Diagnostic Role Of Resistin In Nonalchoholic Fatty Liver Disease

By

Engy Yousry Elsayed, Amal Shawky Mohamed, Hala Abd Elal* and Eman Hamed**

From

Internal Medicine, Clinical Pathology* and Pathology Departments**

Faculty Of Medicine, Ain Shams University, cairo, Egypt.

ashorengy@yahoo.com, amalshawky-mb@hotmail.com,
hala_abdelal@yahoo.com, **imihewedi99@yahoo.com**

Abstract: **Introduction:** Nonalcoholic fatty liver disease (NAFLD) is a major cause of liver-related morbidity and mortality. Insulin resistance is believed to be a key factor in the development of fatty liver. Moreover, insulin resistance states characterized by elevated expression and production of several cytokines; of particular adiponectin, leptin, resistin. Leptin and adiponectin have been implicated in the pathogenesis and progression of NAFLD but direct evidence of the role of resistin in NAFLD is lacking. **The aim of this study** was to determine the circulating resistin level in patients affected by NAFLD and to correlate resistin level with insulin sensitivity, liver function and histologic feature. **Subjects and methods**: This study included 100 subjects divided in to: Forty patients with NAFLD, forty obese person with BMI >30 with normal transaminases and normal liver ultrasound and twenty controls with BMI <20, for all subjects serum resistin was measured, Homeostasis model assessment (HOMA) was calculated and liver profile was assessed. Liver biopsy was done in NAFLD patients. **Results**: Serum resistin was higher in patients with NAFLD (16.2 ± 4) compared to obese and control groups (6.8 ± 4.1 and 3.4 ± 1.1) respectively ($p < 0.01$), serum resistin was higher in advanced cases of NAFLD compared to mild cases (19.2±3.6 vs. 13.5±2.7) respectively ($P < 0.01$). Moreover serum resistin was positively correlated to BMI, HOMA , highly sensitive CRP, AST and ALT. **Conclusion and recommendation**: Resistin has a role in pathogenesis of NAFLD, resistin level is a predictive of histology in NAFLD, so the use of serum resistin assay as a simple diagnostic biomarker for NAFLD is recommended. [Nature and Science 2010;8(4):64-68]. (ISSN: 1545-0740).

Key word: NAFLD, NASH, Obesity and Resistin.

Introduction: Nonalcoholic fatty liver disease (NAFLD) is increasingly recognized as a potential serious condition, which can progress to cirrhosis, liver failure and hepatocellular carcinoma and has a worldwide distribution.[1]The biological basis of variability in histological progression of NAFLD is unknown, consequently, it has become extremely important to understand the patho-physiology of NAFLD to develop sound therapeutic interventions. It is now recognized that non hepatic mechanisms are largely responsible for the development of insulin resistance, which causes hepatic steatosis.[2] Insulin resistance is believed to be a key factor in the development of fatty liver. Moreover, insulin resistance states characterized by elevated expression and production of several cytokines; of particular adiponectin, leptin, resistin[3] Resistin is a recently discovered signal molecule, which could help elucidation of the patho-physiology of the insulin resistance and its correlation with obesity.[4] Leptin and adiponectin have been implicated in the pathogenesis and progression of non-alcoholic steatohepatitis (NASH) and chronic hepatitis C (CHC), but little is known about the role of resistin in chronic liver diseases.[5] **The Aim of this work** was to determine the circulating resistin level in patients affected by NAFLD and to correlate resistin level with insulin sensitivity , liver function and histologic feature. **Subjects and methods**: 100 subjects recruited form the hepatology clinic of Ain Shams university hospital were enrolled in this study, they were divided into:

Group 1: Forty patients with NAFLD, diagnosis was based on chronic elevation of transaminases (>1.5 times the upper normal value for 3 months or longer), absence of hepatitis B and C virus markers, absence of autoantibodies indicative of autoimmune hepatitis , absent alcohol consumption and bright liver at ultrasound scanning, with body mass index (20-35 kg/m^2). In all patients, diagnosis was confirmed by liver biopsy .

Group 2: Forty obese persons with body mass index above 30 kg/m^2 with normal transaminases values and normal liver ultrasound.

Group 3: Twenty age and sex matched healthy subjects with body mass index 20-25 kg/m^2.

None of patients and control subjects were taking lipid-lowering medications, met-formin or thiazolidinediones. Written informed consent was obtained from all participants.

For all subjects the following was done:

1: *Full history taking***.** 2: *Clinical examination* **with special emphasis on calculation of body mass index,**

local abdominal examination.

3: Laboratory examination: (CBC, ESR, fasting and 2 h pp blood glucose, fasting insulin, renal function(S cr, BUN, Na, K) , liver profile(ALT, AST, GGT, ALP, bilirubin(total, direct), albumin, total proteins, PT, INR), lipid profile(total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol and triglycerides), high-sensitivity C-reactive protein (hs CRP). HCV Ab , HBs Ag, HBV C Ab, ANA, AMA, ASMA,LKM, ferretin level).

4: The level of serum resistin was measured by ELISA method.

5: Insulin resistance was estimated by homeostasis model assessment (HOMA) =Fasting insulin X Fasting glucose / 22.5.[6]

6. Abdominal Ultrasonography **using real time scanning device Toshiba, vision 200 (SSA, 320A) with convex probe 3.5-5 uHz, focusing on liver size, texture, visualization of intra-hepatic vessels and diaphragm, liver to kidney contrast ratio.**[7]

7:Percutaneous Liver biopsy was done only for NAFLD patients (group 1). It was performed under ultrasound guidance using 16-gauge needles. Specimens of at least 2.5 cm in length, including a minimum of 12 portal tracts. Thin serial sections (4 micrometers thick) from formalin-fixed, paraffin-embedded blocks of core liver biopsies were stained with hematoxylin & eosin then assessed for detection of fat globules to identify and quantify hepatic steatosis, inflammation, necrosis and fibrosis**.** A NAFLD activity score (NAS), which includes features of active injury, has been defined as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3) and ballooning (0-2).

According to this scale, cases with scores ≥5 are diagnosed as NASH, and scores <3 are diagnosed as not NASH, 3-5 are diagnosed as borderine NASH . It has been clearly emphasized that the NAS is not intended to be used as a diagnostic tool, but rather to provide a uniform tool for assessing disease severity[8].

8: Statistical analysis: All the collected data were expressed as mean ± SD and analyzed by using SPSS version 13 using the following tests: student t, ANOVA, Perason correlation coefficient. P > 0.05 was considered non significant, P < 0.05 was considered significant P < 0.01 was considered highly significant.

Results: This study included 100 subjects they were divided into:

Group (1) forty patients with (NAFLD), they were 15 males and 25 females their mean age was 42± 13 with BMI 28.2 ±5.2 kg/m^2.

Group (2) forty obese patients, they were 13 males and 27 females their mean age was 39± 12 with BMI 32.7 ±1.4 kg/m^2.

Group (3) twenty healthy volunteers (controls), they were 6 males and 14 females their mean age was 40± 12 with BMI 25.2 ±2.6 kg/m^2.

NAFLD and obese patients had higher FBS, 2h PP, fasting insulin and HOMA compared to controls(P<0.01). NAFLD patients had higher serum resisrtin compared to obese and controls (16.2± 4vs.6.8± 4.1and 3.4 ±1.1) respectively p (<0.01) as shown in table 1.

Table(1): Comparison between the studied groups as regard blood glucose, insulin and resistin.

Prameter	Group1	Group2	Group3	1vs.2	1vs.3	2vs.3
FBS(mg/dl)	158±24	157±20	89 ±14	>0.05	**<0.01**	**<0.01**
2h pp(mg/dl)	255±58	250±680	127± 9	>0.05	**<0.01**	**<0.01**
Insuin(**µiu/ml**)	19.3 ±17	13.1± 6	12.1 ±4.1	>0.05	**<0.01**	**<0.01**
HOMA	135.2±123	104± 38	49.4 ±20.5	>0.05	**<0.01**	**<0.01**
Resistin **(ng/ml)**	16.2± 4	6.8± 4.1	3.4 ±1.1	**<0.01**	**<0.01**	>0.05

NAFLD patients were subsequently divided according to histological diagnosis using Kleiner et al., [8] scoring system in to:

Group 1a: Ten cases were classified as 'Not NASH' they were 4 males and 6 females with mean age 46±8.6.

Group 1b: Twelve cases were classified as 'Borderline NASH' they were 8 males and 4 females with mean age 51.3±9.6.

Group 1c: Eighteen cases were classified as 'NASH **'** they were 8 males and 10 females with mean age 32.7±11.4.

Group 1c (NASH) had higher BMI, Hs CRP as well as resistin compared to group1a'Not NASH' and group 1b'Borderline NASH' P < 0.01 as shown in table 2.

Table (2): Comparison between pathological grades of NAFLD among group one as regard different parameters.

	Group 1a(10)	Group 1b(12)	Group 1c(18)	Annova	P
BMI(kg/m^2)	21.8 ± 1.7	27.3± 2.5	33.6 ± 1.3	64.4	<0.01
ALT(U/L)	95 ± 11	96.3 ± 17.2	101.5 ±1 5.5	0.38	>0.05
AST(U/L)	71.1±12.5	76 ±7.8	82.2 ±15.4	1.3	>0.05
FBG(mg/dl)	150±20	153±27	173±19	1.06	>0.05
2hpp(mg/dl)	156.6±60	190±90	273±65	2.7	>0.05
Resistin (ng/ml)	13.5±2.7	14±2.2	19.2±3.6	7.8	<0.01
CRP (mg/dl)	2.3±2.2	3.9 ±1.5	5.9±1.7	6.5	<0.01

Resistin had positive correlation to blood glucose, insulin, HOMA , liver enzymes, LDL cholesterol, TG and Hs CRP as shown in table 3

Table3: Correlation of serum resistin to different parameters.

parameter	r	P	parameter	R	P
BMI	0.68	<0.05	T cholesterol	0.75	<0.01
FBG	0.66	<0.05	LDL	0.77	<0.01
2hpp	0.68	<0.05	TG	0.66	<0.01
Insulin	0.67	<0.05	AST	0.91	<0.01
HOMA	0.75	<0.05	ALT	0.91	<0.01
Hs CRP	0.73	<0.01			

Discussion:

This study essentially showed that NAFLD patients had higher serum resisrtin compared to obese and controls (16.2± 4 vs. 6.8± 4.1 and 3.4 ±1.1) respectively p (<0.01) and this increase was positively correlated with BMI, blood glucose and insulin resistance. The strong association between insulin resistance and NAFLD has been extensively demonstrated [9]. Available evidence suggests that insulin resistance affects hepatic fat accumulation by increasing release of free fatty acids from adipose tissue, increasing fatty acid and triglycerides synthesis in the liver, reducing fatty acid oxidation and reducing very low-density lipoprotein (VLDL) production. Binding of adiponectin to its receptors stimulates phosphorylation of PPARα activity and fatty acid oxidation in liver and reducing fatty acid synthesis through inhibition of acyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) expression and activity[10] , and this mechanism is inhibited by resistin, therefore increased resistin in NAFLD could result in increased fatty acid synthesis, accumulation of triglycerides, and reduced fatty acid oxidation via insulin resistance and inhibiting adiponectin action. **Al-Harithy and Al-Ghamdi** [11]found that serum resistin concentrations increased from lean (11.59 +/- 2.08) to OW/OB non-diabetic (16.29 +/- 2.29) to diabetic (19.42 +/- 3.60 ng/mL) (P<0.001). Furthermore, resistin correlated significantly and positively with insulin and HOMA in diabetic and non-diabetic subjects. **Baranova et al** [12]stated that serum resistin was higher in patients with insulin resistance than patients without insulin resistance and obese patients with insulin resistance have decreased serum adiponectin and increased serum resistin. **Ciba and Widhalm**[13] found an association between insulin resistance and NAFLD in obese children indicating that markers of insulin sensitivity could be useful screening parameters for NAFLD.

This study showed that advanced NAFLD was strongly associated with higher serum resistin, as Group 1c (NASH) had higher resistin compared to group1a'Not NASH' and group 1b'Borderline NASH' P < 0.01. The previous studies of the relationship between resistin and NAFLD were conflicting as **Aller et al**[14] confirmed that blood levels of resistin were higher in patients with a high grade of steatosis, on the other hand **Cho etal**[15] found that serum resistin levels were similar in Group I (normal liver), Group II (mild fatty liver)and Group III (moderate to severe fatty liver), while leptin levels increased with increasing degree of hepatic fat infiltration, moreover **Lee et al**[16] found that there were no significant differences in serum leptin and resistin levels between two normal and increased ALT groups, while serum adiponectin levels were lower in the increased ALT group than in the normal ALT group, furthermore **Tsochatzis et al**[5] **s**tated that there was no significant association between steatosis or necroinflammation and levels of adipokines, while the presence of moderate/severe fibrosis (stages 4-6) was associated lower resistin.

We found a positive correlation between serum resistin and AST,ALT, hs CRP(P<0.01). It is well known that inflammation is a key mechanism in the progression of fatty liver to hepatitis and cirrhosis. [17] Adipokines are believed to act through their effects on insulin

sensitivity. Insulin resistance and hyperinsulinemia are also associated with the inflammatory and fibrotic reaction that complicates advanced stages of the disease [18], but new lines evidence indicate an important action on stimulation/ inhibition of the inflamatory process. [19] **Mojiminiyi and Abdella**[20]stated that resistin may represent a link between obesity and insulin resistance via pro-inflammatory pathways. In NAFLD a self-perpetuating pathway between insulin resistance and inflammation may explain the necro-inflammation observed in the subset of patients with NASH. [21] in contradiction to our results **Pagano et al**[22] found no correlation between resistin and high-sensitivity C-reactive protein and positive correlation between resistin and histological inflammatory score. **Roberto et al**[23]confirmed the significant direct association between hs-CRP and resistin which might explain the inflammatory pathogenic role of resistin which aggravate liver histology at more severe stages in NAFLD.

Conclusion and recommendation: Resistin has a role in pathogenesis of NAFLD, resistin level is a predictive of histology in NAFLD, so it can be used as a simple diagnostic biomarker for NAFLD.

Correspondence to:
Engy Yousry El Sayed .
Faculty Of Medicine, Ain Shams University, cairo, Egypt.
Telephone: 0106905243
Emails: **ashorengy@yahoo.com**, amalshawky-mb@hotmail.com, **hala_abdelal@yahoo.com, imihewedi99@yahoo.com**

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