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Serum Adipokines in Patients with Non-alcoholic Fatty Liver Disease - Is there a Role for Predicting the Severity of Liver Disease?

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Abstract

Introduction: Non-alcoholic fatty liver disease (NAFLD) is considered to be among the most common liver diseases world-wide. NAFLD encompasses a broad spectrum of pathological conditions ranging from Simple Steatosis (SS) to steatohepatitis (NASH), fibrosis and finally even cirrhosis. Adiponectin (A) has been associated with inhibition of fibrogenesis and liver protection while Leptin (L) contributes to fibrogenesis in various chronic liver diseases, notably in NASH.

The aim of work: To determine the validity of serum adipokines including leptin, adiponectin, and A/L ratio to act as potential markers for NAFLD and to discriminate NASH from SS.

Patients and methods: Eighty four patients who have bright liver on abdominal ultrasonography and 28 healthy individuals served as control group. Serum Leptin and Adiponectin were estimated by ELISA technique. Liver biopsy was done for 46 patients and according to histopathological examination they were divided into 21 patients with SS and 25 patients with NASH.

Results: The serum concentration of adiponectin was significantly lower in NASH than SS group (P<0.001). There was no significant difference between serum concentration of leptin in both groups (P=0.4). A/L ratio in NASH group was significantly lower than SS group (P<0.001). Adiponectin was negatively correlated with BMI, total cholesterol and LDL-C in both groups. A/L ratio in NASH group was significantly positively correlated with adiponectin (P<0.001) while it was significantly negatively correlated with leptin (P<0.001). In SS group A/L ratio was significantly negatively correlated with leptin (r=-0.863, P<0.001).

Conclusion: In patients with NAFLD, the serum adiponectin and A/L ratio can discriminate simple steatosis from NASH and predict the severity of liver injury.

Keywords: NAFLD; Leptin; Adiponectin

Introduction

Non-alcoholic fatty liver disease (NAFLD) is considered the most common cause of chronic liver disease in the world with an estimated prevalence of 20-30% in the United States and other Western populations. NAFLD is a slowly progressive disease and encompasses a spectrum of varying liver histology, ranging from simple steatosis to hepatocyte necro-apoptosis, and non-alcoholic steatohepatitis (NASH) which leads to variable grades of fibrosis and ultimately cirrhosis with its complications including hepatocellular carcinoma (HCC) [1,2]. It is important to distinguish NAFLD from NASH, as NAFLD seems to run a clinically benign course in absence of coexisting liver disease, in contrast to NASH which carries an increased risk for cardiovascular disease and mortality [3,4].

NAFLD is closely associated with obesity and insulin resistance which are both key features of the metabolic syndrome. Subjects with metabolic syndrome have an increased prevalence of developing NAFLD compared with those without the disease and an average of 30% of subjects with NAFLD have the metabolic syndrome [5]. Insulin resistance in particular plays a pivotal role in NAFLD pathogenesis. NAFLD may even develop in insulin-resistant subjects who are not obese and have a normal glucose tolerance [6].

Although the pathogenesis of NAFLD has not been fully elucidated, a multiple-hit hypothesis has been proposed. Insulin resistance results in both increased free fatty acids (FFA) flux to the liver by decreased inhibition of adipose tissue lipolysis, and an increased denovo hepatic lipogenesis resulting in lipid accumulation in the hepatocytes mainly in the form of triglycerides (TG) [7,8]. The accumulation of TG in the liver, mediated by insulin resistance, is considered the primary insult or the 'first hit' which sensitizes the liver to injury mediated by 'second hits' such as oxidative stress, inflammatory cytokines like tumour necrosis factor (TNF-α) and interleukin-6 (IL-6), adipocytokines and mitochondrial dysfunction which in turn leads to steatohepatitis and fibrosis [9].

Currently there is abundant evidence that the accumulation of FFA alone is sufficient to induce liver toxicity by activation of inflammatory pathways and increasing oxidative stress, without recourse for a second hit [10]. TG accumulation in the form of steatosis may therefore be protective rather than being harmful by preventing unesterified FFA-induced toxic effects. Oxidative stress inhibits the proliferation of mature hepatocytes which results in recruitment of hepatocyte progenitor cells (HPC) and thus an impaired proliferation of hepatocytes progenitors is additionally considered the 'third hit' hypothesis in the pathogenesis of NAFLD [11].

Adipose tissue is considered an endocrine organ that regulates body metabolism [12,13]. The imbalanced production of pro- and

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anti-inflammatory adipocytokines secreted from the adipose tissue contributes to the pathogenesis of NAFLD and its progress [14]. Adiponectin is a plasma protein, which is secreted abundantly from adipose tissue and has been shown to be a key component in the relationship between adiposity, insulin resistance and inflammation [15].

Hypoadiponectinemia has been implicated in the pathogenesis of NAFLD. Adiponectin circulates at relatively high levels in the bloodstream and is a hepatic insulin sensitizer- by opposing intrahepatic lipid accumulation- and is also an inhibitor of TNF [2,16], therefore is capable of increasing FA oxidation in the liver and exerting anti-inflammatory effects [17].

Leptin, another adipokine, is supposed to be an essential mediator of liver fibrosis, as it increases TNFα, TGF-β and type I collagen expression in the liver [18]. The inflammatory marker CRP is elevated in chronic inflammatory states and in subjects with central obesity. It lacks specificity for hepatic inflammation and has shown inconsistent results for NASH. There were significant increases in hs-CRP in NASH patients in some studies [19,20] and no differences in another [21]. Recent studies have suggested a potential predictive role for hs-CRP in NAFLD [21,22]. The aim of the present study was to determine the validity of these biomarkers as potential biomarkers for NAFLD and to determine whether they can discriminate NASH from Simple Steatosis (SS).

Patients and Methods

A total of eighty four patients (19 males and 65 females) with NAFLD were enrolled in the study from November 2011 to July 2013. They were prospectively recruited from Internal Medicine Out-patients Clinic in Cairo University Hospital. Their age ranged from 22-60 years. Twenty eight healthy volunteers without liver disease, who were gender and age-matched with the study group, were included as the control group. An informed consent was obtained from all participants prior to enrolment. The diagnosis of NAFLD was based on ultrasonographic finding of bright liver (the diagnosis of bright liver was based on abnormally intense, high level echoes from the hepatic parenchyma with amplitude similar to those echoes arising from the diaphragm) according to the standard criteria accepted by the American Gastroenterology Association. In only forty-six patients a confirmatory liver biopsy was done after a written consent was obtained. Subsequently NAFLD patients were divided into three groups; Group IA (NASH), Group IB (Simple Steatosis), and Group IC (NAFLD patients who have not done a liver biopsy) with Group II being the control group.

Inclusion criteria for NAFLD patients were age above 18 years and bright liver on abdominal ultrasound. Patients were excluded from the study if one of the following criteria were present: any liver disease other than NAFLD such as hepatitis B or hepatitis C, autoimmune hepatitis, alpha one antitrypsin deficiency or Wilson's disease, alcohol consumption, use of amiodarone, corticosteroids, tamoxifen, methotrexate, oral contraceptives, pregnancy, diabetes, hypertension, thyroid disease, malignancy and decompensated liver disease. Also any subjects with evidence of systemic or local infection on physical examination or an abnormal urine analysis were excluded from the study because high- sensitivity C-Reactive Protein (hs-CRP) concentrations are altered by infections. In all controls, the absence of any current or past liver disease was established based on the presence of normal liver biochemistry, lack of any evidence from physical examination of any chronic liver disease, and the presence of a normal abdominal ultrasound.

All subjects included in the study were subjected to detailed history taking, complete clinical examination including anthropometric evaluation (weight, height, body mass index (BMI),waist and hip circumference as well as waist to hip ratio were included). BMI between 25-30 kg/m² (<30) and ≥30 kg/m² were defined as overweight and obesity respectively. Laboratory investigations included complete blood count (CBC), liver function tests, fasting plasma glucose, lipid profile, urea and creatinine, hepatitis markers including hepatitis B surface antigen and hepatitis C virus antibodies, and high- sensitivity C- reactive protein (hs-CRP). Serum Adiponectin and Leptin levels were measured by the ELISA technique. Serum adiponectin levels were determined using the BioSource ELISA assay (Europe S.A Nivelles, Belgium) for adiponectin, a so-called Sandwich Assay using two specific and high affinity antibodies. The serum levels of Leptin were determined using also ELISA kits supplied by BioSource (Europe S.A Nivelles, Belgium).

All subjects were subjected to abdominal ultrasonography using probe 3.5 MHZ of TDI Philips machine. Patients were examined after at least 8 hours fasting and were examined in the supine, right and left lateral positions. Scanning was done through several longitudinal, oblique and transverse scans. Ultrasound examination was performed by a single experienced radiologist to avoid inter-observer variability. The diagnosis of fatty liver was based on the following criteria: abnormally intense high level echoes arising from the hepatic parenchyma with the liver being significantly more echogenic than the kidney; smooth surface together with rounded contours of inferior margin of the right lobe and biconvexity of the left lobe; increase in size of liver and changes in shape as volume of infiltration increases; posterior acoustic attenuation due to fatty infiltration; blurring of margins of hepatic veins due to increased refraction and scattering of sound together with pushing apart of vessels with increased infiltration [23].

After a fully informed consent, liver biopsies were obtained from only 46 cases, using an automated gun device and under complete aseptic precautions. Platelets count and prothrombin concentration were assessed prior to the procedure and the steps of the procedure were explained to all cases including all potential complications. The specimens obtained were stained with hematoxylin and eosin stains and were assessed by a senior hepatopathologist blinded to the clinical and laboratory characteristics of the patients. The biopsies were graded according to the NAFLD scoring system proposed by the National Institute of Diabetes and Digestive and Kidney Diseases NASH Clinical Research Network and reported as NAFLD activity score (NAS) [24].

Total NAS score represents the sum of scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2), and ranges from 0-8. Diagnosis of NASH (or, alternatively, fatty liver not diagnostic of NASH) should be made first, then NAS is used to grade activity. The stage of fibrosis was assessed separately from NAS using a four-point scale: 0=no fibrosis; 1=mild/moderate zone 3 perisinusoidal fibrosis or portal/periportal fibrosis only; 2=perisinusoidal and portal/periportal fibrosis; 3=bridging fibrosis and 4=cirrhosis.

In the reference study, NAS scores of 0-2 occurred in cases largely considered not diagnostic of NASH, scores of 3-4 were evenly divided among those considered not diagnostic, borderline, or positive for NASH. Scores of 5-8 occurred in cases that were largely considered diagnostic of NASH. So our NAFLD patients with NAS ≤ 2 were classified as Simple Steatosis (SS) whereas patients with NAS ≥ or fibrosis stage >2 considered as NASH.

Statistical Analysis

All collected questionnaires were revised for completeness and

consistency. Pre-coded data was entered on the computer using "Microsoft Office Excel Software" program (2010) for windows. Data was then transferred to the Statistical Package of Social Science Software Program, version 21 (SPSS) to be statistically analyzed. Data was summarized using mean, and standard deviation for quantitative variables and frequency and percentage for qualitative ones. Comparison between groups was performed using independent sample t-test or one way ANOVA for quantitative variables and Chi square or Fissure exact test for qualitative ones. Pearson or Spearman correlation coefficients were calculated to test the association between parametric and non-parametric variables respectively. P values less than 0.05 were considered statistically significant, and less than 0.01 were considered highly significant.

Results

The demographic, anthropometric and laboratory data of NAFLD patients and their age- and sex- matched controls are shown in Table 1. NAFLD patients showed a statistically significant higher BMI, serum levels of AST and ALT, serum levels of triglycerides, total cholesterol and LDL-C, serum hs-CRP and serum leptin levels. Serum adiponectin concentration was significantly lower in NAFLD patients compared to their matched controls with a mean value of 3.7 ± 1.2 µg/ml and 7.6 ± 0.6 µg/ml respectively (p value <0.001) . Patients with NAFLD showed a significantly higher serum leptin concentration compared to controls (mean value 18.5 ± 8.3 ng/mL vs. 9.7 ± 1.4 ng/mL respectively, p value<0.001).

The NAFLD group was further divided into three groups: Group IA (NASH) and Group IB (Simple Steatosis) according to histologic diagnosis and Group IC (NAFLD patients who have not done a liver biopsy). There were no statistically significant differences between the NASH group and simple steatosis group as regards the mean age or anthropometric measures. The NASH group showed higher mean AST levels and significantly lower adiponectin levels (Figure 1) and A/L ratio (Figure 2) compared to the simple steatosis group), however no significant differences in the serum concentrations of leptin and hs-CRP were observed Table 2.

Correlation of serum adiponectin and leptin levels with clinical and laboratory parameters within the three subgroups of NAFLD patients were performed (Tables 3 and 4). Adiponectin levels were inversely correlated with BMI, total cholesterol and LDL-C in the three NAFLD subgroups (Figures 3 and 4), however no significant correlations between adiponectin and serum leptin or liver enzymes (ALT, AST) were observed. A significant positive correlation was found between adiponectin and HDL-C in Group IC (p=0.003). Serum leptin levels only showed a significant positive correlation with age in simple steatosis group (r=0.484, p=0.026).

It was also found that adiponectin to leptin (A/L) ratio showed an inverse correlation with BMI, serum cholesterol and LDL-C levels in NASH group (Table 5 and Figure 5), an inverse correlation with leptin and adiponectin in simple steatosis group (Figure 6), and a significant positive correlation with HDL-C levels (r=0.337, p=0.039) in Group IC (NAFLD patients who have not done a liver biopsy). There were no significant correlations between hs-CRP and the liver enzymes, lipid profile or serum biomarkers in this present study.

There were no statistically significant differences between males and females in serum concentrations of leptin, adiponectin and A/L ratio in the three studied subgroups of NAFLD patients (Table 6).

Table 7 represents ROC curve analysis conducted to explore the ability of adiponectin, leptin and A/L ratio to discriminate patients with fatty liver disease from controls. As regards adiponectin, it showed an area under the receiver operating characteristic curve (AUC) of 1.0 (95% CI:1.0-1.0, p<0.001) for distinguishing between patients with fatty liver disease and controls, the best cut off value was 6.4 with sensitivity100% and specificity 100% . For leptin, AUC was 0.89 (95%CI: 0.84-0.95, p<0.001), the best cut-off value was 12.0 with sensitivity 82.4% and

	NAFLD (N=84) Group(I)	Controls (N=28) Group(II)	P value
Age(years)	43.6 ± 8.6	40.3 ± 10.4	0.1
Weight(Kg)	96.6 ± 17.2	68.4 ± 10.0	<0.001*HS
Height(meters)	1.60 ± 0.09	1.66 ± 0.07	<0.001*HS
BMI	38.2 ± 7.5	24.8 ± 3.8	<0.001*HS
ALT(U/L)	32.6 ± 26.5	18.8 ± 6.9	<0.001* HS
AST(U/L)	29.7 ± 18.5	18.9 ± 6.2	<0.001*HS
AST/ALT ratio	1.08 ± 0.47	1.07 ± 0.32	0.9
Cholesterol(mg/dL)	206.2 ± 46.0	166.2 ± 29.0	<0.001*HS
HDL-C(mg/dL)	42.2 ± 8.0	42.0 ± 8.0	0.9
LDL-C(mg/dL)	129.2 ± 38.2	109.6 ± 23.8	0.01*S
TGs(mg/dL)	167.8 ± 102.3	86.8 ± 32.4	<0.001*HS
Adiponectin(µg/ml)	3.7 ± 1.2	7.6 ± 0.6	<0.001*HS
Leptin(ng/ml)	18.5 ± 8.3	9.7 ± 1.4	<0.001*HS
A/L ratio	0.25 ± 0.18	0.80 ± 0.12	<0.001*HS
hs-CRP(µg/ml)	2.6 ± 1.3	1.1 ± 0.4	<0.001*HS

All data are expressed as mean ± SD.

Table 1: Clinical and biochemical characteristics in NAFLD patients and controls.

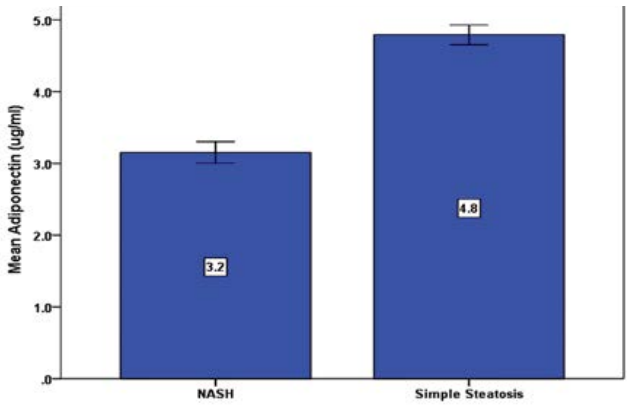

Figure 1: Mean serum concentration of adiponevtin in NASH and SS.

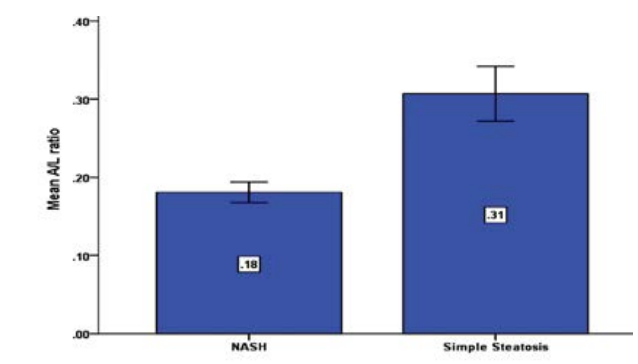

Figure 2: Mean A/L ratio in NASH and SS.

	NASH (N=25) (GROUP IA)	SS (N=21) (GROUP IB)	P Value
Age(years)	41.9 ± 8.1	41.5 ± 10.9	0.9
Weight(Kg)	89.5 ± 15.3	89.3 ± 20.0	1.0
Height(meters)	1.62 ± 0.09	1.60 ± 0.11	0.6
BMI	34.6 ± 6.6	35.3 ± 9.1	0.7
ALT(U/L)	40.5 ± 32.8	35.0 ± 32.6	0.6
AST(U/L)	37.5 ± 24.0	27.1 ± 15.7	0.09
AST/ALT ratio	1.09 ± 0.44	0.98 ± 0.45	0.4
Cholesterol(mg/dL)	205.1 ± 43.1	213.4 ± 67.7	0.6
HDL-C(mg/dL)	42.3 ± 9.5	41.6 ± 6.2	0.8
LDL-C(mg/dL)	120.5 ± 34.8	133.8 ± 54.9	0.3
TGs(mg/dL)	177.3 ± 139.0	170.3 ± 91.9	0.8
Adiponectin(µg/ml)	3.2 ± 0.8	4.8 ± 0.6	<0.001*HS
Leptin(ng/ml)	19.0 ± 5.2	17.8 ± 5.4	0.4
A/L ratio	0.18 ± 0.07	0.31 ± 0.16	<0.001*HS
hs-CRP(µg/ml)	2.8 ± 1.0	2.4 ± 0.7	0.08

All data are expressed as mean ± SD.

Table 2: Comparison between NASH (Group IA) and SS group (Group IB) as regard the clinical and biochemical parameters.

		Adiponectin (µg/ml)		
		NASH (N=25) Group IA	SS (Simple Steatosis) (N=21) Group IB	Group IC (N=38)
Age	r	0.295	0.005	0.2
	p	0.101	0.982	0.228
Weight	r	-0.73	-0.401	-0.73
	p	<0.001*	0.072	<0.001*
Height	r	-0.208	0.275	-0.423
	p	0.254	0.228	0.008
BMI	r	-0.516	-0.471	-0.500
	p	0.003	0.031	0.001
ALT	r	0.009	0.154	0.005
	p	0.961	0.505	0.976
AST	r	-0.127	0.164	-0.003
	p	0.488	0.477	0.988
AST/ALT	r	-0.233	0.172	0.035
	p	0.199	0.455	0.834
TG	r	-0.065	0.062	-0.068
	p	0.722	0.791	0.683
Cholesterol	r	-0.461	-0.803	-0.381
	p	0.008	<0.001*	0.018
HDL-C	r	-0.26	-0.22	0.469
	p	0.151	0.399	0.003
LDL-C	r	-0.568	-0.777	-0.439
	p	0.001	<0.001*	0.006
Leptin	r	-0.242	0.301	-0.194
	p	0.181	0.186	0.244
A/L ratio	r	0.741	-0.102	0.606
	p	<0.001*	0.66	<0.001*
hs-CRP	r	-0.104	0.073	0.17
	p	0.571	0.753	0.308

Table 3: Correlation of Adiponectin with clinical and other biochemical parameters within the studied NAFLD subgroups.

specificity 92.9%. Regarding the A/L ratio, the AUC was 0.98 (95% CI: 0.95-1.0, p<0.001), the best cut-off value was 0.57 with sensitivity 96.7% and specificity 100%.

Table 8 represents the ROC curve analysis exploring the ability of adiponectin, leptin and A/L ratio to distinguish between NASH and simple steatosis. It revealed that only adiponectin and the A/L ratio were able to discriminate NASH from simple steatosis. Adiponectin showed an AUC of 0.92(95% CI: 0.86-0.99, p<0.001), the best cut-off point was 3.9 with sensitivity 81.3% and specificity 90.5%. As regards the A/L ratio, the AUC was 0.84(95% CI: 0.73-0.94, p<0.001), the best cut-off value was 0.21 with sensitivity 71.9% and specificity 81.0%. Therefore from the previous two ROC curve analyses, it can be concluded that both adiponectin and A/L ratio can significantly distinguish patients with fatty liver from those without, as well as distinguish NASH from Simple Steatosis (SS) (Figure 7).

Discussion

NAFLD represents a broad spectrum of disorders ranging from simple steatosis to steatohepatitis, fibrosis which can progress to cirrhosis and its complications [25]. The mechanisms responsible for liver injury and progression of NAFLD/NASH still remain incompletely understood [26]. Increasing evidence indicates that the interplay between the pro- and anti-inflammatory adipocytokines, the most important of which are leptin and adioponectin, contributes to the pathogenesis and severity of NAFLD [27]. Our study attempted to determine whether serum adipokines levels would be related to the severity of liver injury in NAFLD patients and whether their determination could be useful as a non-invasive marker to discriminate between NASH and simple steatosis.

		Leptin (ng/ml)		
		NASH(N=25) Group IA	SS(SimpleSteatosis) (N=21) Group IB	Group IC (N=38)
Age	r	0.054	0.484	-0.159
	p	0.768	0.026	0.341
Weight	r	0.237	0.235	0.117
	p	0.191	0.306	0.484
Height	r	-0.079	-0.091	0.267
	p	0.668	0.695	0.105
BMI	r	0.249	0.306	-0.063
	p	0.169	0.177	0.705
ALT	r	-0.081	-0.093	0.044
	p	0.66	0.688	0.795
AST	r	-0.07	-0.012	0.049
	p	0.702	0.957	0.77
AST/ALT ratio	r	0.164	0.012	0.051
	p	0.368	0.957	0.762
TG	r	0.021	0.011	-0.017
	p	0.91	0.963	0.919
Cholesterol	r	0.239	-0.227	-0.01
	p	0.188	0.323	0.953
HDL-C	r	-0.085	-0.043	-0.191
	p	0.645	0.853	0.251
LDL-C	r	0.198	-0.254	-0.112
	p	0.279	0.267	0.502
Adiponectin	r	-0.242	0.301	-0.194
	p	0.181	0.186	0.244
A/L ratio	r	-0.767	-0.829	-0.558
	p	<0.001*	<0.001*	<0.001*
hs-CRP	r	0.338	-0.108	0.063
	p	0.054	0.642	0.705

Table 4: Correlation of Leptin with clinical and other biochemical parameters within the studied NAFLD subgroups.

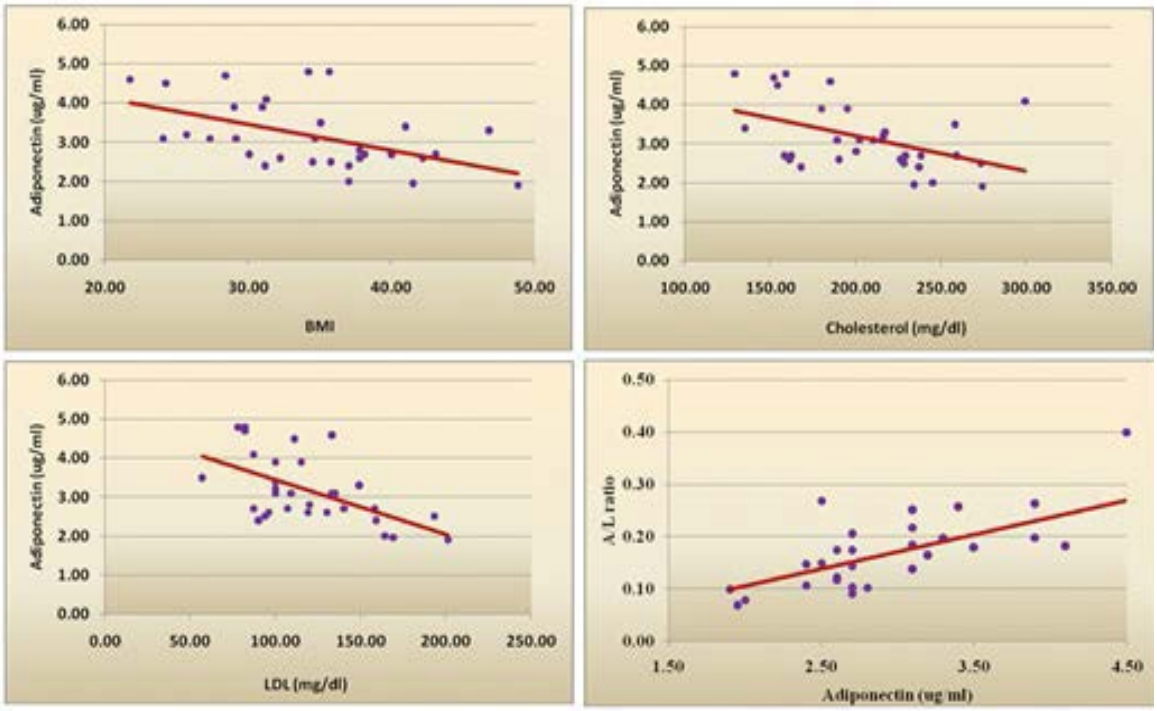

Figure 3: Correlations of adiponectin in NASH group.

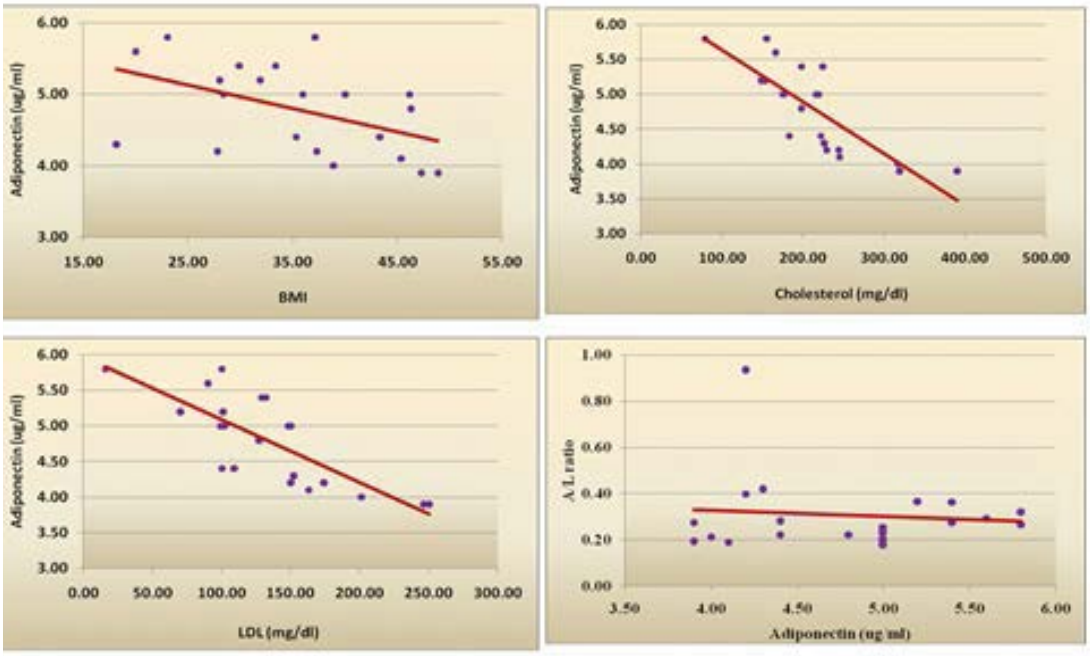

Figure 4: Correlation of serum adiponectin in SS group.

Several studies have demonstrated the association between hypoadiponectinemia and NAFLD. The present study revealed that serum adiponectin concentration was significantly lower in NAFLD patients compared to those of the controls. This is concordant to the results of previous studies by Tsochatzis et al., Bugianesi et al., Pagano et al., and Yoon et al. [27-29] A study by Hui et al. stated that hypoadiponectinemia is a feature of NASH independent of insulin resistance; is associated with more extensive necroinflammation and may contribute to the development of necroinflammatory forms of NAFLD [21].

Moreover, we observed significantly lower serum levels of adiponectin in NASH group in comparison to the simple steatosis

		A/L ratio		
		NASH (N=25) Group IA	**SS(Simple Steatosis) (N=21) GroupIB**	**Group IC (N=38)**
Age	r	0.14	-0.222	0.133
	p	0.443	0.333	0.426
Weight	r	-0.578	-0.287	-0.091
	p	**0.001**	0.207	0.585
Height	r	-0.108	-0.018	-0.049
	P	0.557	0.939	0.771
BMI	r	-0.441	-0.265	-0.074
	p	**0.012**	0.246	0.661
ALT	r	0	0.243	-0.196
	p	0.998	0.289	0.239
AST	r	-0.074	0.102	-0.148
	p	0.688	0.66	0.376
AST/ALT ratio	r	-0.204	-0.137	0.082
	p	0.263	0.553	0.624
TG	r	-0.092	0.3	-0.015
	p	0.615	0.186	0.927
Cholesterol	r	-0.489	-0.001	0.152
	p	**0.005**	0.996	0.362
HDL-C	r	-0.171	-0.165	0.337
	p	0.348	0.476	**0.039**
LDL-C	r	-0.466	0.067	0.152
	p	**0.007**	0.771	0.362
Adiponectin	r	0.741	-0.102	0.606
	p	**<0.001**	0.66	**<0.001**
Leptin	r	-0.767	-0.829	-0.558
	p	**<0.001**	**<0.001**	**<0.001**
hs-CRP	r	-0.254	0.199	0.025
	p	0.16	0.386	0.883

Table 5: Correlation of A/L ratio with other parameters within the studied NAFLD subgroups.

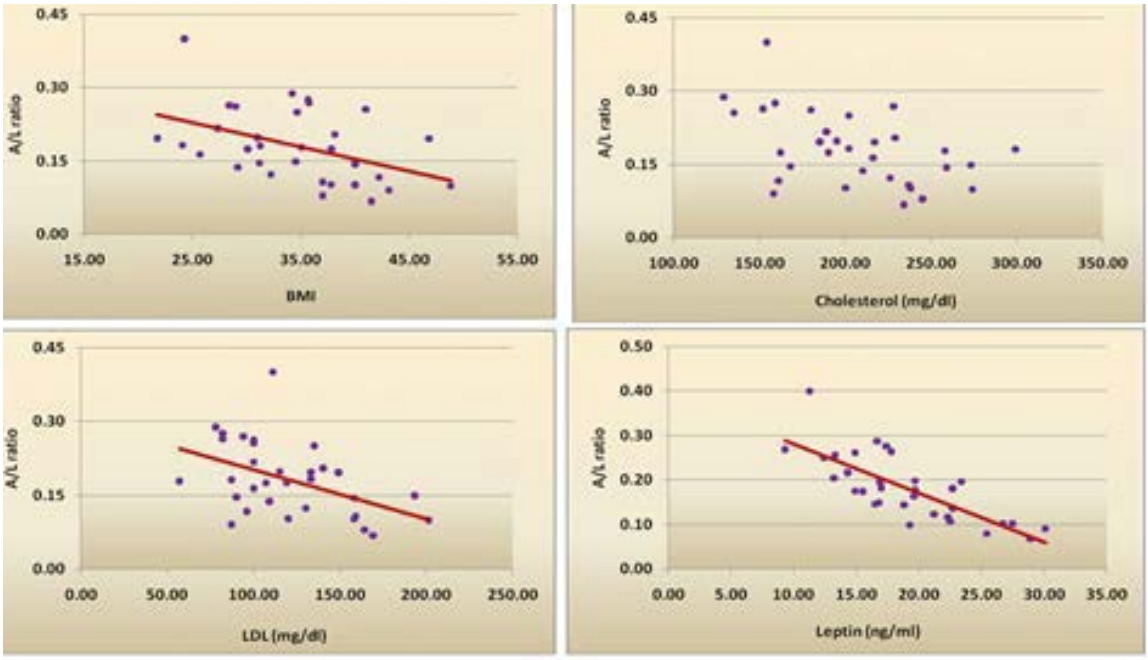

Figure 5: Correlation of A/L ratio in NASH group.

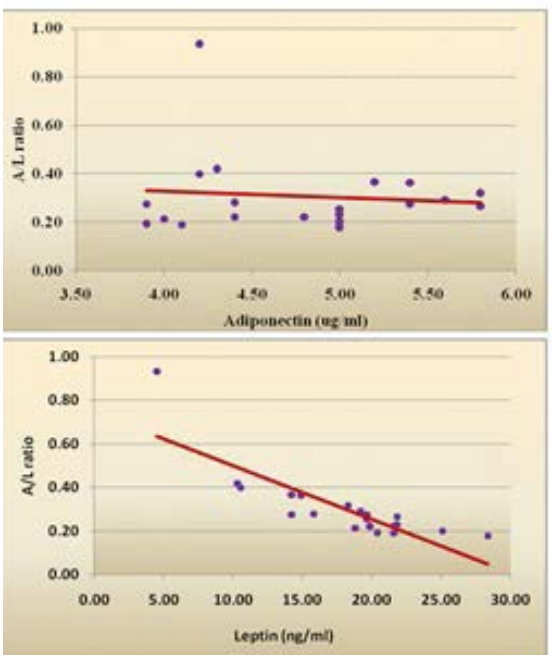

Figure 6: Correlation of A/L ration in SS group.

		Male	Female	p value
NAFLD (n=84) Group I	Adiponectin(µg/ml)	4.0 ± 1.3	3.7 ± 1.2	0.4
	Leptin(ng/ml)	16.2 ± 6.1	19.1 ± 8.7	0.2
	A/L ratio	0.27 ± 0.12	0.24 ± 0.19	0.5
NASH Group IA	Adiponectin(µg/ml)	3.2 ± 0.8	3.1 ± 0.9	0.8
	Leptin(ng/ml)	16.7 ± 4.5	19.6 ± 5.3	0.3
	A/L ratio	0.21 ± 0.07	0.18 ± 0.08	0.4
SS (Simple Steatosis) Group IB	Adiponectin(µg/ml)	5.2 ± 0.5	4.6 ± 0.6	0.05
	Leptin(ng/ml)	17.0 ± 5.1	18.2 ± 5.7	0.6
	A/L ratio	0.33 ± 0.08	0.30 ± 0.19	0.7
Group IC (NAFLD patients who haven't undergone a biopsy)	Adiponectin(µg/ml)	3.5 ± 1.5	3.7 ± 1.3	0.8
	Leptin(ng/ml)	15.1 ± 8.4	19.1 ± 11.9	0.4
	A/L ratio	0.29 ± 0.16	0.27 ± 0.24	0.8

Table 6: Effect of gender on serum leptin and adiponectin concentrations among the studied NAFLD patients and subgroups.

	AUC	95% CI	p value	Cut-off	Sensitivity	Specificity
Adiponectin	1.0	1.0-1.0	<0.001	6.4	100.0%	100.0%
Leptin	0.89	0.84-0.95	<0.001	12.0	82.4%	92.9%
A/L ratio	0.98	0.95-1.0	<0.001	0.57	96.7%	100.0%

Table 7: Area under the curve (AUC) of the studied serum adiponectin, leptin and A/L ratio in NAFLD patients and controls.

group. Both groups showed lower levels than those of the controls and this therefore means that high levels of adiponectin have a protective effect against fatty liver [30,31]. Our findings agree with those of previous studies by Musso et al. and Shimada et al. who reported that serum adiponectin levels were significantly lower in patients with NASH than in the control group [32,33].

Another finding of the present study was that patients with NAFLD showed a significantly higher mean serum leptin concentration compared to controls (18.5 ± 8.3 ng/mL vs. 9.7 ± 1.4 ng/mL respectively; p value<0.001). This however, disagrees with the results of a study by ElAttar and El Melegy, which revealed that serum leptin levels showed no significant difference between NAFLD patients and controls, however significant higher levels were observed on subgrouping of these patients according to gender, BMI and AST/ALT ratio [34].

No gender difference was observed in serum adiponectin levels in NAFLD patients, even after subgrouping them. These findings disagree with those of a study by ElAttar and El Melegy, where lower serum levels of adiponectin were observed in female patients. They attributed this to the fact that women tend to have less visceral fat than subcutaneous fat tissue. Adiponectin levels in this study were mainly determined by visceral fat, not by subcutaneous fat. Therefore, they postulated that the difference in plasma adiponectin levels in women and men might be due to the contribution of sexual dimorphism of body fat distribution [34].

In the present study, there was a linear inverse relationship between serum adiponectin and fasting TG levels, but this did not reach statistical significance as this could be because of low number of studied sample. Absence of a significant relationship between serum adiponectin and HDL-C levels was also observed in NASH and Simple Steatosis (SS) group. However, a significant positive correlation between HDL-C and adiponectin was only observed in Group IC. These findings are in concordance with those of Mohamed et al., which also revealed significant negative correlation between adiponecin and each of TG, LDL-C and total cholesterol , while positive non significant regarding HDL-C. These findings suggest that determination of TG levels and HDL-C are inappropriate predictors for serum adiponectin [14].

In dyslipidemic and insulin resistant patients with NAFLD, most of the liver damage is thought to be attributable to the accumulation of hepatic TGs, and adiponectin might be able to preserve hepatic function by preventing the accumulation of lipids in hepatocytes. Adiponectin also modulates inflammatory response and is a potent insulin sensitizer [14].

The present study also revealed a nonsignificant correlation between adiponectin and liver enzymes (ALT, AST). This is disconcordant to the results of the study by Mohamed et al., which demonstrated a weak negative correlation between adiponectin and the liver enzymes. A study by Sargin et al. conducted on 35 non-diabetic patients with NAFLD also found a significant correlation between adiponectin and liver function tests like AST, ALT and GGT [14]. On the other hand, Lopez-Bermejo et al. reported that adiponectin levels were significantly

	AUC	95% CI	p value	Cut-off	Specificity	Specificity
Adiponectin	0.92	0.86-0.99	<0.001	3.9	81.3 %	90.5%
Leptin	0.54	0.38-0.70	0.6	18.8	50.0 %	52.4%
A/L ratio	0.84	0.73- 0.94	<0.001	0.21	71.9 %	81.0%

Table 8: Area under the curve (AUC) of the studied serum adiponectin, leptin and A/L ratio in NASH group and SS (simple steatosis) group

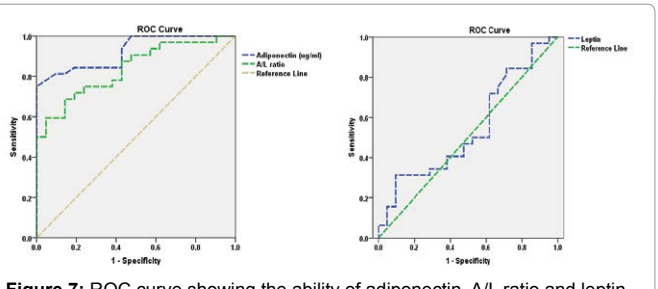

Figure 7: ROC curve showing the ability of adiponectin, A/L ratio and leptin to discriminate NASH from simple steatosis.

correlated with ALT independently of sex, age, BMI and insulin resistance and therefore suggesting a greater role for adiponectin in maintenance of liver integrity [35].

Another finding in our present study was that serum leptin levels were significantly higher in NAFLD patients in comparison to those of controls. The role of leptin in pathogenesis and severity of NAFLD in humans still remains controversial because of conflicting results in various studies. The dysfunction in the production of leptin or its receptor, affect the metabolic pathway of the triglycerides with PPAR-α down regulation being the link between the abnormalities observed in the visceral adipose tissue and the hepatic features of NASH [36]. This therefore indicates that decreased lipid breakdown occurs in addition to increased fatty acids inflow to the liver, with subsequent activation of different metabolic pathways like ceramide biosynthesis resulting in increased apoptosis and other deleterious effects on hepatocytes [37].

A report by Tsochatzis et al. also showed significantly higher leptin levels in NAFLD patients compared to healthy controls [38], however other studies by Angulo et al. and Musso et al. found no differences between serum leptin levels in patients with NASH and controls [30,32]. Another study by Kumar et al. observed significantly lower leptin levels in NAFLD patients as compared to healthy controls and found no significant association between either leptin or adiponectin and any of disease variables [39].

On subgrouping the NAFLD patients in our study, we did not note significantly higher serum concentration of leptin in NASH group as compared to simple steatosis group. These findings are in concordance with that of a study by Chitturi et al who found that leptin was not an independent predictor of hepatic inflammation or fibrotic severity [40]. On the contrary, the study by El-Attar and El-Melegy, showed no significant difference between NAFLD patients and controls as regards serum leptin levels, however significantly higher levels of this adipokine were observed on subgrouping the patients according to gender, BMI and AST/ALT ratio [34].

Data of the present study suggest that serum leptin concentrations are substantially higher in women than men. This gender difference in serum leptin concentration has been reported in previous studies by Nobeili et al. and Huang et al. [41,42]. The gender difference in leptin concentration can be attributed to the well known gender difference in adipose tissue distribution as increased leptin production has been reported in subcutaneous versus intra- abdominal adipose tissue deposits [43]. The role of sex steroid hormones has also been implicated. Elevated androgen concentrations tend to lower lptin levels while estrogen and/or progesterone can affect leptin levels causing an increase in leptin concentrations [44].

We did not observe significant correlations between serum leptin concentration and total cholesterol, LDL-C, or HDL-C levels in our present study. This agrees with a study by Park et al. who failed to find a correlation between leptin and lipid profile. These data confirm that leptin is a marker of the adipose tissue mass, but it does not play the major role in determining metabolic syndrome. Our results, however, disagree with those of El-Attar and ElMelegy, 2010, as they found that serum leptin levels were significantly positively correlated with each of TG, TC, LDL-C, non-esterified fatty acid(NEFFA)and showed a significant inverse correlation with HDL-C, particularly in female patients with BMI ≥30 and those with AST/ALT ratio>1 [45].

Adiponectin and leptin generally exhibit opposite variations, so we determined the adiponectin/leptin (A/L) ratio in order to sensitize the adipokine changes. This ratio was lower in patients with NAFLD patients compared to the controls. Most importantly, this ratio was able to discriminate between patients with NASH and those with Simple Steatosis (SS) as A/L ratio in SS group was significantly higher than the NASH group and this agrees with the results of the study by Lemoine et al. A/L ratio in NAFLD patients was significantly positively correlated with adiponectin while it was significantly negatively correlated with leptin and this was concordant to study done by Abd ElMoety et al. [46,47].

Our results also revealed a significant inverse correlation between A/L ratio and BMI in NASH group and this was in concordance with those of Lemoine et al. There was also a significant negative correlation between A/L ratio and cholesterol and LDL-C in NASH group and significant positive correlation with HDL-C in GroupIC [46].

Another important finding in the present study was that the level of hs-CRP was found to be significantly higher in NAFLD patients compared to the controls. These findings are consistent with those of previous studies by Kogiso et al., El-Attar et al., and Mohamed et al. [14,34,48]. The study by Koruk et al. pointed out that increased levels of hs-CRP could be helpful in the diagnostic workup of patients with NAFLD [49].

We did not, however, observe a significant difference in the levels of hs-CRP between NASH group and Simple Steatosis (SS) group. These findings agree with those of Wieckowska et al., as they found no significant difference in the levels of CRP among NAFLD patients [50]. Our results are also consistent with those of Ether Zimmermann et al, who reported that it is the accumulation of fat – both in adipose tissue and in liver that leads to increased hs-CRP among obese patients. Thus, hs-CRP may be a marker of steatosis but not of severity of NAFLD, in obese patients [51].

It is still controversial whether CRP can be used to differentiate between NASH and simple steatosis. Oruk et al. demonstrated significantly higher hs-CRP in patients with simple steatosis or hepatosteatosis than in healthy controls, however the study was limited by lack of histologic diagnosis as NAFLD was diagnosed based on elevated ALT levels and sonographic evidence of fatty liver [52]. A study by Yoneda et al. also added that hs-CRP could be a clinical feature that not only distinguishes NASH from simple non-progressive steatosis, but also indicates the severity of hepatic fibrosis [20]. In a Romanian study, CRP had an excellent performance in predicting the presence of NASH using a cut-off value of 3.5 mg/L (AUROC 0.906, sensitivity 82%, specificity 88%) [53].

One of the most important results of our study is that adiponectin and A/L ratio were able to significantly discriminate NASH from simple steatosis. The AUC for adiponectin was 0.92 (95% CI: 0.86-0.99); the best cut-off point was 3.9 with sensitivity 81.3% and specificity 90.5%. As regards the A/L ratio, the AUC was 0.84 (95% CI: 0.73-0.94); the best cut-off point was 0.21 with sensitivity 71.9% and specificity 81.0%. We can therefore conclude that serum adiponectin levels and A/L ratio can be used to predict the severity of liver injury.

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