Adiponectin: A Differential Marker Between Steatosis And Steatohepatitis

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ABSTRACT

Background: *Nonalcoholic fatty liver disease (NAFLD) becoming a world - wide public health problem.It represents a spectrum of disease ranging from simple steatosis to steatohepatitis (NASH). Adipocytokines refer to adipocyte-derived biologically active molecules TNF-α, leptin and adiponectin, all been implicated in development of hepatic inflammation and fibrosis in NAFLD patients. This new hormone differ from its predecssors in import-ant feature, production and concentration acutully decrease in obesity, and all adipose-derived hormone are increased. It is possible that adiponectin expression is activated during adipogenesis, a feed back inhibition on its production may occur during the development of obesity. Adiponectin may exert a hepatic protective effect.*

Objective*: Was to evaluate adiponectin level as a differential marker between steatosis and Steatohepatitis.*

Methods: *Twenty NAFLD patients, twenty biopsy proved NASH and twenty control subjects, matched for age,sex and BMI.All the subjects were subjected to an abdominal ultrasonography, routine biochemical evaluation: liver function ALT& AST, lipid profile (cholesterol, triglycerides, HDL-C), CRP& Adipocytokines (TNF-α, IL-6, LEPT-IN, & Adiponectin).*

Results: *Plasma adiponectin levels were significantly lower in NAFLD patients than control gp (6.15± 1.39ng/ml vs12.03±3.46ng/ml). Adiponctin was significantly lower in NASH than NAFLD (1.80 0±0.96 ng/ml vs 6.15±1.39 ng/ml). leptin level was significantly higher in NAFLD than NASHgp (69.50±18.70ng/ml vs 43.20±6.93ng/ml). adiponectin ROC curve showed an AUROC curve in NAFLD gp (o.945 p=0.049) while inNASH was(0.995 p=0.007).TNF-α & IL-6 was significantly higher in NASH than NAFLD gp (79.25±13.89 pg/ml vs41.25±17.53 pg/ ml)and (110.20±55.34 pg/ml vs 43.85±16.13). Plasma adiponectin level in NAFLD gp was inversely correlated with T.G (r=-0.368 p=0.111). GOT (r=-0.037 p=0.878) & GPT (r=-0.022 p=0.926) while it was +ve correlated in NASH gp with Cholesterol (r=0.317 p=0.174) & T.G (r=0.042 p=0.861).*

Conclusion: *This data support a role for low circulating adiponectin in the pathogenisis of NAFLD and hypoadiponectinemia found to be a feature of NASH. ADIPONECTIN found to be a non –invasive differential marker between NAFLD &NASH.*

Key words: *Adiponectin, Leptin, IL-6, TNFα, NAFLD, NASH.*

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is rapidly becoming a world-wide public health problem. NAFLD is the most frequent cause of abnormal liver function tests.[1-2] "It represents a spectrum of disease ranging from simple steatosis, which is considered relatively benign, to nonalcoholic steatohepatitis and to NAFLD-associated cirrhosis and end-stage liver disease."[3]

The prevalence of NAFLD is not well known, but according to various studies, it ranges between 3% and 24%.[4-5] The exact pathogenic mechanisms involved are still not well known. However, theory of pathogenesis to be proposed involving two stages, known as "two-hit theory".[6-7] The first hit of steatosis, giving rise to the first lesions is caused by excess free fatty acids (FFA) in the liver, which are esterified to triglycerides (TG).[8] These initial lesions make the liver vulnerable to aggressive factors of the second hit, which is caused by the oxidative stress and proinflammatory cytokines (TNF-α, TGF-beta, IL-6, IL-8). This leads to the occurrence of inflammation and fibrosis, and consequently the evolution of hepatic steatosis to steatohepatitits.[9-11] Adipocytokines (tumour necrosis factor-α[TNF-α], leptin and adiponectin), free fatty acids, mitochondrial dysfunction, bacterial endotoxin and vascular disturbance have all been implicated in the development of hepatic inflammation and fibrosis in patients with NAFLD.[9-10,12-15] New advances in biomedical field are continuously changing our view on the role of different tissues and organ in human body. It is now generally accepted that in addition to its classical function as an energy storage depot, adipose tissue represents an important and very active endocrine organ that produces a number of hormones that control metabolism.[16] "The term adipocytokines refer to a series of adipocyte-derived biologically active molecules which may influence the function as well as the structural integrity of other tissues."[15] Recently, adiponectin, a physiologically active Polypeptide secreted by adipose tissues has been the focus of research intrest. A description of the cDNA encoding adiponectin was first reported in 1995 by Scherer et al.[18] It was also independently cloned

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and named Adipo Q by Hue et al.[19] Human adiponectin gene is located on chromosome 3q27, is a protein of 247 amino acids, also referred to as gelatine-binding protein -28 (GBP-28), the product of the apM1 gene which is specifically and highly expressed in human adipose cells, consisting of 4 domains, an amino-terminal signal sequence, a variable region, a collagenous domain (cAd) and a carboxy–terminal globular domain (gAd).[18] Adiponectin is most similar to C1q protein a member of the complement- related family of proteins.[19] The adiponectin receptor-1 (AdipoR1) was found to be predominantly expressed in skeletal muscle, whereas the expression of AdiopR2 was most abundant in the liver. Both receptors are related to G-protein coupled seven transmembrane domain receptors.[20-22] Also it is a physiologically active polypeptide, has been shown to demonstrate anti-diabetic, anti-inflammatory and anti-atherogenic effects. The mechanism through which adiponectin exerts its action are largely controversial, it stimulat glucose utilisation and fatty acid oxidation in skeletal muscle and liver through activation of 5-AMP kinas which is belived to play a crucial role in the regulation of energy expenditure and glucose and lipid metabolism.[23-24] Adiponectin has now been added on the list as a new and very exiting player in the field of obesity. This new hormone produced exclusively by adipocytes differ from its predecssors in at least one important feature, where adiponectin production and concentration acutully decrease in obese subjects, the all currently known adipose-derived hormone are increased by obesity.[25] In the liver, low doses of adiponectin decreased the expression of proteins involved in fatty acid transport, leading to reduced fatty influx into the liver and hepatic T.G. It is possible that although adiponectin expression is activated during adipogenesis, a feed back inhibition on its production may accure during the development of obesity.[26] Adipocyte expression and secretion of adiponectin has been shown to be reduced by TNF- α. Therefore it may be reasonable that increased TNF- α and other adipocytokines that are expressed in increased amount in the obese state may at least be responsible partially for the decreased adiponectin production in obesity and accelerating sever histological necro-inflammatory processes in patients with non-alcoholic steatohepatitis (NASH).[27-29]

Recently, Xu et al.[35] demonstrated that adiponectin resulted in alleviation of both steatosis and hepatomegaly. It is also likely that some of these adipocytokines mediate the systemic effects of obesity on health. As a matter of fact, leptin is considered to be a fundamental signal of satiety to the brain and has a variety of actions, ranging from interference with sympathetic activity to hematopoiesis and reproductive function.[31]

Aim of the Work

Evaluation of adiponectin level as non-invasive adifferential marker between steatosis and Steatohepatitis.

METHODS

The studied subjects were recruited from participants in routine health examination at the hepatology department in faculty of medicine and the medical research institute, Alexandria university. Written confined consent was obtained from all the participants before commencing the study.

Twenty stestosis (NAFLD) patients; (11 males and 9 females).

Twenty biopsy proved steatohepatitis (NASH) (7males and 13 females) both had median age (33.1±9.4 years).

Twenty control subjects with median age (29.1± 9.2 years); (8 males and 12 females), matched for age, sex and body mass index (BMI=body weight (Kg)/ (body height)^2 were included in this study. Statistical analysis were performed using The SPSS soft ware.[32]

All the subjects were subjected to an abdominal ultrasonography

The diagnosis of fatty liver was based on the following criteria; increased echogenicity, with liver significantly more echogenic than kidney, smooth surface, rounded contours of inferior margin of right lobe and biconvexity of 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.[38-40] Diagnosis of NAFLD was confirmed by absence of hepatitis B& C viral markers, absence of auto-antibodies indictive of autoimmune hepatitis, celiac disease or wilson's disease and no evidence of genetic, drug-induced, or cholestatic liver disease & are negative for CRP, while all the NASH group of patients are positive for CRP.

The control group was age, weight and sex matched and was free from hepatic, neoplastic and endocrine diseases.

A routine biochemical evaluation was performed:

Liver function tests; including serum aspartate and alanine aminotranseferases (AST& ALT), serum gamma glutamyl transpeptidase (GGT).[41-42]
Fasting blood glucose levels [35]

Hepatitis virus markers; Hepatitis B surface antigen was estimated using ELISA technique& hepatitis C virus was done by polymerase chain reaction (PCR).[43-44]

Lipid profile: (12-14 hours overnight fasting) including; serum cholesterol, triglycerides, HDL-C.[45-47]

C-reactive protein (CRP).[48]

Adipocytokines (TNF-α, IL-6, LEPTIN, Adiponectin were estimated by ELISA technique).[49-52]

RESULTS

The studied groups comprised (11males 55%) and (9 females 45%) in Steatosis (NAFLD) group & biopsy proven steatohepatitis (NASH) group (7 male 35%) and (13 female 65%). Serum concentration of Adiponectin was significantly lower in steatohepatitis (NASH) gp and ranged from (0.77- 4.50ng/ml) with a mean value of (1.80±0.96 ng/ml) than in steatosis (NAFLD) gp which ranged from (3.70-8.64ng/ml) with a mean value of (6.15±1.39ng/ml) (p<0.001). Mean serum concentration of adiopnectin was significantly lower in both groups than the controls (6.15± 1.39ng/ml vs12.03 ± 3.46ng/ml) (z=4.815 p<o.oo1) & (1.80±0.96ng/ml vs12.03±3.46ng/ml) (z=5.357 p<0.001) (table VI). Adiponectin was negatively correlated with TG in the NAFLD gp (r=-0.368 p=0.111) and in the NASH gp (r=-0.042 p=0.861). (Table III). Adiponectin was significantly positively correlated with TNFα & IL-6 in steatosis (NAFLD) gp which was (r=0.811p<0.001) & (r=0.678 p=0.001) while it was negatively correlated with IL-6 (r=-0.229 p=0.331) & positivly correlated with TNFα (r=0.359 p=0.120) in NASH gp. (Table V). Adiponectin ROC curve in both patients groups revealed that AUROC curve at a cutoff < 8.0ng/ml was (0.995 p=0.007) in NASH gp with high diagnostic performance of 100%, while the AUROC in NAFLD gp was lower (0.945 p=0.049) with 95% sensitivity 100% specificity & PPV, 95.24% NPV & 97.5% accuracy.(Fig 6) TNFα in NAFLD group ranged from (20.00-80.00pg/ml) while in NASH group was (35.00-95.00pg/ml) had a significant mean level of (41.25±17.53 pg/ml) and (79.25± 13.89 pg/ml) (p<0.001). Serum levels of TNF-α in both patient groups were significantly higher than the control group (41.25±17.53 pg/ml vs 6.06±2.60 pg/ml) (5.414 p<0.001) & (79.25±13.89 pg/ml vs 6.06±2.60 pg/ml) (5.429 p<0.001). (Table VI). TNFα in steatosis gp (NAFLD) was negatively correlated with GOT (r= - 0.074 p=0.758) and GPT (r= -0.061 p=0.798) .While in the steatohepatitis gp (NASH) TNFα showed positive correlation with GOT (r= 0.017 p=0.942) but it showed a significant negative correlated with GPT (r= -0.493 p=0.027) (table III). IL-6 ranged from (15.00-70.00pg/ml) & (40.00-230.00pg/ml) & showed a significant mean level of (43.85±16.13 pg/ml) and (110.20 ± 55.34pg/ml) (p<0.001). Serum levels of IL-6 were significantly higher than the control group (43.85±16.13pg/mlvs7.10±3.67pg/ml (5.411p<0.001) & (110.20±55.34pg/ml vs 7.10± 3.67 pg/ml) (5.411 p<0.001). (Table VI). IL-6 in NAFLD gp was negatively correlated with GOT (r= -0.181 p=0.446) and GPT (r= -0.254 p=0.280), while in NASH gp IL-6 showed negative correlation with GPT (r= -0.361 p=0.118) & positive correlation with GOT (r=0.149 p=0.531). (Table III) serum concentration of Leptin ranged from (40.00-95.00ng/ml) in steatosis (NAFLD) with a mean value of (69.50 ± 18.70ng/m) which was significantly higher than steatohepatitis which ranged from (30.00-55.00ng/ml) & had mean value of (43.20±6.93ng/ml) (p<0.001). Mean serum concentration of leptin was significantly higher in both patients gp than the controls (69.50±18.70ng/ml vs16.31±10.53ng/ml) (r=5.419 p<0.001) & (43.20±6.90ng/ml vs 16.31± 10.53ng/ml) (r=5.290 p<0.001). (Table VI). Both group of patients showed no significant correlation between leptin & adiponectin (r=0.054p=0.822) & (0.093p=0.695) respectively. (Table V). AUROC for leptin at a cutoff (>15ng/ml for males & >40ng/ml for females) was higher in NAFLD gp (1.000 p<0.001) with 100% diagnostic performance. While in NASH gp leptin had a lower AUROC of (0.988 p=0.012) with 70% sensitivity, 100% specificity & PPV, 76.92% NPV & 85.0% accuracy. (fig6). adiponectin / leptin (A/L) ratio in NAFLD gp had a mean value of (0.10±0.04) which was significantly higher than NASH gp which was (0.04±0.02) (p<0.001). (Table I). A/L ratio in steatosis gp was significantly positivly correlated with adiopnectin (r=0.564 p=0.01) while it was significantly negatively correlated with leptin (r=-0.74 p<0.001). Also A/L ratio in NASH gp showed positive significant correlation with adiponectin (r=0.942 p<0.001) while it was negatively correlated with leptin (r=-0.226 p=o.338) (table IV). A/L ratio in th NAFLD gp showed negative correlation with cholesterol, TG and GOT (r= -0.002 p=0.993), (r=-0.387 p=0.092) and (r=-0.165 p=0.488) respectively, while it showed positive correlation with GPT (r=0.102 p=0.669). A/L ratio in NASH gp showed positive correlation with cholesterol, TG & GPT (r=0.339 p=0.144), (r=0.145 p=0.541) and (r=0.311 p=0.182) rescpectivly while it was negatively correlated with GOT (r=-0.370 p=0.109). (Table III)

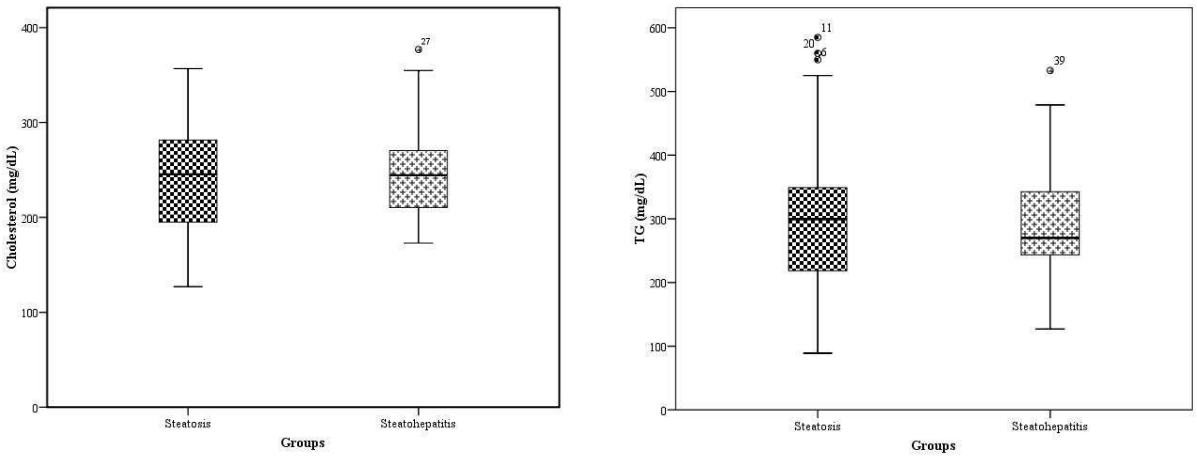


Fig 1: BOX Plot of Cholesterol and Triglycerides in different studied groups

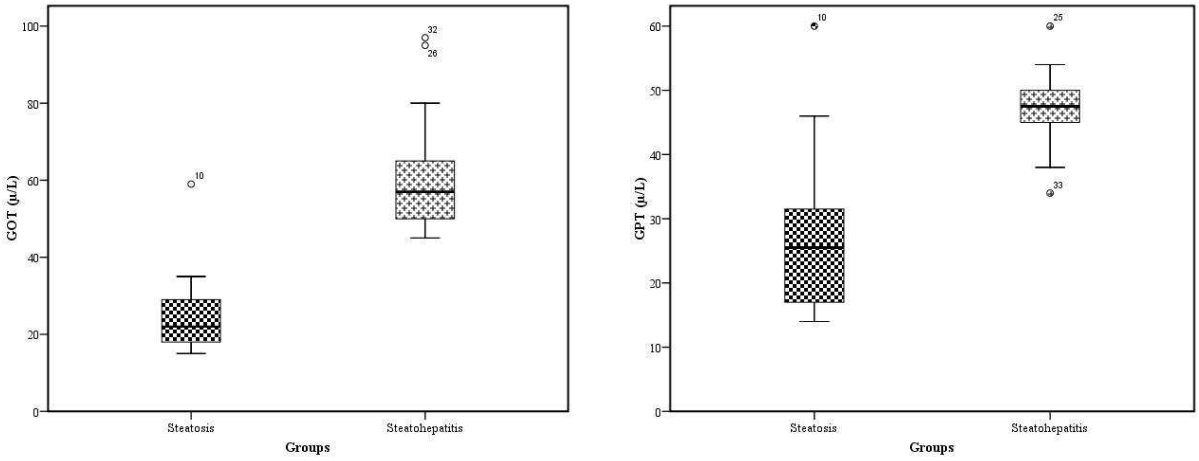


Fig 2: Box Plot presentation of GOT and GPT in different studied groups

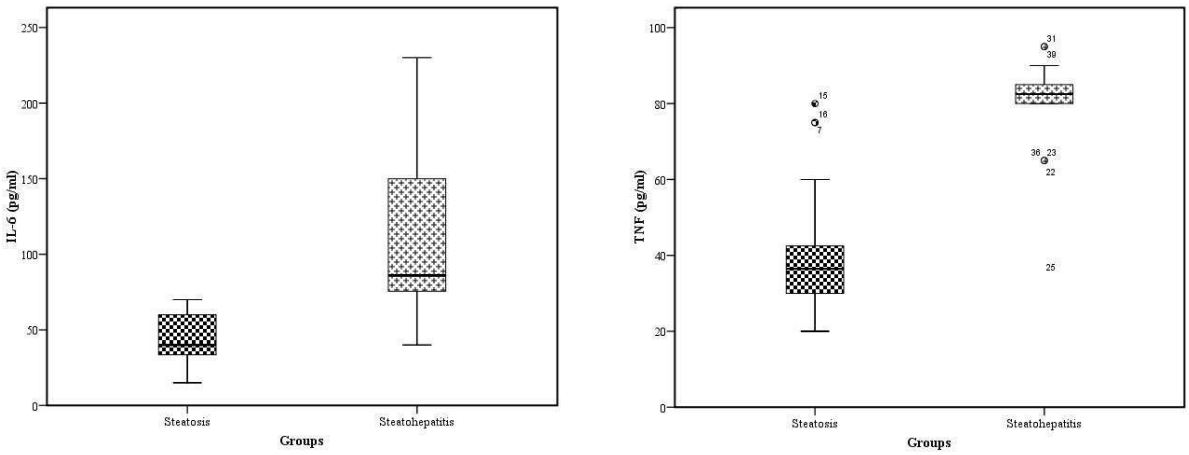


Fig 3: Box Plot presentation of IL-6 and TNF-α in different studied groups

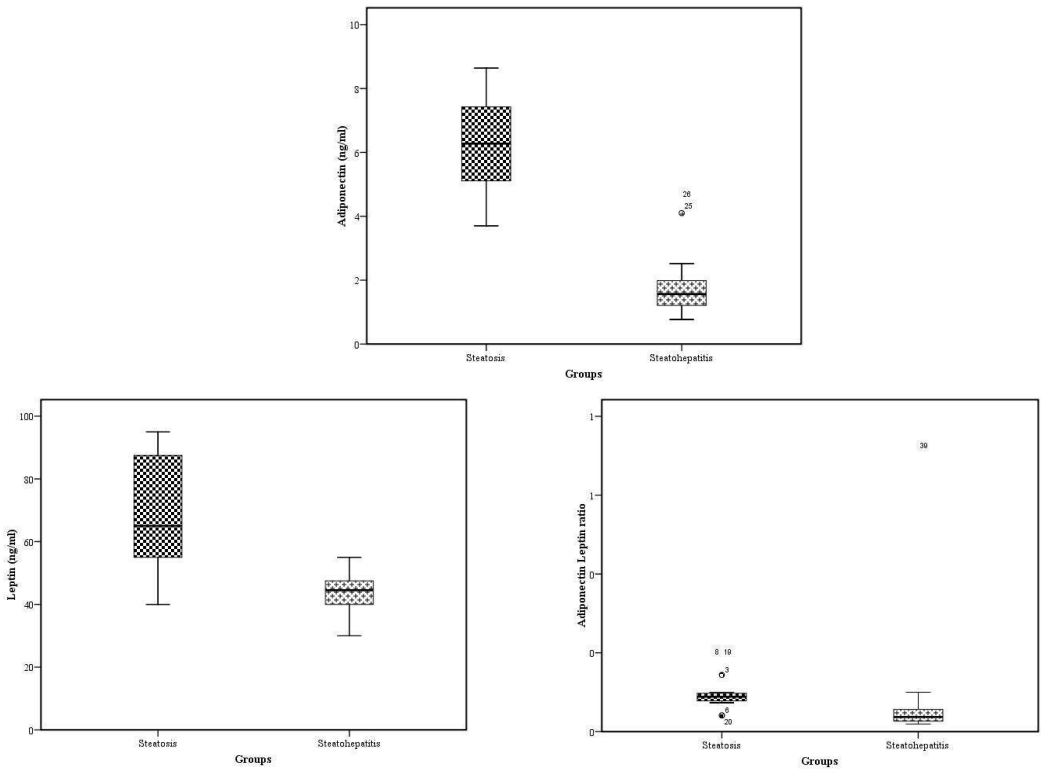


Fig 4: Box Plot presentation of adiponectin, leptin and A/L ratio in different studied groups.

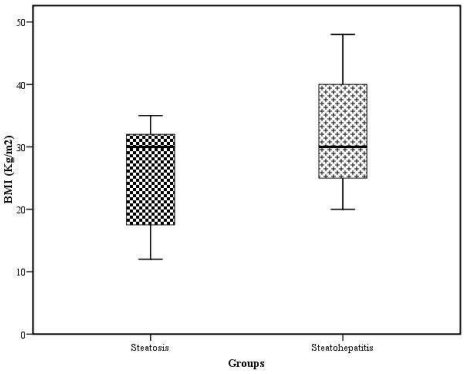
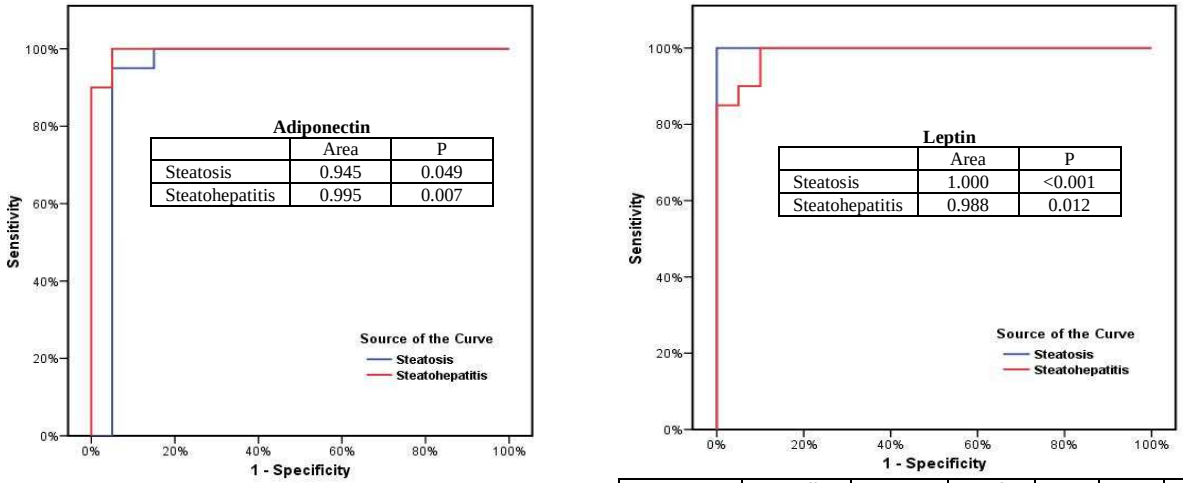


Fig 5: Box Plot presentation of BMI in different studied groups.



	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
Steatosis	<8.0	95.0	100.0	100.0	95.24	97.5
Steatohepatitis		100.0	100.0	100.0	100.0	100.0

	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
Steatosis	>15(male)	70.0	100.0	100.0	76.92	85.0
Steatohepatitis	>40(female)	100.0	100.0	100.0	100.0	100.0

Fig 6: ROC curve presentation of adiponectin and leptin in different studied groups.

Table I: Comparison between the two studied groups (steatosis NAFLD & steatohepatitis NASH) according to different variables

	Groups				Test of sig.
	Steatosis		Steatohepatitis		
	No.	%	No.	%	
Sex					
Male	11	55.0	7	35.0	$\chi^2 = 1.616$
Female	9	45.0	13	65.0	p = 0.204
Cholesterol(mg/dl)					
Range	127.00 – 357.00		89.00 – 585.00		Z = 0.189
Mean ± SD	244.40 ± 64.20		312.75 ± 141.14		p = 0.850
Median	245.50		299.50		
TG (mg/dl)					
Range	173.00 – 377.00		127.00 – 533.00		Z = 0.203
Mean ± SD	249.65 ± 54.73		291.00 ± 98.04		p = 0.839
Median	244.50		270.00		
CRP					
-ve	20	100.0	0	0.0	FEp <0.001*
+ve	0	0.0	20	0.0	
GOT (U/L)					
Range	15.00 – 59.00		45.00 – 97.00		Z = 5.147*
Mean ± SD	25.20 ± 10.03		60.85 ± 14.87		p <0.001
Median	22.00		57.00		
GPT (U/L)					
Range	14.00 – 60.00		34.00 – 60.00		Z = 4.605*
Mean ± SD	27.50 ± 12.07		47.20 ± 5.71		p <0.001
Median	25.50		47.50		
Adiponectin /leptin ratio					
Range	0.04 – 0.19		0.02 – 0.10		Z = 4.437*
Mean ± SD	0.10 ± 0.04		0.04 ± 0.02		p <0.001
Median	0.09		0.03		
BMI					
Range	12.00 – 35.00		20.00 – 48.00		Z = 1.502*
Mean ± SD	26.00 ± 7.87		31.15 ± 8.50		p <0.001
Median	30.00		30.00		

Table II: Correlation between BMI with adiponectin and leptin in the two studied groups

			Adiponectin	Leptin
BMI	Steatosis group	r	-0.670*	0.136
		p	0.001	0.568
	Steatohepatitis group	r	0.015	-0.164
		p	0.948	0.489

Table III: Correlation between different parameters in the two studied groups

			Cholesterol	TG	GOT	GPT
Steatosis group	TNF	r	0.341	-0.127	-0.074	-0.061
		p	0.141	0.595	0.758	0.798
	Adiponectin	r	0.095	-0.368	-0.037	-0.022
		p	0.690	0.111	0.878	0.926
	Leptin	r	0.244	0.304	0.086	-0.306
		p	0.300	0.192	0.718	0.190
	IL6	r	0.529*	0.125	-0.181	-0.254
		p	0.016	0.600	0.446	0.280
	Adiponectin /leptin ratio	r	-0.002	-0.387	-0.165	0.102
		p	0.993	0.092	0.488	0.669
Steatohepatitis group	TNF	r	-0.124	0.242	0.017	-0.493
		p	0.601	0.304	0.942	0.027*
	Adiponectin	r	0.317	-0.042	-0.338	0.409
		p	0.174	0.861	0.145	0.073
	Leptin	r	0.053	-0.307	-0.112	0.188
		p	0.826	0.188	0.639	0.427
	IL6	r	0.016	0.499*	0.149	-0.361
		p	0.946	0.025	0.531	0.118
	Adiponectin /leptin ratio	r	0.339	0.145	-0.370	0.311
		p	0.144	0.541	0.109	0.182

Table IV: Correlation between adiponectin / leptin ratio (A/L) with adiponectin and leptin in the two studied groups

			Adiponectin	Leptin
Adiponectin/ leptin ratio	Steatosis group	r	0.564*	-0.740*
		p	0.010	<0.001
	Steatohepatitis group	r	0.942*	-0.226
		p	<0.001	0.338

r: Pearson coefficient *: Statistically significant at $p \leq 0.05$

Table V: Correlation between leptin, adiponectin, TNF and IL-6 in the two studied groups

			Leptin	Adiponectin	TNF	IL-6
Steatosis group	Leptin	r		0.054	0.419	0.354
		p		0.822	0.066	0.126
	Adiponectin	r			0.811*	0.678*
		p			<0.001	0.001
	TNF	r				0.765*
		p				<0.001
	IL-6	r				
		p				
Steatohepatitis group	Leptin	r		0.093	0.338	-0.232
		p		0.695	0.145	0.325
	Adiponectin	r			0.359	-0.229
		p			0.120	0.331
	TNF	r				0.671*
		p				0.001
	IL-6	r				
		p				

r: Pearson coefficient *: Statistically significant at $p \leq 0.05$

Table VI: Comparison between the controls & the different studied groups according to TNF, adiponectin, leptin and IL 6

	Groups		
	Control	**Steatosis**	**Steatohepatitis**
TNFα (pg/ml)			
Range	1.60 – 10.00	20.00 – 80.00	35.00 – 95.00
Mean ± SD	6.06 ± 2.60	41.25 ± 17.53	79.25 ± 13.89
Median	6.35	36.50	82.50
Z_1 (p)		5.414* (<0.001)	5.429* (<0.001)
Z_2 (p)			4.787* (<0.001)
Adiponectin (ng/ml)			
Range	3.50 – 17.40	3.70 – 8.64	0.77 – 4.50
Mean ± SD	12.03 ± 3.46	6.15 ± 1.39	1.80 ± 0.96
Median	11.75	6.28	1.57
Z_1 (p)		4.815* (<0.001)	5.357* (<0.001)
Z_2 (p)			5.276* (<0.001)
Leptin(ng/ml)			
Range	3.50 – 37.50	40.00 – 95.00	30.00 – 55.00
Mean ± SD	16.31 ± 10.53	69.50 ± 18.70	43.20 ± 6.93
Median	13.95	65.00	44.50
Z_1 (p)		5.419* (<0.001)	5.290* (<0.001)
Z_2 (p)			4.404* (<0.001)
IL_6(pg/ml)			
Range	1.30 – 13.90	15.00 – 70.00	40.00 – 230.00
Mean ± SD	7.10 ± 3.67	43.85 ± 16.13	110.20 ± 55.34
Median	6.45	40.00	86.00
Z_1 (p)		5.411* (<0.001)	5.411* (<0.001)
Z_2 (p)			4.900* (<0.001)

Z_1: Z for Mann Whitney test between controls and other groups
Z_2: Z for Mann Whitney test between steatosis and steatohepatitis
*: Statistically significant at $p \leq 0.05$.

Table VII: Comparison between HDL & the different studied groups

	Groups		
	Control	**Steatosis**	**Steatohepatitis**
HDL			
Range	52.50 – 69.0	40.10 – 65.60	31.70 – 59.50
Mean ± SD	60.56 ± 10.14	52.74 ± 9.16	44.07 ± 8.16
Median	60.25	52.25	42.0
Z_1 (p)		2.733* (0.006)	4.897* (<0.001)
Z_2 (p)			2.787* (0.005)

Z_1: Z for Mann Whitney test between control and other groups
Z_2: Z for Mann Whitney test between steatosis and steatohepatitis
*: Statistically significant at $p \leq 0.05$

DISCUSSION

The pathogenesis of NAFLD/NASH and, in particular, the mechanisms responsible for liver injury and disease progression remain still incompletely understood.[53] Recent studies have focused on the adipokines, bioactive proteins secreted by adipose tissue, including leptin, adiponectin, tumor necrosis factor alpha and interleukin 6.[54-57] Recently, adipokines which are central factors in the development and progresion of NAFLD and inflammation have been investigated.[58-59] Increasing evidence indicates that they might play important roles in the NASH pathogenesis.[57,60] A number of studies have demonstrated the association between hypoadiponectinemia and NAFLD.[57] In our study we observed significantly lower serum concentration of adiponectin in patients with NASH than in NAFLD group & both groups were lower than in healthy subjects, this mean that high levels of adiponectin are associated with a protective effect against fatty liver.[61-63]

Our finding are in accordance with the recent report by Hui *et al*.,[64] Musso *et al*.[65] and Shimada *et al*.[66] They reported that serum adiponectin level was significantly lower in patients with NASH than in the control group. Moreover, Hui *et al.*[64] observed that lower serum adiponectin level in NASH patients was associated with more extensive necroinflammation.

On the other hand, Wong *et al*.[57] and Bugianesi *et al*.[67] did not find the correlation between serum adiponectin concentration and the disease severity, what is in contradictory with our data, where there was negative correlation between adiponectin & GOT, GPT in both patient groups. Studies link hypoadiponctinemia & NAFLD in adults and childern and in particular, with necroinflammatory NASH.[58]

In the present study we observed negative correlation between adiponectin and IL-6 in NASH group. Because this cytokine inhibit adiponectin messenger RNA in adipose tissue.[59] On the other hand, adiponectin induces its anti-inflammatory properties by suppression of IL-6.[58] Studies of Ota *et al*.[56] support the idea that excessive production of IL-6 versus the defective production of adiponectin may provide a link between inflammation in NASH. Our study also demonstrated significant higher TNF-α serum levels in patients with NASH than in control, what is in agreement with Jarrar *et al*. Study.[60] In the present study, TNF-α levels significantly increased in simple steatosis compared to controls, and even higher in NASH . Similarly, Crespo *et al*.[69] and Wai-Sun Wong *et al*.[57] showed increased expression of TNF- α and its type 1 receptor in patients with NASH compared with patients with simple steatosis.Another interesting issue in our study was the significant differences in serum leptin levels in patients with NASH &NAFLD and controls. Our data does not correspond with those of Musso *et al*.[65] and Angulo *et al*.,[61] where they found no differences between serum leptin levels in patients with NASH and controls .On the other hand, Chitturi *et al*.[62] found that leptin levels were significantly higher in NASH patients than in controls. However, we noted significantly higher serum concentration of leptin in NAFLD patients than the controls&even more than in advanced inflammation (NASH) gp ,what is in agreement with Angulo *et al*,[61] Havel et al,[70] Diehel etal,[71] data showing the correlation between serum leptin levels and liver fat accumulation, and agreement with the present study positive correlation between leptin, cholesterol & TG in NAFLD group.earlier workers suggested that elevated serum leptin levels might promot steatosis and steatohepatitis.[72-74] However subsequent studies found that leptin levels are independent perdictors for the severity of hepatic steatosis but not of necroinflammatory liver changes,[75] ruling out a direct role of leptin in the pathogenesis of NASH[76] and finally excluding a role of serum leptin in the pathogenesis of NAFLD.,[77-79] which in agerement with our results where leptin showed no significant correlation with the liver GOT &GPT function testes in both patient groups. Another issue in our research is that we have observed a negative significant correlation between the concentration of adiponectin with triglyceride in NAFLD patient group. However it showed positive

association with concentration of HDL-C. These current results agree with those reported by owecki and associates.[80-82]

The present study showed that the best cut–off value for adiponectin was <8.0ng/ml & the diagnostic preformance in NASH was 100% with an AUROC of (0.995 p=0.007) while in the NAFLD group was lower (0.945 p=0.049) with diagnostic sensitivity 95%, specificity & PPV was 100%, NPV was 95.24% & diagnostic accuracy was 97.5%. AUROC for leptin at a cutoff (>15 ng/ml for males & >40ng/ml for females) was higher in NAFLD gp (1.000 p<0.001) with 100% diagnostic performance, While in NASH gp leptin had a lower AUROC of (0.988 p=0.012) with 70%sensitivity, 100% specificity & PPV, 76.92% NPV & 85.0% accuracy.

Based on our study we concluded that;

Changes of adipohormones levels provide an additional role in the pathogenisis of steatosis (NAFLD) & progression of steatohepatitis (NASH). Our data also suggest that hypoadiponectenemia may be associated with more advanced form of NASH & has a role in the pathogenesis and progression of non alcoholic steatohepatitis. Serum leptin is significantly elevated in steatosis (NAFLD) than steatohepatitis (NASH) ruling out a direct role of leptin in the pathogenesis of NASH. Adiponectin serum level provides a non- invassive differential marker between steatosis (NAFLD) & steatohepatitis (NASH).

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