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Study of Serum Vaspin Level in Patients with Nonalcoholic Fatty Liver Disease and Its Relation to Insulin Resistance and Some Anthropometric and Metabolic Measures

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Abstract

Introduction Nonalcoholic fatty liver disease (NAFLD) is a public health problem with a spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) that may be associated with fibrosis and cirrhosis. The presence of steatosis is tightly associated with hepatic Inflammation where the role of hepatocyte cytokine production in the progression of steatosis to NASH is proved. Visceral adipose tissue–derived serine protease inhibitor (vaspin) is a novel adipocytokine that may link obesity, IR, and type 2 DM. Aim of work To study the significance of serum vaspin level in patients with NAFLD and NASH. Also, the relationship between its level and different laboratory, metabolic and anthropometric parameters was assessed. Subjects and Methods 50 age and sex matched subjects were included and divided into: Group I: 20 NAFLD patients, Group II: 20 NASH patients and 10 healthy control subjects (Group III). Results Serum vaspin level was the highest among NASH patients in comparison to NAFLD patients and control subjects, an evident statistical significant difference was observed among different studied groups. Conclusion Serum vaspin level is markedly elevated among NASH patients, this proves its role as a laboratory marker for the diagnosis of NASH.

Keywords Nonalcoholic fatty liver disease (NAFLD), Nonalcoholic steatohepatitis (NASH), Body mass index (BMI), Vaspin.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is an emerging health problem worldwide with an estimated prevalence of up to 40% in Western countries. The likelihood of developing NAFLD increases substantially with body mass index (BMI), where NAFLD is considered as the hepatic manifestation of the metabolic syndrome. (1)

Mild NAFLD involves less than 30% of hepatocytes, whereas severe form involves more than 60% of hepatocytes. (2) NAFLD can progress to a severe form of the disease, namely nonalcoholic steatohepatitis (NASH), which has a prevalence of 2-3% in lean subjects, (3) 20% in obese and 50% in morbidly obese patients. (4) Whereas NAFLD is a benign condition with low risk of developing cirrhosis (lower than 4% over a period of 1-2 decades), 5-8% of patients with NASH

will develop liver cirrhosis within five years, thus; NASH is currently recognized as a leading cause of cryptogenic cirrhosis. [5, 6]

NAFLD is characterized by the accumulation of triglycerides, which are formed from the esterification of free fatty acids (FFAs) and glycerol within the hepatocytes. FFAs arise in the liver from three distinct sources; lipolysis within adipose tissue (60%), dietary sources (15%), and denovo lipogenesis DNL (25%). [7, 8]

Insulin has a potent action to suppress adipose tissue lipolysis. However, in situations of insulin resistance (IR), this suppression is impaired resulting in an increased efflux of FFAs from adipose tissue. [9] The hyperinsulinemia associated with IR leads to: (i) up-regulation of the transcription factor sterol regulatory element binding protein-1c (SREBP-1c), which is a key transcriptional regulator of genes involved in DNL, [10] and (ii) Inhibition of β-oxidation of FFAs thus promoting hepatic lipid accumulation. [11]

The presence of steatosis is tightly associated with chronic hepatic Inflammation which is associated with elevated hepatic expression of inflammatory cytokines such as tumor necrosis factor- α (TNF-α), interleukin-6 (IL-6), interleukin 1-beta (IL-1β), and activation of Kupffer cells. [12] The Iκκ-β/NF-κB pathway in hepatocytes can also be activated directly by FFAs, providing a further mechanism by which central obesity with consequent increased hepatic FFAs supply can contribute to inflammation. [13]

Cytokines have a key role in the progression of steatosis to NASH where they induce neutrophil chemotaxis, hepatocyte apoptosis/necrosis, Mallory body formation and stellate cell activation. Additionally, data suggests that inflammation and NF-κB activation can promote carcinogenesis, thus; the chronic inflammatory state associated with NASH may also play a key role in HCC development. [14, 15]

The role of oxidative stress and mitochondrial dysfunction in NASH is well established, with more advanced disease correlating with greater degrees of oxidative stress. [16] β-oxidation takes place in the mitochondria, but in the context of NAFLD this process giving rise to reactive oxygen species (ROS). ROS induce oxidative stress, with subsequent activation of inflammatory pathways, and also mitochondrial damage. [17] Also, structural mitochondrial abnormalities and a reduction in mitochondrial respiratory chain activity have been observed in human studies of NASH. [18]

Moreover, NASH pathogenesis includes endoplasmic reticulum (ER) stress and gut-derived endotoxaemia. ER stress can be caused by a variety of biological stresses, including hyperinsulinemia and hyperlipidemia, and can result in activation of various pathways leading to IR, inflammation, apoptosis and mitochondrial dysfunction. [19] Evidence is also emerging for the role of bacterial overgrowth in the pathogenesis of NASH. Bacterial overgrowth results in production of ethanol and release of bacterial lipopolysaccharides, [20] both of which can activate TNF-α production in Kupffer cells and thus induce hepatic inflammation. [21] Small intestinal bacterial overgrowth and increased gut permeability found more in patients with NASH when compared with controls. [22]

This may explain the onset of NASH and liver fibrosis as a complication of jejuno-ileal bypass surgery. [23] This hypothesis is further supported by evidence that alteration of gut flora with antibiotics and probiotics can reduce hepatic inflammation in humans. [24, 25]

Visceral adipose tissue–derived serine protease inhibitor (vaspin) was identified as a member of serine protease inhibitor family. [26] It is an adipokine that has been isolated from both visceral and subcutaneous white adipose tissue. Visceral vaspin expression is significantly correlated with BMI, percentage of body fat and the level of plasma glucose after 2 h of oral glucose tolerance testing, whereas its subcutaneous expression is significantly correlated with waist-to-hip ratio, fasting plasma insulin concentration and glucose infusion rate during the steady state of a euglycemic-hyperinsulinemic clamp. [27, 28]

Vaspin expression was shown to decrease with worsening of diabetes and body weight loss, whereas its serum levels could be normalized by insulin or pioglitazone treatment. [26] Human vaspin mRNA expression in adipose tissue of obese subjects is fat depot specific and not detectable in lean normal glucose-tolerant individuals. [29] It was postulated that induction of vaspin mRNA expression in human adipose tissue could represent a compensatory mechanism associated with obesity, severe insulin resistance, and type 2 DM. [30] Also, vaspin suppresses leptin, TNFα and resistin expression. Administration of recombinant vaspin significantly improved insulin sensitivity and glucose tolerance. [31]

Aim of work: The present work aimed to study serum vaspin level in patients with simple steatosis (NAFLD) and patients with nonalcoholic steatohepatitis (NASH). Also, to assess the relationship between serum vaspin level and different laboratory, metabolic and anthropometric parameters.

Subjects

The present study included 50 age and sex matched subjects presented to the outpatient clinic of the Internal Medicine Department, Alexandria Main University Hospital. They were divided into 3 groups:

- **Group I:** 20 patients with NAFLD and BMI more than 30 kg/m^2. NAFLD was diagnosed by abdominal ultrasonography.
- **Group II:** 20 patients with NASH and BMI more than 30 kg/m^2. NASH was diagnosed by elevated liver enzymes (ALT, AST) in NAFLD patients.
- **Group III:** 10 healthy control subjects with BMI ranged from18.5 to 24.9 kg/m^2.

Exclusion criteria: Patients with DM, hypertension and/or manifest heart disease, liver diseases, renal diseases, history of alcohol intake and any metabolic abnormalities were excluded. All the participants included were informed about the nature of the study, and written consents obtained.

Methods

All subjects included in this study were subjected to the following:

- Complete history taking and full clinical examination focusing on: exclusion criteria, waist circumference and body mass index (BMI) where BMI = Weight (kg)/Height (m)^2.

- Laboratory investigations including: fasting blood glucose and serum insulin levels, complete lipid profile, liver enzymes (ALT, AST), and serum vaspin level. [32]
- Abdominal ultrasonography stressing on the sonographic criteria of NAFLD; including the presence of bright echoes, increased hepatorenal contrast, vessel blurring, posterior beam attenuation and non-visualization of diaphragm.

Statistical analysis of the data:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test, If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed data, comparison between more than two population were analyzed F-test (ANOVA) to be used and Post Hoc test (LSD), For abnormally distributed data, Kruskal Wallis test was used to compare between different groups and pair wise comparison was assessed using Mann-Whitney test. Significance of the obtained results was judged at the 5% level. [33, 34]

Results

Age in NAFLD patients **(Group I)** had a mean value of 45.50±5.69 years, in NASH patients **(Group II)** was 44.45±6.90 years and in control subjects **(Group III)** was 44.70±5.96 years. As regards sex, males predominated females in all studied groups. There was no statistical significant difference between the three studied groups according to demographic data.

BMI showed a median of 40.5 and 39.75 in **Groups I and II** respectively. On the other hand, its median in control subjects **(Group III)** was much lower (20.85). A statistical significant difference was reported between NAFLD and NASH groups in comparison to the control group ($p < 0.001$), while there was no statistical significant difference between NAFLD and NASH groups. **Table (1)**

Waist circumference had close medians of 110.5 cm and 110 cm in NAFLD and NASH groups respectively. On the other hand, a much lower median of 76 cm was reported among control subjects **(Group III)**. A statistical significant difference was reported between NAFLD and NASH groups in comparison to the control group ($p < 0.001$), while there was no statistical significant difference between NAFLD and NASH groups. **Table (1)**

Table (2) showed multiple studied laboratory parameters among different studied groups. Moreover, the mean of serum vaspin level was the highest among NASH patients **(Group II)** (1.25±0.06 ng/ml) in comparison to NAFLD patients **(Group I)** and control subjects **(Group III)** (1.11±0.09 ng/ml and 0.50±0.20 ng/ml respectively). An evident statistical significant difference was observed among different studied groups ($p<0.001$). **Table (3) Figure (1)**

In our study, there was no correlation between serum vaspin level and different studied laboratory, metabolic and anthropometric parameters in NAFLD and NASH group. **Table (4)**

Discussion

Non-alcoholic fatty liver disease (NAFLD) is a common condition with increasing prevalence globally; it is usually associated with obesity, insulin resistance (IR) and type 2 DM. [35] Up to 2-3% of the general population is estimated to have non-alcoholic steatohepatitis (NASH), which may progress to liver cirrhosis and hepatocellular carcinoma. [36] Vaspin is a novel adipocytokine that may link obesity, IR, and type 2 DM. Some studies have suggested that vaspin could play an important role in the development of obesity and other metabolic disorders. [37]

In our study, age showed close medians and males predominated females in all studied groups. In agreement with our results, Clark M and Marianne J [38] stated that the overall prevalence of NAFLD in all age groups was significantly more common in men compared with women (33% vs 17%). Also, Bacon R et al [39] reported that 58% of their studied patients with NASH were males.

Regarding BMI and waist circumference, our results were supported by those of Marchesini G et al, [40] they found that most patients with NAFLD have the clinical characteristics of IR syndrome including obesity. Also, Angulo P et al [41] reported that obesity (BMI>31.1 kg/m^2 in males, >32.3 kg/m^2 in females) was independent predictor of fibrosis in NASH patients. Moreover, several studies proved the strong link between NAFLD, NASH and obesity with high BMI. [42, 43]

In our study, different laboratory parameters were assessed. Fasting blood glucose (FBG) level showed close values in different studied groups. Regarding fasting insulin (FI) level, it showed highest median in NASH patients **(Group II)**, while its median showed close values in the remaining NAFLD patients **(Group I)** and control subjects **(Group III)**, 7.25 IU/ml; 5.45 IU/ml and 4.40 IU/ml respectively. A statistical significant difference was reported between NASH patients **(Group II)** and control group **(Group III)** ($p<0.05$). In accordance with our results, Lesmana CR et al [44] stated that among NASH patients FI level was significantly higher than the control group.

In the present study, homeostatic model assessment of insulin resistance (HOMA-IR) showed its highest median in NASH patients **(Group II)**; while its median showed close values in the remaining NAFLD patients **(Group I)** and control subjects **(Group III)**, 1.53; 1.18 and 0.85 respectively. A statistical significant difference was reported between NASH patients **(Group II)** and control group **(Group III)** ($p<0.05$). In accordance with our results, Salgado AL et al [45] reported higher HOMA-IR values in NAFLD patients than control subjects.

Regarding triglyceride (TG), cholesterol and low density lipoprotein (LDL) levels, they showed highest median in NASH patients **(Group II)**, while close values were observed in the remaining NAFLD patients **(Group I)** and control subjects **(Group III)** with a statistical significant difference between NASH patients **(Group II)** in comparison to NAFLD and control groups.

In accordance with our results, Agrawal R et al [46] reported hypertriglyceridaemia in 63.7%, hypercholesterolemia in 50%–80%, elevated LDL in 25% of NAFLD patients. Also, Zhuravlyova LV et al [47] reported a

disorder of blood lipid profile in NAFLD and NASH patients.

AST and ALT showed their highest median (46 IU/L, 67 IU/L) among NASH patients **(Group II)** in comparison to NAFLD patients **(Group I)** and control subjects **(Group III)**. A statistical significant difference was reported between NASH patients in comparison to NAFLD patients and control subjects where $p < 0.001$.

In accordance with our results, Targher G et al [48] reported elevated liver enzymes in patients with hepatic steatosis detected by US. Also, Barchetta I et al [49] stated that AST and ALT levels were significantly high in NAFLD patients in comparison to control subjects.

Serum vaspin level showed its highest mean among NASH patients **(Group II)** in comparison to NAFLD patients **(Group I)** and control subjects **(Group III)** with an evident statistical significant difference among different studied groups ($p<0.001$). In agreement with our results, Aktas B et al [50] reported high serum vaspin level in NAFLD patients compared to control subjects. Also, Genc H et al [51] reported high serum vaspin level in NASH patients compared to control subjects. On the contrast, Kukla M et al [52] stated that there was no difference in serum vaspin level between NAFLD patients and control subjects.

Regarding correlation of serum vaspin level with different studied parameters, on the contrast to our findings, Youn B et al [53] reported a significant correlation between serum vaspin level and BMI in NAFLD and NASH patients. Moreover, Buldak R et al [54] stated that serum vaspin level is correlated with ALT and AST in NASH and NAFLD patients.

Conclusion

Serum vaspin level is markedly elevated among NASH patients in comparison to NAFLD patients and control subjects, which proves its role as a laboratory marker for the diagnosis of NASH. However, the ultimate diagnostic utility and implication of serum vaspin in NAFLD and NASH patients need to be validated in a wider scale multicenter population based studies.

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Table (1): Comparison between the three studied groups according to BMI and waist circumference.

	NAFLD (GI, n=20)	NASH (GII, n=20)	Control (GIII, n=10)	^{F}p
BMI				
Min.–Max.	32.70–53.40	32.80–45.30	18.50–24.10	
Mean±SD.	40.91±5.94	39.09±3.96	21.25±2.05	<0.001*
Median	40.50	39.75	20.85	
Sig. bet. grps	**p_1 = 0.219, p_2 <0.001*, p_3 <0.001***			
Waist circumference (cm)				
Min.–Max.	94.0–135.0	100.0–119.0	69.0–85.0	
Mean±SD.	111.50±11.39	108.90±5.58	76.15±6.21	<0.001*
Median	110.50	110.0	76.0	
Sig. bet. grps	**p_1 = 0.339, p_2 <0.001*, p_3 <0.001***			

^{F}p: P value for F test (ANOVA)
p_1: p value for Post Hoc test (LSD) for comparing between NAFLD and NASH
p_2: p value for Post Hoc test (LSD) for comparing between NAFLD and Control
p_3: p value for Post Hoc test (LSD) for comparing between NASH and Control
*: Statistically significant at $p \leq 0.05$

Table (2):Comparison between the three studied groups according to different studied laboratory parameters.

	NAFLD (GI, n=20)	NASH(GII, n=20)	Control(GIII, n=10)	p
FBG (mg/dl)				
Min.–Max.	70.0–97.0	75.0–98.0	70.0–88.0	
Mean±SD.	84.05±7.74	88.40±6.95	79.40±5.25	$^{F}p= 0.006^{*}$
Median	85.0	90.0	80.0	
Sig. bet. Grps	$p_1 = 0.055$, $p_2 = 0.093$, $p_3 = 0.002^{*}$			
Fasting insulin (IU/ml)				
Min.–Max.	1.24–13.04	1.27–12.50	3.94–5.01	
Mean±SD.	6.22±3.17	7.45±3.21	4.38±0.34	$^{KW}\chi^2 p= 0.017^{*}$
Median	5.45	7.25	4.40	
Sig. bet. Grps	$p_1 = 0.156$, $p_2 = 0.187$, $p_3 = 0.002^{*}$			
HOMA-IR				
Min.–Max.	0.29–2.73	0.29–2.77	0.68–1.0	
Mean±SD.	1.35±0.70	1.55±0.70	0.86±0.10	$^{KW}\chi^2 p= 0.021^{*}$
Median	1.18	1.53	0.85	
Sig. bet. Grps	$p_1 = 0.304$, $p_2 = 0.075$, $p_3 = 0.004^{*}$			
TG (mg/dl)				
Min.–Max.	70.31–210.0	103.50–220.70	68.40–130.50	
Mean±SD.	114.29±47.79	156.15±34.22	95.64±19.84	$^{KW}\chi^2 p= 0.001^{*}$
Median	91.05	159.75	89.73	
Sig. bet. Grps	$p_1 = 0.003^{*}$, $p_2 = 0.725$, $p_3 < 0.001^{*}$			
Cholesterol (mg/dl)				
Min.–Max.	132.80–270.90	130.54–232.80	117.0–182.0	
Mean±SD.	174.15±34.35	194.12±27.19	159.40±19.55	$^{F}p= 0.014^{*}$
Median	167.10	195.10	164.5	
Sig. bet. Grps	$p_1 = 0.049^{*}$, $p_2 = 0.200$, $p_3 = 0.005^{*}$			
LDL (mg/dl)				
Min.–Max.	91.20 – 206.90	100.0–199.0	75.0 – 133.0	
Mean±SD.	122.46 ± 29.37	155.65±30.32	102.30 ± 22.10	$^{F}p<0.001^{*}$
Median	117.16	158.52	100.0	
Sig. bet. Grps	$p_1 = 0.001^{*}$, $p_2 = 0.074$, $p_3 < 0.001^{*}$			
AST (IU/L)				
Min.–Max.	18.0–37.0	40.0–65.0	15.0–30.0	
Mean±SD.	26.05±6.04	49.30±7.68	21.70±4.76	$^{F}p<0.001^{*}$
Median	25.50	46.0	21.0	
Sig. bet. Grps	$p_1<0.001^{*}$, $p_2 = 0.093$, $p_3<0.001^{*}$			
ALT (IU/L)				
Min.–Max.	30.0–56.0	45.0–110.0	30.0–65.0	
Mean±SD.	43.13±8.19	70.0 ±12.65	43.50±13.77	$^{F}p<0.001^{*}$
Median	41.50	67.0	39.50	
Sig. bet. Grps	$p_1<0.001^{*}$, $p_2 = 0.932$, $p_3<0.001^{*}$			

^{F}p: P value forF test (ANOVA), Sig. bet. grps was done usingPost Hoc test (LSD)

$^{KW}\chi^2$p: P value for Chi square for Kruskal Wallis test, Sig. bet. grps was done usingMann Whitney test

p_1: p value for comparing between NAFLD and NASH

p_2: p value for comparing between NAFLD and Control

p_3: p value for comparing between NASH and Control

*: Statistically significant at $p \leq 0.05$

Table (3): Comparison between the three studied groups according to serum vaspin level.

	NAFLD (GI, n=20)	NASH (GII, n=20)	Control (GIII, n=10)	P
VASPIN (ng/ml)				
Min.–Max.	0.97–1.23	1.16–1.34	0.20–0.78	
Mean±SD.	1.11±0.09	1.25±0.06	0.50±0.20	<0.001*
Median	1.12	1.25	0.51	
Sig. bet. Grps	p_1<0.001*, p_2<0.001*, p_3 <0.001*			

p_1: p value for Post Hoc test (LSD) for comparing between NAFLD and NASH
p_2: p value for Post Hoc test (LSD) for comparing between NAFLD and Control
p_3: p value for Post Hoc test (LSD) for comparing between NASH and Control
*: Statistically significant at $p \leq 0.05$

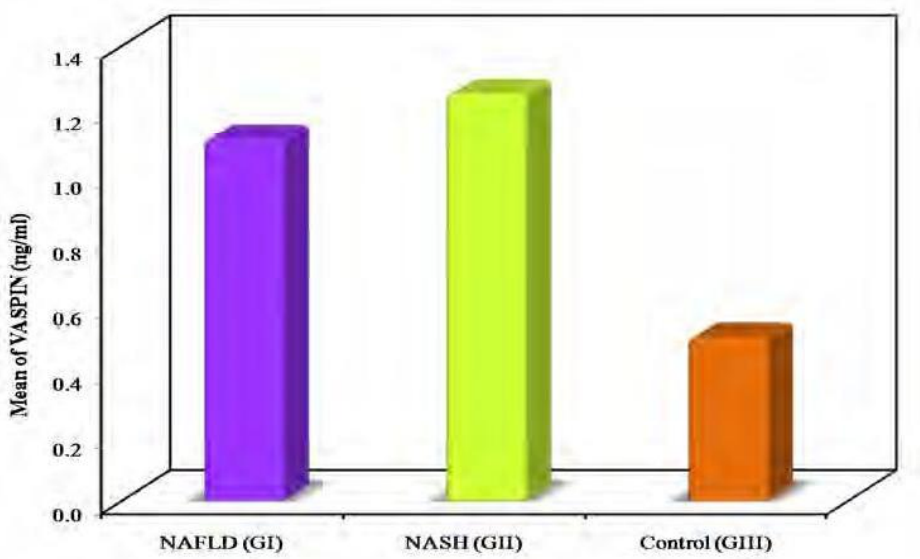

Figure (1): Comparison between the three studied groups according to serum vaspin level.

Table (4): Correlation between serum vaspin level and different studied parameters.

	VASPIN (ng/ml)			
	NAFLD		NASH	
	Coeff.	P	Coeff.	P
Age	0.029	0.904	-0.515	0.020
BMI	0.029	0.903	0.028	0.906
Waist	-0.228	0.333	-0.211	0.373
FBG	-0.061	0.797	0.107	0.652
Fasting insulin	0.119	0.617	-0.051	0.830
HOMA-IR	0.134	0.573	-0.109	0.649
TG	0.217	0.358	0.330	0.155
Cholesterol	0.051	0.831	0.304	0.192
HDL	-0.332	0.153	0.406	0.076
LDL	-0.033	0.890	0.295	0.206
AST	0.279	0.234	0.044	0.854
ALT	-0.193	0.414	0.003	0.991

Coeff.: Pearson or Spearman coefficient
*: Statistically significant at $p \leq 0.05$