤0.6975			Univariate regression analysis for plasma NF-κB levels ≥0.543		
	OR	95% Cl	p	OR	95% Cl	p
Type-2 diabetes mellitus	1.446	0.634-3.299	0.380	1.791	0.775-4.138	0.173
Hypertension	1.679	0.655-4.305	0.281	0.955	0.380-2.397	0.921
Hyperlipidemia	1.654	0.557-4.918	0.365	2.057	0.662-6.396	0.213
BMI ≥35 kg/m^2	1.055	0.458-2.427	0.900	1.103	0.477-2.551	0.819

BMI: Body mass index

knockout and wild type mice. However, serum liver enzyme levels do not correlate with histological findings in the clinical setting and are not helpful in the diagnosis of NAFLD and in determining the severity of the disease. Despite all this, TSP-1 may be an enchanting target for NASH-induced hepatic fibrosis [30]. The hepatocyte-mediated fibrogenic response is regulated by TSP-1 [31]. Several research projects conducted by Inoue et al. [32] and Cui et al. [33] indicated that the TSP-1 deficiency in mice might be preventive against fibrosis in several organs such as muscle and kidney. Many studies have documented the development of fibrosis in NAFLD patients [34, 35]. The TSP-1 expression in arteries increased in aged wild-type mice and older human subjects, promoting deterioration of multiple restorative and homeostatic mechanisms [36]. Our findings, correlated with the decrease in TSP-1 levels compared to the increase in platelet levels makes us think that NF-κB has a suppressive effect on TSP-1, considering the correlation between TSP-1 and NF-κB. But, the role of TSP-1 in the detailed cellular and molecular mechanisms of NAFLD is still unclear. The results are controversial in studies on metabolic mechanisms [31-35]. Although TSP-1 has been reported as a critical modulator in NASH [37], it still needs to be investigated in NAFLD. Therefore, further clinical, and experimental studies are needed.

Nuclear factor kappa B is a transcription factor involved in a number of pathological processes such as inflammation as well as innate and adaptive immune responses. Under normal conditions, NF-κB is sequestered in the cytoplasm and then binds to IκB proteins, which inhibits the nuclear localization of NF-κB. Activation of NF-κB is normally moderate, whereas its expression in liver and adipose tissue is greatly increased under IR conditions [38]. In the current study, the NF-κB level was found to be significantly higher in the NAFLD group compared to the control group. Various molecules activate the NF-κB pathway, increasing local inflammation and may lead to IR. It can be speculated that NF-κB might target the liver as the principal organ of metabolism in conditions such as NAFLD, thereby contributing to hepatic IR. It has been reported that NF-κB expression increased in experimental liver injury [39, 40]. Saadati et al. [41] reported increased NF-κB expression in peripheral blood mononuclear cells Fmet of NAFLD patients. In another study, naringenin inhibited NAFLD by down-regulating the NLRP3/NF-κB signaling pathway in both Kupfer cells and hepatocytes and improved inflammation in mice livers [42]. The TSP-1 monomers have many cell receptors. The species cFLIP-L (FL) forms a complex with procaspase-8 involving DISC formation and promotes the activation of NF-κB [43]. Although the pathogenesis of NAFLD remains unclear, our results suggest that increased NF-κB plays a role in the pathogenesis of NAFLD.

Although our study has strengths, it has some limitations. We diagnosed and graded liver steatosis by ultrasonography, but it is known that the specificity and sensitivity of ultrasonography is decreased when the level of steatosis in the liver is very low, or the patient is morbidly obese. To further strengthen the diagnosis of NAFLD, we calculated the HSI index of the patients, and we included only the patients with an HSI index ≥36. For this cutoff value of HSI, NAFLD positive diagnosis has a specificity of 92.4%. Other limitations might be the inclusion of patients with other metabolic features, apart from NAFLD, that might influence the results of plasma TSP-1 and NF-κB levels. However, the univariate analysis revealed that the presence or absence of each of these comorbid diseases did not cause a change in TSP-1 and NF-κB levels, which are the main parameters of our study. Future studies on NAFLD patients without metabolic comorbidities might clarify this question.

CONCLUSIONS

TSP-1 levels were lower, while NF-κB levels were higher in the NAFLD group compared to the control group. TSP-1 and NF-κB signaling pathways might have a role in the inflammatory and fibrogenic processes of NAFLD. Furthermore, they may be used as a noninvasive marker and could assist as a therapeutic target for NAFLD. In addition, these findings suggest that NAFLD should be investigated in every patient with MetS features and that possible complications such as cirrhosis and hepatocellular carcinoma can be prevented with early diagnosis and treatment approaches. However, more comprehensive studies are required to be able to confirm this issue.

Conflicts of interest: None to declare.

Authors' contribution: İ.E., O.T. and H.U. designed the study. İ.E., G.A., I.Y., M.B., A.C., H.O., I.K.U., M.A., O.T. collected and determined the patients. S.D. and H.U. made ELISA test at laboratory. İ.E. performed the statistical analysis. I.E., H.U. and O.T. wrote the paper. All authors approved the final version of the paper.

REFERENCES

1. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from

the American Association for the Study of Liver Diseases. Hepatology 2018;67:328-357. doi:10.1002/hep.29367
2. Macut D, Božić-Antić I, Bjekić-Macut J, Tziomalos K. MANAGEMENT OF ENDOCRINE DISEASE: Polycystic ovary syndrome and nonalcoholic fatty liver disease. Eur J Endocrinol 2017;177:R145-R158. doi:10.1530/EJE-16-1063
3. Cariou B, Byrne CD, Loomba R, Sanyal AJ. Nonalcoholic fatty liver disease as a metabolic disease in humans: A literature review. Diabetes Obes Metab 2021;23:1069-1083. doi:10.1111/dom.14322
4. Coppack SW. Pro-inflammatory cytokines and adipose tissue. Proc Nutr Soc 2001;60:349-356. doi:10.1079/pns2001110
5. Mota M, Banini BA, Cazanave SC, Sanyal AJ. Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease. Metabolism 2016;65:1049-1061. doi:10.1016/j.metabol.2016.02.014
6. Tsochatzis E, Papatheodoridis GV, Archimandritis AJ. The evolving role of leptin and adiponectin in chronic liver diseases. Am J Gastroenterol 2006;101:2629-2640. doi:10.1111/j.1572-0241.2006.00848.x
7. Mussbacher M, Salzmann M, Brostjan C, et al. Cell Type-Specific Roles of NF-κB Linking Inflammation and Thrombosis. Front Immunol 2019;10:85. doi:10.3389/fimmu.2019.00085
8. Gutierrez LS, Gutierrez J. Thrombospondin 1 in Metabolic Diseases. Front Endocrinol (Lausanne) 2021;12:638536. doi:10.3389/fendo.2021.638536
9. Chatterjee A, Villarreal G Jr, Rhee DJ. Matricellular proteins in the trabecular meshwork:review and update. J Ocul Pharmacol Ther 2014;30:447-463. doi:10.1089/jop.2014.0013
10. Xu X, Zheng L, Yuan Q, et al. Transforming growth factor-β in stem cells and tissue homeostasis. Bone Res 2018;6:2. doi:10.1038/s41413-017-0005-4
11. Matsuo Y, Tanaka M, Yamakage H, et al. Thrombospondin 1 as a novel biological marker of obesity and metabolic syndrome. Metabolism 2015;64:1490-1499. doi:10.1016/j.metabol.2015.07.016
12. Chavez-Tapia NC, Rosso N, Tiribelli C. Effect of intracellular lipid accumulation in a new model of non-alcoholic fatty liver disease. BMC Gastroenterol 2012;12:20. doi:10.1186/1471-230X-12-20
13. Li Y, Turpin CP, Wang S. Role of thrombospondin 1 in liver diseases. Hepatol Res 2017;47:186-193. doi:10.1111/hepr.12787
14. Ferraioli G, Soares Monteiro LB. Ultrasound-based techniques for the diagnosis of liver steatosis. World J Gastroenterol 2019 Oct 28;25:6053-6062. doi:10.3748/wjg.v25.i40.6053
15. Lee JH, Kim D, Kim HJ, et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. Dig Liver Dis 2010;42:503-508. doi:10.1016/j.dld.2009.08.002
16. Novelli EM, Kato GJ, Ragni MV, et al. Plasma thrombospondin-1 is increased during acute sickle cell vaso-occlusive events and associated with acute chest syndrome, hydroxyurea therapy, and lower hemolytic rates. Am J Hematol 2012;87:326-330. doi:10.1002/ajh.22274
17. Gwag T, Reddy Mooli RG, Li D, Lee S, Lee EY, Wang S. Macrophage-derived thrombospondin 1 promotes obesity-associated non-alcoholic fatty liver disease. JHEP Rep 2020;3:100193. doi:10.1016/j.jhepr.2020.100193
18. Frampton G, Reddy P, Jefferson B, Ali M, Khan D, McMillin M. Inhibition of thrombospondin-1 reduces glutathione activity and worsens acute liver injury during acetaminophen hepatotoxicity in mice. Toxicol Appl Pharmacol 2020;409:115323. doi:10.1016/j.taap.2020.115323
19. Starlinger P, Haegele S, Wanek D, et al. Plasma thrombospondin 1 as a predictor of postoperative liver dysfunction. Br J Surg 2015;102:826-836. doi:10.1002/bjs.9814
20. Zhang Y, Xu W, Li S, et al. Expression and significance of the proteins in TSP-1 and NF-κB signal pathways of infantile capillary hemangioma. Zhonghua Zheng Xing Wai Ke Za Zhi 2016;32:441-446.
21. Isenberg JS, Ridnour LA, Perruccio EM, Espey MG, Wink DA, Roberts DD. Thrombospondin-1 inhibits endothelial cell responses to nitric oxide in a cGMP-dependent manner. Proc Natl Acad Sci U S A 2005;102:13141-13146. doi:10.1073/pnas.0502977102
22. Nigam P, Bhatt SP, Misra A, Vaidya M, Dasgupta J, Chadha DS. Non-alcoholic fatty liver disease is closely associated with sub-clinical inflammation: a case-control study on Asian Indians in North India. PLoS One 2013;8:e49286. doi:10.1371/journal.pone.0049286
23. Kucukazman M, Ata N, Yavuz B, et al. Evaluation of early atherosclerosis markers in patients with nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol 2013;25:147-151. doi:10.1097/MEG.0b013e32835a58b1
24. Ozhan H, Aydin M, Yazici M, et al. Mean platelet volume in patients with non-alcoholic fatty liver disease. Platelets 2010;21:29-32. doi:10.3109/09537100903391023
25. Fenkci S, Rota S, Sabir N, Akdag B. Ultrasonographic and biochemical evaluation of visceral obesity in obese women with non-alcoholic fatty liver disease. Eur J Med Res 2007;12:68-73.
26. Toledo FG, Sniderman AD, Kelley DE. Influence of hepatic steatosis (fatty liver) on severity and composition of dyslipidemia in type 2 diabetes. Diabetes Care 2006;29:1845-1850. doi:10.2337/dc06-0455
27. Zhao C, Lou F, Li X, et al. Correlation of CD3+/CD4+, and serum CK-18 fragment levels with glucose and lipid metabolism in elderly type 2 diabetes patients with nonalcoholic fatty liver disease. Am J Transl Res 2021;13:2546-2554.
28. Ratziu V, Giral P, Charlotte F, et al. Liver fibrosis in overweight patients. Gastroenterology 2000;118:1117-1123. doi:10.1016/s0016-5085(00)70364-7
29. Min-DeBartolo J, Schlerman F, Akare S, et al. Thrombospondin-I is a critical modulator in non-alcoholic steatohepatitis (NASH). PLoS One 2019;14:e0226854. doi:10.1371/journal.pone.0226854
30. Panke CL, Tovo CV, Villela-Nogueira CA, et al. Evaluation of thrombocytopenia in patients with non-alcoholic fatty liver disease without cirrhosis. Ann Hepatol 2020;19:88-91. doi:10.1016/j.aohep.2019.05.011
31. Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 2008;88:125-172. doi:10.1152/physrev.00013.2007
32. Inoue M, Jiang Y, Barnes RH 2nd, et al. Thrombospondin 1 mediates high-fat diet-induced muscle fibrosis and insulin resistance in male mice. Endocrinology 2013;154:4548-4559. doi:10.1210/en.2013-1587
33. Cui W, Maimaitiyiming H, Qi X, Norman H, Wang S. Thrombospondin 1 mediates renal dysfunction in a mouse model of high-fat diet-induced obesity. Am J Physiol Renal Physiol 2013;305:F871-F880. doi:10.1152/ajprenal.00209.2013
34. Li Y, Tong X, Rumala C, Clemons K, Wang S. Thrombospondin1 deficiency reduces obesity-associated inflammation and improves insulin sensitivity in a diet-induced obese mouse model. PLoS One. 2011;6:e26656. doi:10.1371/journal.pone.0026656
35. Varma V, Yao-Borengasser A, Bodles AM, et al. Thrombospondin-1 is an adipokine associated with obesity, adipose inflammation, and insulin resistance. Diabetes 2008;57:432-439. doi:10.2337/db07-0840
36. Ghimire K, Li Y, Chiba T, et al. CD47 Promotes Age-Associated Deterioration in Angiogenesis, Blood Flow and Glucose Homeostasis. Cells 2020;9:1695. doi:10.3390/cells9071695

37. Richmond A. Nf-kappa B, chemokine gene transcription and tumour growth. Nat Rev Immunol 2002;2:664-674. doi:10.1038/nri887
38. Rahman MM, McFadden G. Modulation of NF-κB signalling by microbial pathogens. Nat Rev Microbiol 2011;9:291-306. doi:10.1038/nrmicro2539
39. Kelany ME, Hakami TM, Omar AH. Curcumin improves the metabolic syndrome in high-fructose-diet-fed rats:role of TNF-α, NF-κB, and oxidative stress. Can J Physiol Pharmacol 2016;95:140–150. doi:10.1139/cjpp-2016-0152
40. Maithilikarpagaselvi N, Sridhar MG, Swaminathan RP, Zachariah B. Curcumin prevents inflammatory response, oxidative stress and insulin resistance in high fructose fed male Wistar rats: potential role of serine kinases. Chem Biol Interact 2016;244:187–194. doi:10.1016/j.cbi.2015.12.012
41. Saadati S, Sadeghi A, Mansour A, et al. Curcumin and inflammation in non-alcoholic fatty liver disease:a randomized, placebo controlled clinical trial. BMC Gastroenterol 2019;19:133. doi:10.1186/s12876-019-1055-4
42. Wang Q, Ou Y, Hu G, et al. Naringenin attenuates non-alcoholic fatty liver disease by down-regulating the NLRP3/NF-κB pathway in mice. Br J Pharmacol 2020;177:1806-1821. doi:10.1111/bph.14938
43. Wu Q, Finley SD. Predictive model identifies strategies to enhance TSP1-mediated apoptosis signaling. Cell Commun Signal 2017;15:53. doi:10.1186/s12964-017-0207-9