

# Non-alcoholic Fatty Liver Disease and Its Association with Serum Nesfatin-1\*

## Non-Alkolik Yağlı Karaciğer Hastalığı ve Serum Nesfatin-1 İlişkisi

### Abstract

**Aim:** Nesfatin -1 is a novel peptide which is defined as satiety peptide with an anorexigenic action. Studies have shown its association with metabolic syndrome and insulin resistance. With this study, we sought to establish the association between clinicopathologic characteristics of patients with biopsy-diagnosed non-alcoholic fatty liver disease (NAFLD) and Nesfatin-1.

**Materials and Methods:** Serum Nesfatin-1 levels were measured using the enzyme-linked immunosorbent assay in 59 patients with histologically diagnosed NAFLD and a control group comprising 32 healthy subjects.

**Results:** Serum Nesfatin-1 level did not significantly differ between patients with NAFLD and control group ( $p<0.170$ ). Simple correlation analysis showed that nesfatin-1 levels decreased as body mass index increased ( $p=0.043$ ) and nesfatin-1 values increased in proportion to elevations in AST ( $p=0.05$ ). A multiple regression model constructed for assessment showed that while portal inflammation ( $B=10.767$ ,  $p=0.007$ ), body mass index score ( $B=-0.510$ ,  $p<0.001$ ) and HDL cholesterol ( $B=-0.208$ ,  $p<0.001$ ) had a negative association with nesfatin-1, age ( $B=0.120$ ,  $p=0.031$ ), sex (lower levels among males versus females) ( $B=-40.897$ ,  $p<0.001$ ), systolic blood pressure ( $B=0.063$ ,  $P=0.050$ ) and AST ( $B=0.033$ ,  $p=0.019$ ) had a positive and linear significant association with nesfatin-1.

**Conclusion:** In conclusion, the findings point out that serum nesfatin-1 level may be an independent predictor of portal inflammation in NAFLD.

**Keywords:** NAFLD; nesfatin-1; portal inflammation; fibrosis; obesity

### Öz

**Amaç:** Nesfatin -1 anoreksijenik etkisi olan tokluk peptidi olarak tanımlanan yeni bir peptittir. Yapılan çalışmalarda metabolik sendrom ve insülin rezistansı ile ilişkisi olduğu ortaya konmuştur. Biz bu çalışma ile biyopsi tanımlı non-alkolik yağlı karaciğer hastalığı (NAFLD) olan hastaların klinikopatolojik özellikleri ile Nesfatin-1 arasındaki ilişkiyi ortaya koymaya çalıştık.

**Gereç ve Yöntemler:** Histolojik olarak non-alkolik yağlı karaciğer hastalığı tanısı konmuş 59 hasta ve 32 sağlıklı kontrol grubunda serum Nesfatin-1 seviyeleri enzyim-linked immunosorbent assay (ELISA) yöntemiyle ölçülmüştür.

**Bulgular:** Serum Nesfatin-1 seviyesi non-alkolik yağlı karaciğer hastalığı olanlarla kontrol grubu arasında anlamlı farklılık göstermiyordu ( $p<0.170$ ). Basit korelasyon analizinde vücut kitle indeksi (VKİ) arttıkça nesfatin-1 seviyelerinin azaldığı ( $p=0.043$ ) ve AST arttıkça nesfatin-1 değerlerinin de arttığı ( $p=0,05$ ) görüldü. Çoklu regresyon modeli oluşturularak yapılan incelemede ise portal inflamasyon ( $B=10,767$ ,  $p=0,007$ ), VKİ skoru ( $B=-0,510$ ,  $p<0,001$ ) ve HDL kolesterol ( $B=-0,208$ ,  $p<0,001$ ) ile nesfatin-1 arasında negatif yönlü ilişki yaş ( $B=0,120$ ,  $p=0,031$ ), cinsiyet (erkeklerde kadınlara göre daha düşük) ( $B=-40,897$ ,  $p<0,001$ ) sistolik kan basıncı ( $B=0,063$ ,  $P=0,050$ ) ve aspartat aminotransferaz ( $B=0,033$ ,  $p=0,019$ ) arasında pozitif ve doğrusal anlamlı ilişki bulunmuştur.

**Sonuç:** Özet olarak bulgular serum nesfatin-1 seviyesinin non-alkolik yağlı karaciğer hastalığında portal inflamasyonun bağımsız bir prediktörü olabileceğine işaret etmektedir.

**Anahtar Sözcükler:** Non-alkolik yağlı karaciğer hastalığı; nesfatin-1; portal inflamasyon; fibrozis; obezite

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## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in industrialized western countries which affects about 20-40% of the general population (1,2). It has a wide spectrum range from simple steatosis to non-alcoholic steatohepatitis and cirrhosis. Major risk factors include metabolic syndrome components such as obesity, dislipidemia and diabetes mellitus (3,4). Insulin resistance has been reported to be the major key mechanism involved in the pathogenesis of NAFLD (5,6).

Nesfatin-1 is a novel peptide of 82 aminoacids, that is encoded by the nucleobindin-2 (NUCB2) gene and defined as the satiety peptide associated with melanocortin signaling in the hypothalamus (7).

Nesfatin-1 is present in paraventricular nucleus, lateral hypothalamic area, supraoptic nucleus, dorsomedial nucleus and arcuate nucleus of the hypothalamus, solitary tract nucleus, and some peripheral tissues (e.g. adipose tissue) (7- 9). It is a molecule associated with dietary habits and has an anorexigenic action. Following a 24-hour fasting, expression of nesfatin-1/ NUCB2 gene and concentration of nesfatin-1 in the paraventricular nucleus of the hypothalamus is decreased (7). Studies demonstrate that centrally or peripherally applied nesfatin-1 depresses food intake, because of that inhibiting weight gain. Also, intravenous nesfatin-1 administration was shown to decrease glucose level in db/db mice (10).

This is the first clinical study which measured serum nesfatin-1 levels using ELISA method among subjects with biopsy-diagnosed NAFLD and a healthy control group. In this study we aimed to determine whether there was a relationship between Nesfatin-1 and insulin resistance, body mass index, histopathologic characteristics, metabolic syndrome components and biochemical parameters.

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## MATERIALS AND METHODS

### Ethics

This study was approved by the local ethics committee (Uludag University, Medical Faculty, Bursa, Turkey) (2011-13/15). Informed consent was obtained from each patient included in the study.

### Study participants

This study was conducted in 2011 at Uludag University, Bursa/Turkey. This study is a case-control study. Fifty-nine subjects with NAFLD (30 males, 29 females, mean age  $45.97 \pm 8.257$  years) and a control group of 32 healthy controls (12 males, 20 females, mean age  $38.41 \pm 7.015$  years) were enrolled. Patients diagnosed with NAFLD were being followed as outpatients for over 12 months in a university hospital and all of them were shown to have Grade I or worse steatosis ultrasonographically. Patients with disease other than NAFLD, diagnosis of malignancy, prior abdominal surgery and patients with daily alcohol consumption exceeding 20 grams were not included in the study. Patients using steroids, estrogen, amiodarone, tamoxifen and lipid-lowering drugs were also excluded. All control group subjects were healthy people with normal liver function tests and no signs of fatty liver as demonstrated by ultrasonography. Individuals with daily alcohol consumption exceeding 20 grams and those receiving any medical treatment were excluded.

### Clinical and biochemical characterization

Biochemical and anthropometric analyses and physical examination were performed for all participants. Body mass index (BMI) was measured from height and weight measurements. Diagnosis of diabetes mellitus was made by criteria of the American Diabetes Association (ADA) (11). National Cholesterol Education Program Adult Treatment Panel III (ATP III) criteria were used for diagnosing metabolic syndrome (12). Insulin resistance was evaluated using the homeostasis model assessment method for insulin resistance (HOMA-IR):  $\text{Insulin resistance} = \frac{\text{fasting plasma insulin (microunits/ml)} \times \text{fasting plasma glucose (FPG) (mmol/L)}}{22.5}$  ( $<2.7$  normal,  $\geq 2.7$  insulin resistance) (13). Blood pressure measurement was performed in a quiet room by using a sphygmomanometer after 10 minutes of rest. Blood biochemistry were analysed by the biochemistry laboratory of university hospital.

### Liver histology

Ultrasonography-guided percutaneous liver biopsy was performed by using a 16 gauge needle under conscious sedation. All biopsy tissue specimens were

transferred into formaldehyde solution for fixation and embedded in paraffine blocks. Liver cross-sections were stained with hematoxylin–eosin and Masson's trichrome dyes.

Scoring was performed in 4 categories including definite steatohepatitis, borderline steatohepatitis zone 3, borderline steatohepatitis zone 1 and not steatohepatitis with steatosis according to recommendations for current non-alcoholic steatohepatitis clinical studies (14). For the purpose of analysis, borderline steatohepatitis zone 3 and borderline steatohepatitis zone 1 were combined and assessed as a single group (borderline steatohepatitis) (Table 1).

NAFLD activity score was assigned by a blinded expert pathologist according to the NASH-CRN scoring system. Steatosis was scored by a 4-stage scoring system from 0 to 3, where S0 indicated no steatosis or less than 5%, S1 between 5-33%, S2 between 33-66% and S3 greater than 66%. Lobular inflammation was staged as follows: stage 0: no foci, stage 1: perisinusoidal or periportal fibrosis, Stage 1A: mild, zone 3, perisinusoidal; 1B: intermediate, zone 3, perisinusoidal fibrosis and 1C: portal/periportal fibrosis; stage 2: perisinusoidal and portal/periportal fibrosis, stage 3: bridging fibrosis and stage 4: cirrhosis (15).

### Serum nesfatin-1 measurement

Samples of blood were obtained from the vein after night fasting in the morning between 08.00 and 09.00. Blood was centrifuged for ten minutes at 2500 rpm and serum samples were stored at -80°C until the time of analysis. Serum nesfatin-1 level was determined by using ELISA kit (Phoenix Pharmaceuticals, Burlingame, California, USA) according to the protocol of procedures recommended by the manufacturer. Also, all measurements were performed in a blinded fashion without any knowledge about clinical status of participants.

### Statistical analysis

Descriptive data for control and NAFLD patient groups were expressed in mean  $\pm$  SD, median value, minimum and maximum values, numbers and % frequencies. Kolmogorov-Smirnov test was used to determine whether numerical values showed normal distribution. Independent samples t-test was used for

comparing two groups with respect to the numerical attribute showing normal distribution. Mann-Whitney U test was used to compare control and NAFLD patient groups in terms of variables not showing normal distribution. Simple linear associations between nesfatin-1 and other numerical attributes in patient and control groups were assessed by Pearson's correlation analysis. A multiple linear regression model was used to determine risk factors which affected nesfatin-1 results among patients and backward variable elimination method was utilized for model construction. Relations between categorical characteristics and groups were assessed using appropriate chi-square analysis (Pearson's chi-square or Fisher exact chi-square tests). Statistical significance level was set at  $P < 0.05$  and Predictive Analytics Software (SPSS version 18) was used for calculations.

## RESULTS

NAFLD patient group had 59 (male/female: 30/29) patients and control group had 32 (male/female: 20/12) subjects. Mean age was  $45.96 \pm 8.25$  years and  $38.40 \pm 7.01$  years for patients in NAFLD group and control group, respectively ( $P < 0.0001$ ). Age was significantly higher in NAFLD group (Table 2). Descriptive data for numerical attributes with normal distribution are presented in the Table 2 for patient and control groups. When categorical attributes were assessed, there was no difference in two groups with respect to sex ( $P < 0.273$ ). However, presence of insulin resistance, diabetes mellitus or metabolic syndrome differed significantly in favor of NAFLD group ( $P < 0.0001$ ). As seen from the Table 2, while only high-density lipoprotein cholesterol (HDL-C) was significantly lower, mean values for other characteristics were significantly higher among patients. Descriptive values are shown for hemoglobin A1c (HbA1c) and ferritin because they were only measured in patients.

Descriptive values for numerical characteristics that show normal distribution are shown in Table 3 below for patient and control groups. From this table, it can be seen that while a significant association could not be identified for HDL-cholesterol, mean values for the remaining characteristics were significantly greater among patients versus those of control group.

**Table 1.** Histological features according to steatohepatitis status

Histology variable	Entire cohort	Definite SH	Borderline SH	Steatosis without SH
N	59	56	2	1
Steatosis grade				
0 (<5%)	0	0	0	0
1 (5-33%)	14	14	0	0
2 (34-66%)	27	26	1	0
3 (>66%)	18	17	1	1
Steatosis location				
0 (zone 3)	11	11	0	0
1 (zone 1)	1	1	0	0
2 (azonal)	12	9	2	1
3 (panacinar)	35	35	0	0
Lobular inflammation				
0 (none)	1	1	0	0
1 (<2)	14	13	0	1
2 (2-4)	36	35	1	0
3 (>4)	8	7	1	0
Chronic portal inflammation				
0 (none)	24	23	0	0
1 (mild)	26	24	2	0
2 (>mild)	9	9	0	1
Ballooning				
0 (none)	3	0	2	1
1 (few)	22	22	0	0
2 (many)	34	34	0	0
Fibrosis				
0 (none)	19	19	0	0
1a	13	13	0	0
1b	3	3	0	0
1c	3	3	0	0
2	10	7	2	1
3 (bridging)	8	8	0	0
4 (cirrhosis)	3	3	0	0

Data are reported as counts. SH: steatohepatitis

Analysis of descriptive values for numerical attributes without normal distribution demonstrated that there was not any difference between NAFLD patient group (2.199±3.923 ng/ml and median=0.8864 ng/ml) and control group (1.847±2.553 ng/ml and median=1.0381ng/ml) with respect to mean nesfatin-1 concentration ( $P<0.170$ ).

Descriptive values for numerical characteristics that do not show normal distribution are shown in

Table 4 for patients and control groups. As can be seen from the table, systolic and diastolic blood pressures were significantly higher in patients but no significant difference was detected between two groups in nesfatin-1.

In our study, there was a significant difference between BMI and sex of both groups. In case when there is a significant difference, the effects of these variables can be eliminated with use of multiple statistical mod-

**Table 2.** General characteristics of control and patient groups

	Patients with non-alcoholic fatty liver disease (n=59)	Control group (n=32)	P value
Sex (male/female)	30/29	12/20	<0.273
Age (years)	45.96±8.25	38.40±7.01	<0.0001
Body mass index (kg/m <sup>2</sup> )	31.13±3.67	24.83±2.72	<0.0001
Metabolic syndrome (yes/no)	40/19	0/32	<0.0001
Diabetes mellitus (yes/no)	24/35	0/32	<0.0001
HOMA-IR (yes/no)	47/12	3/29	<0.0001
Systolic blood pressure (mmHg)	126.86±14.16	116.41±8.25	<0.0001
Diastolic blood pressure (mmHg)	80.42±10.87	74.06±5.74	=0.002
AST (U/L)	63.76±34.23	18.44±5.63	<0.0001
ALT (U/L)	99.02±48.00	15.22±6.56	<0.0001
Total cholesterol (mg/dl)	220.80±46.72	180.06±38.79	<0.0001
HDL cholesterol (mg/dl)	41.75±9.27	46.47±10.75	=0.003
LDL cholesterol (mg/dl)	142.75±43.78	112.00±25.06	<0.0001
Triglyceride (mg/dl)	204.37±120.611	116.13±80.84	<0.0001

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

**Table 3.** Descriptive values for numerical characteristics that show normal distribution in patients and control groups

	Groups	N	Mean	Standard Deviation	P value
Age	Control	32	38.4063	7.01546	<0.0001
	NAFLD	59	45.9661	8.25659	
Body Mass Index score	Control	32	24.8376	2.72775	<0.0001
	NAFLD	59	31.1334	3.67465	
AST (U/L)	Control	32	18.44	5.639	<0.0001
	NAFLD	59	63.76	34.233	
ALT (U/L)	Control	32	15.22	6.564	<0.0001
	NAFLD	59	99.02	48.001	
Triglyceride (mg/dl)	Control	32	116.13	80.844	<0.0001
	NAFLD	59	204.37	120.611	
Total Cholesterol (mg/dl)	Control	32	180.06	38.790	<0.0001
	NAFLD	59	220.80	46.722	
HDL Cholesterol (mg/dl)	Control	32	46.47	10.755	0.003
	NAFLD	59	41.75	9.271	
LDL Cholesterol (mg/dl)	Control	32	112.00	25.061	<0.0001
	NAFLD	58	142.75	43.789	
HbA1c	Control	---	---	---	---
	NAFLD	58	6.3328	1.43361	
Ferritin (mcg/ml)	Control	---	---	---	---
	NAFLD	59	126.41	89.802	

AST: aspartate aminotransferase; ALT: alanine aminotransferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HbA1c: glycosylated hemoglobin

els, i.e., matched groups may be obtained using statistical methods. However, in the present study, the difference between groups in nesfatin-1 was assessed

using a univariate Mann-Whitney test because no significant or considerable association was found between sex or BMI score and nesfatin in either groups

**Table 4.** Descriptive values for numerical characteristics that do not show normal distribution in patients and control group

Numerical characteristics without normal distribution	Control group (n:32)	NAFLD group (n:59)	P value
	Mean ± Standard Deviation	Mean ± Standard Deviation	
Nesfatin-1	1.847±2.55	2.199±3.92	0.170
Systolic blood pressure (mmHg)	116.41±8.25	126.86±14.16	0.000
Diastolic blood pressure (mmHg)	74.06±5.74	80.42±10.87	0.002
Erythrocyte Sedimentation Rate (mm/h)	---	20.36±17.10	---
Ultrasonography	---	2.03±0.66	---
Histological steatosis	---	2.07±0.74	---
Lobular inflammation	---	1.86±0.65	---
Portal inflammation	---	0.75±0.70	---
Ballooning	---	1.54±0.62	---
Fibrosis	---	4.64±4.91	---
NASH score	---	5.51±1.29	---

NAFLD: Non-Alcoholic Fatty Liver Disease; NASH: Non-Alcoholic Steatohepatitis

(borderline significance was detected for the association of nesfatin-1 and BMI score only among patients but since the significance was weak, it was considered non-significant).

The results of regression analysis also failed to demonstrate any association after making correction for BMI and sex. Thus, in the current study, association of nesfatin-1 and other individual characteristics were evaluated separately for each group in order to determine the factors affecting nesfatin-1. Matching the groups for BMI or sex was not needed because each group was analyzed separately. Two study groups were assessed individually with the anticipation that different associations with nesfatin-1 would be observed in each group.

Simple linear relations (correlation coefficient "r" and significance level "p") between Nesfatin-1 and other numerical attributes are shown in the table for control and patient groups separately. From the Table 5, it was observed that nesfatin-1 values were significantly decreased as BMI increased ( $P=0.043$ ) and nesfatin-1 values increased in proportion to increases in AST ( $P=0.05$ ) among patients. However, no other significant associations were found for patients or controls.

For patients, a multiple regression model was constructed in order to find out the factors which influence nesfatin and backward variable elimination method was used to determine the variables to be included in

the model. The model is presented in the Table 6 and includes the variables that have a significant linear association with nesfatin. As seen in the table a positive linear and significant association was found between age, systolic blood pressure and AST and nesfatin-1.

## DISCUSSION

Our findings are the first to measure and evaluate serum nesfatin-1 levels in NAFLD patients diagnosed with biopsy. The weight-reducing effect of Nesfatin-1 which has anorexigenic action led us to the hypothesis that Nesfatin-1 is involved in NAFLD pathogenesis and associated with factors involved in metabolic syndrome, insulin resistance and many other similar etiologies. Su et al. showed that Nesfatin-1 has a critical role for the control of glucose metabolism via its antihyperglycemic action (10).

Gonzales et al. reported that insulin producing beta cells coexist with pre-pronesfatin in rodent islet cells (16). Li et al. demonstrated that fasting nesfatin-1 concentrations in type 2 DM patients were lower than healthy subjects and type 1 DM patients (17). However, in the this study we did not obtain a significant association between serum nesfatin-1 level and type 2 DM patients on oral antidiabetics or diabetic patients newly diagnosed by oral glucose tolerance test.

Basar et al. found statistically significantly lower nesfatin-1 levels among patients without a biopsy

**Table 5.** Correlations between serum Nesfatin-1 and other variables in 59 patients with biopsy proven non-alcoholic fatty liver disease

	<b>r</b>	<b>P value</b>
Sex	-0.244	0.063
Age	0.030	0.822
Body mass index	-0.264	0.043
Diabetes mellitus	0.158	0.232
Metabolic syndrome	-0.193	0.144
HOMA-IR	-0.024	0.855
Systolic blood pressure	-0.019	0.889
Diastolic blood pressure	0.018	0.891
AST	0.250	0.050
ALT	0.135	0.307
Total cholesterol	-0.064	0.631
HDL cholesterol	-0.126	0.342
LDL cholesterol	-0.008	0.953
Triglyceride	-0.117	0.376
HbA1c	0.128	0.337
hs-CRP	-0.020	0.880
Sedimentation	-0.137	0.304
Steatosis grade	0.050	0.709
Lobular inflammation	0.173	0.190
Chronic portal inflammation	0.111	0.401
Ballooning	0.207	0.115
Fibrosis	-0.103	0.436

HOMA-IR: homeostasis model assessment of insulin resistance; AST: aspartate aminotransferase; ALT: alanine aminotransferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein; HbA1c: glycosylated hemoglobin

diagnosis but considered to have NAFLD based on elevated liver function tests and grade 2 or grade 3 steatosis ultrasonographically (0.26±0.14 ng/ml and 0.38±0.18 ng/ml respectively,  $P=0.008$ ) (18). Also, in that study serum nesfatin-1 level was found to be lower in the obese group versus non-obese group based on BMI and significantly lower in the group who had insulin resistance compared to the group who did not. In this study, there was no difference between NAFLD patient group (2.199±3,923 ng/ml and median=0.8864 ng/ml) and control group (1,847±2,553 ng/ml and median=1.0381 ng/ml) in nesfatin-1 levels ( $P<0.170$ ). By contrast, in this study there was not any significant association between NAFLD patients with insulin resistance and nesfatin-1.

Atsuchi et al. showed that central administration of nesfatin-1 resulted in lower food intake and inhibition of gastroduodenal motility in mice and suggested that nesfatin-1 had an influence on gut motility and dietary habits (19). Intracerebroventricular (ICV) nesfatin-1 infusion was shown to reduce food intake in a dose-dependent manner within approximately 6 hours (20). Contrastingly, appetite was shown to be stimulated when nesfatin-1 antibody was given by ICV route. In rats, decreased body weight and reduced size of white adipose tissue were observed following chronic nesfatin-1 infusion into third ventricle. This suggests that nesfatin/NUCB2 is associated with physiological regulation of eating habits in rats. Subcutaneous injection of nesfatin-1 inhibited food intake and anorexigenic

**Table 6.** The factors that effecting the Nesfatin-1 level in the NASH patients according to multiple regression analysis

<b>Model</b>	<b>Regression coefficient</b>			<b>p</b>
	<b>B</b>	<b>Standart Error</b>	<b>t</b>	
(Constant)	140.067	50.555	20.533	0,015
Age	0.120	0.054	20.217	0.031
Sex	-40.897	10.119	-40.378	0.000
Systolic blood pressure	0.063	0.032	10.957	0.050
Body mass index	-0.510	0.128	-30.980	0.000
AST	0.033	0.014	20.427	0.019
HDL cholesterol	-0.208	0.052	-30.975	0.000
Erythrocyte Sedimentation Rate	-0.086	0.028	-30.116	0.003
Chronic portal inflammation	10.767	0.625	20.829	0.007

NASH: Non-Alcoholic Steatohepatitis; AST: aspartate aminotransferase; HDL:high-density lipoprotein

action was maintained for 14 hours after the injection (20). Central injection of alpha MSH ( $\alpha$ -melanocyte-stimulating hormone) results in increased expression of nesfatin/NUCB2 in the paraventricular nucleus. This indicates that there is a relation between nesfatin signaling pathway and melanocortin signaling pathway in the hypothalamus (7).

Tsuchiya et al. showed a negative correlation between BMI and nesfatin-1 and suggested that nesfatin-1 contributed to energy homeostasis (21).

In this study, simple correlation analysis of nesfatin-1 and other numerical data for control and patient groups showed that nesfatin-1 values decreased as BMI increased ( $P=0.043$ ) and nesfatin-1 values increased as AST elevated ( $P=0.05$ ). On the other hand, while a positive linear and significant relationship was found between age, systolic blood pressure and AST in the multiple regression analysis, other characteristics including BMI, HDL cholesterol, erythrocyte sedimentation rate and portal inflammation showed a negative significant relationship with nesfatin-1.

In one study, it was suggested that in addition to its anorexigenic action, nesfatin-1 also had a hypertensive role via its melanocortin<sub>3/4</sub> receptors in the hypothalamus, and subsequently, an increase in arterial blood pressure was shown in another study following intravenous administration of nesfatin-1 (22,23). In our study, a significant increase in systolic blood pressure was observed among patients with higher serum nesfatin-1 levels.

Another remarkable finding of our study was that Nesfatin-1 level increased significantly as age increased.

## CONCLUSION

For the first time, with this study, a negative correlation between nesfatin-1 and increased portal inflammation was shown histologically in NAFLD patients. Also, a significant association was demonstrated between metabolic syndrome components and nesfatin-1 including systolic blood pressure and HDL cholesterol.

Chronic portal inflammation has significance in predicting the progression of disease among NAFLD patients. Chronic portal inflammation concurrently occurs with ductular reaction and progression of fi-

brosis. Increased portal inflammation is an indicator of disease progression in NAFLD patients. However, Brunt et al. showed that chronic portal inflammation is not associated with steatosis or lobular inflammation but may be associated with fibrosis (24).

This study has some limitations. First, the study was conducted in a defined population; thus, studies on different populations would be valuable. Also, the number of subjects enrolled in the study was rather small; we believe that, prospective studies with larger number of subjects would help to clarify the mechanisms in question. Yet, data available provide us some important facts.

While there is an unrelenting quest for pharmacologic therapy, lifestyle modifications and exercise programs tailored for reduction of abdominal obesity (a major risk factor) are still at the forefront of efforts to decrease the prevalence of NAFLD. The recommendations for development of exercise programs specifically designed for amelioration of the adipose tissue, skeletal muscle and liver are important to decrease the severity and incidence of NAFLD (25,26).

## Conflict of Interest

The authors declare no conflict of interest.

## Financial Disclosure

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