Original Article

Usefulness of combined validation of insulin growth factor 1 and serum Adiponectin level to anticipate the early stage of nonalcoholic Steatohepatitis.

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is considered one of the most common causes of chronic liver disease globally. NAFLD prevalence is increasing in parallel with other manifestations of metabolic syndrome. Diagnosis of liver fibrosis depends on imaging and biopsy. Adiponectin and insulin growth factor 1 (IGF-1) are metabolic markers associated with liver dysfunction.

Aim of the study: Was to determine the possibility of using adiponectin and IGF-I in the diagnosis of early-stage nonalcoholic steatohepatitis (NASH) and the validity of the results in older age groups.

Methodology: Comparative cross-sectional study included 60 hepatic patients: 30cases with NASH compared to 30cases with simple steatosis. Both IGF-1 and serum adiponectin were assessed in serum via Enzyme-linked Immunosorbent assay (ELISA). Levels of both analytes were correlated with clinical condition, abdominal ultrasonography (US), immunological and virological data.

Results: Adiponectin and IGF-1 levels were significantly lower in the NASH group; compared to the simple steatosis group ($p < 0.01$). Also, the significant negative correlation between both of them and liver enzymes ($p < 0.01$) reveals that liver and parenchymal dysfunction is linked with lower serum level of IGF-I and adiponectin. Older patient's subgroup analysis showed similar results.

Conclusion: Insulin growth factor 1 and Serum adiponectin are less in patients with NASH compared to those with simple steatosis so they can possibly be used in early laboratory diagnosis of NASH. These results are also valid in older age patients.

Keywords: Serum adiponectin level, IGF1 levels, NASH-NAFLD, Simple steatosis

Introduction

The most frequent form of chronic liver disease, nonalcoholic fatty liver disease (NAFLD), impacts 30% of Western populations. It is a metabolic disorder linked with metabolic syndrome, associated with excessive triglyceride accumulation in the hepatocytes [1], and is a frequent indication for liver transplantation [2]. Histologically NAFLD is categorized into two groups; the first group is simple steatosis group, where there is hepatic steatosis with no hepatocellular damage's proof [3]. However, in the second group, nonalcoholic steatohepatitis (NASH), there is hepatocyte injury (ballooning) associated with inflammation and steatosis, with or without fibrosis [4].

Diagnosis of hepatic steatosis is dependent on imaging and histology only, with the elimination of the reasons for secondary hepatic fat buildup as steatogenic therapy's utilization, hereditary syndromes, consumption of alcohol, or metabolic insulin-resistance syndrome where most patients are suffering from frank obesity, hypertension, dyslipidemia with uncontrolled glucose level. Insulin resistance states are correlated with great levels of adipokines. Adipokines are cytokines released via adipose tissue as adiponectin, Tumor necrosis factor α (TNFα), leptin, Transforming growth factor-β (TGF-β), and resistin [4,5].

Routine liver biopsy is an invasive technique, and it is not preferable to diagnose NAFLD. Also, it has several limitations concerning cost, sampling variability, multiple complications, and inter-observer skills [6]. Still, ultrasonography represents the first line in the diagnosis of NAFLD, among other imaging studies. It has many advantages concerning availability, safety, also its convenience, and relatively low cost [7].

Diagnosis of fatty liver by contrast-enhanced CT has limited sensitivity. However, MRI is useful to exclude fatty infiltration. It can detect the quantity of fatty infiltration across the whole liver [8]. Although, liver enzymes and previous imaging tests (ultrasound, CT, and MRI) can't be reliable for assessing steatohepatitis and fibrosis degree in cases with NAFLD. Therefore, new non-invasive biomarkers are necessary to recognize steatohepatitis in patients with NAFLD [6].

High concentrations of adiponectin circulate in the serum [9], behaving as insulin in insulin-sensitive tissues (muscles & liver) by stimulation of adenosine monophosphate-activated protein kinase to increase oxidation of fatty acid and glucose utilization [10-12]. Many previous studies investigate the role of adiponectin in decreasing insulin resistance and also its role in attenuating liver fibrosis and inflammation, as it can suppress inflammation by direct inhibition of hepatic TNF-α [13]. Deficiency of adiponectin was associated with high aminotransferase levels and liver disease progression [14,15].

In 2010, Polyzos et al. detected level changes of adiponectin throughout the adipose tissue's expansion leading to metabolic syndrome progression and the concomitant progression of NAFL to NASH, leading to NASH-correlated cirrhosis [16,17]. Increased inflammatory cytokines in the liver will result in collagen deposition, liver injury, and finally fibrosis [14].

Insulin-like growth factor-1 (IGF-1) is a hormone that affects metabolism, growth, and development. The liver secretes large amounts of IGF-1 and its binding protein stimulated by growth hormone so, the metabolism of lipids, protein, and carbohydrates is affected by growth hormone through, IGF-1 which increases levels of circulating free fatty acids (FFA) and lipolysis [18]. Increased level of

IGF-I stimulates the uptake of peripheral glucose with decrease synthesis of hepatic glucose improving insulin sensitivity. Dal et al. and Takahashi Y detected a decrease in levels of IGF-1 and its binding proteins in chronic liver diseases, which was associated with GH resistance [19,20]. Many authors hypothesized that insulin resistance increased by IGF-1 deficiency leading to NAFLD development [23-25]. Several data are available concerning the protective role of IGF-1 and adiponectin in fatty liver, mostly in mice, not humans [26].

This study aims to examine the possible usage of adiponectin and IGF1 as noninvasive diagnostic and prognostic tools in NASH and to see if these results apply to older age group.

Methodology

Study design and Setting:

This study was a comparative cross-sectional study that was conducted on 60 patients who attended the outpatient clinic of Ain Shams University Hospitals. Their ages ranged from thirty to seventy years, and they were divided into 2 groups: 30 cases with NASH in one group and 30 cases with simple steatosis in the other group.

Inclusion criteria:

NAFLD patients are diagnosed by evidence of excessive hepatic fat accumulation in the liver parenchyma by abdominal sonar with no other causes of steatosis nor history of significant alcohol consumption [48].

NAFLD was subdivided into 2 groups: the first group included NASH patients who were diagnosed by abdominal ultrasonography and elevated liver enzymes. The second group included patients with simple steatosis diagnosed by abdominal ultrasonography with normal liver enzymes [48].

Exclusion criteria:

Causes of elevated liver enzymes other than NALFD i.e., history of alcohol consumption (>20 g day for women and >30 g/day for men), history of use of medications known to precipitate steatohepatitis (e.g., valproate, amiodarone, or prednisone), viral hepatitis (hepatitis B, or C), metabolic causes of steatohepatitis, e.g., Hemochromatosis and Wilson disease and autoimmune liver disease.

Methods

All patients were subjected to full medical history, clinical examination and laboratory investigations including:

- Liver function tests: aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum albumin, serum bilirubin, alkaline phosphatase (Alk. Phosphatase).
- Total proteins.
- Serum creatinine, blood urea nitrogen.
- Fasting blood sugar.
- Lipid profile (serum triglycerides, serum cholesterol).
- Serum ferritin.
- Serum ceruloplasmin.
  (All previous laboratory tests were done by Synchron CX5 clinical analyzer Beckman Synchron CX5 Clinical Analyzer, 900 Seventh Street, Washington.).
- Prothrombin profile (BIO-TP, BIOLABO SAS Les Hautes Rives, Maizy, France).
- Complete blood picture using an automated blood cell counter (Beckman Coulter Diagnostics, 900 Seventh Street, Washington, U.S.).
- Viral markers: Hepatitis B surface antigen (HBSAg) and Hepatitis C virus antibody (HCV Ab) (BIOTECH,13855 Stowe Drive, Poway U.S).
- Antinuclear antibody (ANA) and Anti-smooth muscle antibody (ASMA) by Indirect immunofluorescence (IIF) using commercial INOVA slides.

- Serum adiponectin (Normal adiponectin level (2-37 microgram per milliliter).
- Serum IGF1: Normal insulin growth factor (34 -329 ng/ml) differs according to sex, age by ELISA (Bioassay Technology Laboratory, BT LAB -419 Shanghai, Korain, Biotech Co., Ltd, China).
- Abdominal ultrasonography with the evidence of excessive hepatic fat accumulation in the liver parenchyma [48].

Statistical Analysis:

For statistical analysis, MedCalc ver. 20 (MedCalc, Ostend, Belgium) was utilized. Non-numerical data were presented as frequency and percentage, while non-parametric numerical data were expressed as Inter-quartile range (IQR) and Median. For comparing between various groups of non-numerical factors, the Chi-square test was utilized. In the case of non-parametric numerical records, the Mann-Whitney U assay was utilized for contrasting between two groups. Spearman's rank correlation coefficient (r) was utilized to validate the correlation degree between adiponectin and IGF-1 levels and basic clinical, radiological, laboratory, and hepatic markers variables. Forward logistic regression analysis was utilized to detect the independent parameters that correlate with NASH occurrence. The diagnostic performance of IGF-1 and adiponectin levels were validated in aspects of their diagnostic specificity and sensitivity using the ROC Curve (receiver operating characteristic) to find out the best cut-off value.

Results

This study included 60 NAFLD patients divided into 2 groups (30 patients in each group): NASH and simple steatosis. As regards demographic and clinical characteristics: 65% of patients were males, while 35% were females. Their ages ranged from 33 to 68 years. Older age subgroup included 26.7% and 23.3% of NASH and simple steatosis respectively. There was no significant difference between the 2 groups concerning age, BMI, and gender.

All patients were negative for HCV, HBV, and ANA antibodies. However, there was a greatly significant elevation in many parameters in the NASH group compared to the simple steatosis group as AST was (45.5 vs19.5), ALT (82 vs. 24), total bilirubin (1.1 vs. 1), direct bilirubin (0.4 vs. 0.3), alkaline phosphatase (82.5 vs. 76.5), and ferritin (99 vs. 50.5), ($p < 0.01$ consecutively) between the 2 groups. **(Table 1)**.

The average Adiponectin level was (10.3 ± 7.5 μg/mL), and the average IGF-1 was (45.65 ± 52.7) ng/mL. A highly significant decrease in Adiponectin (6 vs. 13.7) and IGF-1 levels (21.5 vs. 47.5) in the NASH group; compared to the simple steatosis group ($p < 0.01$), **(Table 2).**

There was a highly significant positive correlation between Adiponectin level and IGF-1 ($p = 0.0002$), but there was no significant correlation with age even among older subgroup. However, a highly significant negative correlation was detected between adiponectin level and other parameters as ALT, AST, direct and total bilirubin ($p < 0.01$). Also, a highly significant negative correlation was detected between IGF-1 and the same hepatic parameters (ALT, AST, total, and direct bilirubin) ($p < 0.01$) **(Table 3).**

ROC curve analysis was applied to anticipate NASH reveling that adiponectin level at a cutoff point (≤9.1) anticipated cases with NASH, with good sensitivity= 93%, (83%) accuracy, and specificity= 73% ($p < 0.01$). IGF-1 level at a cutoff point (≤38) anticipated cases with NASH, with fair sensitivity= 86%, (77%) accuracy, and specificity= 66% ($p < 0.01$) **(Table 4).**

Discussion

This comparative cross-sectional study was done on 60 NFALD patients to correlate severity of fatty liver and levels of serum adiponectin and IGF 1. The average age of our patients was 42 to 44, which is most likely the result of a sedentary lifestyle without exercise and a nutritious diet. Many prior authors as Jamali et al. and Pandey et al. showed the same observation concerning the relatively young age of patients [27, 28]. On the other hand, our study showed similar results and correlations in older patients. This implies that the same statistical conclusions can be generalized to those participants.

Prevalence of NAFLD is male-predominant; (65% males) compared to (35% females) were detected in our investigation. This is in accordance with prior studies performed by Arun J et al., Balmer et al., and Salvoza NC et al., which showed NAFLD in 58.62% males versus 41.3% females [29-31]. However, another study done in 2013, by Alam et al. was inconsistent with our finding because they discovered that among Bangladeshi women NAFLD is more common [32].

Also, we observed that more than two thirds of our patients had high BMI which is suggested to be associated with inflammatory change in liver as showed by Savvidou et al. who had similar observation [33].

Adiponectin has shown a very encouraging performance in our study of differentiating simple steatosis from NASH. As there was a

highly significant reduction in adiponectin in NASH group compared to simple steatosis group. (p value < 0.01), this data was in line with Arvaniti et al., Bugianesi et al., and Shimada et al. who confirmed that overall adiponectin levels are low in NASH cases [34-36]. The same was indicated in a work done by Mendez-Sanchez et al. who found that cases with hepatic steatosis exhibit hypoadiponectinemia [37].

A comparable study by Musso et al., observed that adiponectin has a protective role against liver fibrosis; they found that advanced fibrosis with insulin resistance and high levels of fat accumulation was associated with lower adiponectin levels [38]. In 2004, Hui et al. found that both low level of adiponectin and insulin resistance could predict the intensity of necroinflammation and steatosis but they couldn't detect fibrosis [39]. Finally, a study by Van der Poortenet al. found that burnt-out NASH was associated with an increased level of adiponectin [40].

Our study also demonstrated a highly significant reduction in IGF-1 levels in NASH group compared to simple steatosis group. (p< 0.01). This data is similar to that found by Fusco et al., García et al., and Sumida et al., who noted that NASH was associated with lower levels of IGF-1, they hypothesized that a decreased level of IGF-1 would increase insulin resistance leading to NAFLD development. They found also that IGF-1 decreased with the development of NASH and increased in simple steatosis in NAFLD cases [25,41-42]. Another research, however, produced conflicting findings, as demonstrated by Dichtel et al., who discovered that steatosis was not connected to low IGF-1 levels [43].

Additionally, we found a highly significant negative correlation between liver enzymes (ALT and AST) and both adiponectin and IGF-1. The same was observed by Arturi et al., and Yatsuzuka Y et al., who stated a negative correlation between liver enzymes and IGF-I, indicating that parenchymal dysfunction in NASH was associated with a low level of IGF-I [44-45]. Contrary to our results Mustafa et al. noted a significant variation in adiponectin level between NASH and simple steatosis group accompanied with high ALT level [46].

To sum up; Lower levels of serum adiponectin and IGF1 were correlated with greater levels of ALT and AST propose a protective function of adiponectin and IGF-1 in preventing liver damage.

The older age group sub-analysis in our study, showed similar results and correlations to those seen in the adults under 55 years of age. This implies that the same statistical conclusions can be generalized to older age patients.

Conclusion

Insulin growth factor 1 and Serum adiponectin can be used in early diagnosis of NASH (rather than simple steatosis) these results can also be applied to older age groups. There might be a protective or even a therapeutic role for IGF-1 and adiponectin versus the progression of NASH; this role needs to be further investigated.

List of abbreviation:

- (NAFLD) Non-alcoholic fatty liver disease
- (IGF-1) insulin growth factor 1
- (NASH) nonalcoholic steatohepatitis
- (TNFα) Tumor Necrosis Factor Alpha
- (TGF-β) Transforming growth factor beta
- (CT) computed tomography
- (MRI) magnetic resonance imaging
- (GH) growth hormone
- (HBSAg) hepatitis b surface antigen
- (HCV Ab) hepatitis c virus antibody
- (ANA) antinuclear antibody

- (ASMA) anti-smooth muscle antibody
- (IIF) Indirect immunofluorescence
- (BMI) body mass index
- (US) ultrasound
- (ALT) alanine aminotransferase
- (AST) aspartate aminotransferase
- (ELISA) Enzyme-linked Immunosorbent assay

Table (1): Comparison between the 2 groups as per basic clinical data.

Demographic data	**Simple steatosis group (30)**	**NASH group (30)**	**Mann-Whitney's U test**
	Median (IQR)	**Median (IQR)**	**P value**
Age (years)	44.5 (34 – 68)	42.5 (33 – 61)	= 0.3139
Number of Patients ≥ 55 years	8 (26.7%)	7 (23.3%)	= 0.7630
BMI	31.3 (30.1 – 33.7)	32.5 (29.4 – 36.1)	= 0.7448
Laboratory investigations			
Hb(g/dL)	13.9 (12 – 14.4)	14.6 (13 – 15)	= 0.0733
PLT (10^3/µL)	222.5 (199 – 302)	242.5 (214 – 275)	= 0.8591
TLC (10^3/µL)	7.4 (6.5 – 10)	7.5 (6.2 – 8.9)	= 0.4776
T. Cholesterol (mg/dL)	170 (161 – 227)	212.5 (185 – 230)	= 0.1118
TGs (mg/dL)	156 (92 – 200)	160.5 (136 – 181)	= 0.5152
FBS (mg/dL)	134.5 (100 – 150)	108 (102 – 124)	= 0.1759
Creat. (mg/dL)	0.8 (0.8 – 1)	0.9 (0.8 – 0.98)	= 0.3662
AST (U/L)	19.5 (17 – 22)	45.5 (42 – 59)	< 0.0001*
ALT (U/L)	24 (19 – 30)	82 (67 – 97)	< 0.0001*
T. Bil. (mg/dL)	1 (0.8 – 1)	1.1 (1 – 1.1)	< 0.0001*
D. Bil. (mg/dL)	0.3 (0.3 – 0.4)	0.4 (0.4 – 0.5)	= 0.00013
Alkaline Phosphatase (mg/dL)	76.5 (65 – 90)	82.5 (72 – 115)	= 0.02
Alb. (g/dL)	4.2 (4 – 4.3)	4.25 (4.1 – 4.5)	= 0.0867
INR	1 (0.9 – 1)	1 (0.9 – 1.1)	= 0.0858
PT (sec)	12.4 (12 – 13)	12.7 (12 – 13)	= 0.1775
Ferritin (ng/dL)	50.5 (30 – 92)	99 (80 – 116)	= 0.0016

*IQR: inter-quartile range, BMI: body mass index, Hb: hemoglobin, PLT: platelets, TLC: total leucocytic count, TG: triglycerides, FBS: fasting blood sugar, Creat: creatinine, ALT: alanine aminotransferase, AST: aspartate aminotransferase, T. Bil: total bilirubin, D. Bil: direct bilirubin, Alb.: albumin, INR: international normalized ratio, PT: prothrombin time. *=statistically significant.*

Table (2): Comparison between the 2 groups as per hepatic markers utilizing Mann-Whitney's U test.

Variable	simple steatosis group (30)	NASH group (30)	Mann-Whitney's U test
	Median (IQR)	Median (IQR)	P value
Adiponectin (µg/mL)	13.7 (9 – 17)	6 (5 – 8)	< 0.0001*
IGF-1 (µg/mL)	47.5 (30 – 80)	21.5 (10 – 32)	= 0.00026

**=statistically significant. IQR: inter-quartile range.*

Table (3): Correlation analysis of adiponectin and IGF-1 with clinical and laboratory criteria.

Associated Factor		Adiponectin level		IGF-1 level	
		Rho	P	Rho	P
Clinical	**Age** (years)	0.195	=0.1344	0.0283	=0.8302
	Number of Patients ≥ 55 years	0.162	=0.1007	0.0192	=0.7164
	BMI	0.125	=0.3399	0.193	=0.1387
Laboratory	**Hb**(g/dL)	-0.0710	=0.5899	-0.131	=0.3170
	PLT (10^3/µL)	-0.115	=0.3799	-0.0586	=0.6567
	TLC (10^3/µL)	-0.0710	=0.5898	0.239	=0.0656
	T. Cholesterol (mg/dL)	-0.240	=0.0646	-0.0200	=0.8797
	TGs (mg/dL)	-0.0210	=0.8736	0.208	=0.1109
	FBS (mg/dL)	0.208	=0.1106	0.0342	=0.7951
	Creat. (mg/dL)	-0.0162	=0.9024	-0.0807	=0.5398
	AST (U/L)	-0.414	=0.001**	-0.509	<0.0001**
	ALT (U/L)	-0.435	=0.0005**	-0.480	=0.0001**
	T. Bil. (mg/dL)	-0.430	=0.0006**	-0.407	=0.0012**
	D. Bil. (mg/dL)	-0.419	=0.0009**	-0.493	=0.0001**
	Alkaline Phosphatase (mg/dL)	-0.130	=0.3239	-0.248	=0.0558
	Alb. (g/dL)	0.0368	=0.7801	-0.246	=0.0577
	INR	-0.115	=0.3826	-0.181	=0.1657
	PT (sec)	-0.110	=0.4046	-0.286	=0.026*
	Ferritin (ng/dL)	-0.239	=0.0655	-0.241	=0.0631
Hepatic markers	**IGF-1** (µg/mL)	0.456	=0.0002**		

Rho: Spearman's rho (correlation coefficient). IQR: inter-quartile range, BMI: body mass index, Hb: hemoglobin, PLT: platelets, TLC: total leucocytic count, TG: triglycerides, FBS: fasting blood sugar, Creat: creatinine, ALT: alanine aminotransferase, AST: aspartate aminotransferase, T. Bil: total

*bilirubin, D. Bil: direct bilirubin, Alb.: albumin, INR: international normalized ratio, PT: prothrombin time. *=statistically significant.*

Table (4): Roc-curve of hepatic markers to anticipate patients with NASH.

Variable	AUC	SE	Best Cut off point (Criterion)	Sensitivity (%)	Specificity (%)	P value
Adiponectin (μg/mL)	0.831	0.0573	≤9.1	93.33	73.33	<0.0001**
IGF-1 (μg/mL)	0.774	0.0629	≤38	86.67	66.67	<0.0001**

AUC= Area under curve, SE= Standard Error, ROC (Receiver operating characteristic).

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