Serum resistin levels in nonalcoholic fatty liver disease and their relationship to severity of liver disease

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Abstract

Background: Resistin is a hormone that is linked to the development of insulin resistance (IR), but information on the direct relationship of resistin levels in humans with nonalcoholic fatty liver disease (NAFLD), and their effect on the histological severity of NAFLD, is lacking.

Objective: The aim of the current study is to determine the circulating resistin levels obtained from patients with NAFLD and to correlate them with insulin resistance and hepatic histological features.

Methods: Blood samples were collected from 30 consecutive patients with liver-biopsy-proven NAFLD and 30 subjects as controls. Serum resistin levels were measured. Body mass index (BMI) was calculated for all subjects, and serum insulin, C-peptide, and lipoprotein levels were also measured.

Results: Mean serum resistin level and BMI in the NAFLD group were significantly higher than in the controls (both $P < 0.001$). Both men and women in the NAFLD group had higher mean serum resistin levels than did the men and women in the control group (all $P < 0.001$). Multivariate analysis showed that the percentage of hepatic steatosis, sex, BMI, and homeostasis model assessment of insulin resistance [HOMA(IR)] were related to serum resistin levels.

Conclusion: These data suggest increased resistin levels in NAFLD patients which are related to histological severity of the disease. These findings support the link between resistin, insulin resistance and BMI in these patients.

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Introduction

Resistin is a 10 kDa protein composed of 94 amino acids. It was cloned in 2001 and was shown to be a thiazolidinedione (TZD)-regulated cytokine expressed in adipose tissue.[1,2,3] The effect of resistin on insulin action has been extensively investigated in laboratory models.[4] It was shown to be involved in hepatic glucose and lipid metabolism and appears to play a pivotal role in hepatic insulin resistance (IR) induced by high-fat diet.[5,6] Further studies on animal models suggested that resistin could represent a link among obesity, insulin resistance, and diabetes.[7] Data on the role of this adipokine in insulin sensitivity and obesity in human studies are controversial.[8] Some studies indicated that high serum resistin levels are associated with increased obesity, visceral fat, insulin resistance, and type 2 diabetes, while others failed to show the same observations.[9,10,11]

Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of disorders characterised by macrovesicular hepatic fat accumulation occurring in individuals without alcohol consumption. It is a complex metabolic condition in which both lifestyle and genetic factors play a pathogenic role.[12,13] Moreover, NAFLD has been convincingly associated with the metabolic syndrome and insulin resistance.[14] Insulin resistance, through inhibition of lipid oxidation and increased fatty acid and triglyceride synthesis, is believed to be a key factor in the development of fatty liver. Moreover, insulin resistance states, such as obesity and diabetes, are also characterised by altered production of adipokines (adiponectin, leptin, resistin, TNF, TGF, and plasminogen activator inhibitor-1). Because activation of inflammatory mechanisms was found in adipose tissue, it is possible that changes in the resistin concentration may be involved in the pathogenesis of NAFLD.[15] Pagano *et al* reported that patients with NAFLD were characterised by higher serum resistin levels in association with the nonalcoholic steatohepatitis (NASH), whereas no correlation was found with insulin resistance.[16] The aim of our study is to to determine circulating resistin levels obtained from patients with NAFLD and to correlate its levels with insulin resistance and hepatic histological features.

Materials and methods

Patients

A total of 30 consecutive patients with liver-biopsy-proven NAFLD from March 2007 to September 2007 at Cairo University Hospital (Kasr

El Aini Hospital) were enrolled in this prospective clinical trial. The 30 persons recruited as a control group included healthy subjects who were matched with the studied patients in terms of age, sex and BMI. All study patients had had a liver biopsy either during laparoscopic or open cholecystectomy. All patients signed an informed consent document. The clinical trial protocol and the consent form were approved by the Human Investigational Board of Cairo University Hospital. The open liver biopsies were taken from the inferior border of the liver, while laparoscopic biopsies were taken from the epigastric port using scissors and diathermy for adequate haemostasis.

All subjects underwent a detailed clinical and laboratory evaluation, including liver function tests, markers for hepatitis markers, and autoantibodies, in addition to upper abdominal ultrasonography. Alcohol consumption was absent in all subjects. Subjects with a previous or current history of acute or chronic viral hepatitis, malignant disease, acute infections, pituitary, adrenal, thyroid and pancreatic disease, or evidence for any other endocrine disorder, or prolonged use of corticosteroids or sex hormones were excluded. The controls had normal liver enzymes and no clinical, laboratory or imaging evidence of liver disease. Bright liver at ultrasound scanning was defined as an evident sonographic contrast between hepatic and renal parenchyma, vessel blurring, focal sparing, and narrowing of the lumen of the hepatic veins, according to international guidelines.[17]

Clinical and laboratory evaluation

Overnight (12-hour) fasting samples of serum were obtained from the study subjects and stored in aliquots at -70 ˚C until assayed. Clinical and laboratory data were collected on the date that the liver biopsy was performed. A complete medical history and physical examination was accomplished in all patients and controls. BMI was calculated using the following formula: BMI = weight (kg)/height $(m)^2$. Laboratory evaluation included tests for liver enzymes, glucose, complete blood count, total cholesterol, triglycerides, viral serology for hepatitis B and C, and autoantibodies. C-peptide and insulin were measured by direct chemiluminescent technique, using an ADVIA centre immunoassay system, Bayer, Germany. The IR index was calculated on the basis of fasting values of plasma glucose and insulin according to the homeostasis model assessment for insulin sensitivity (HOMA) model formula: HOMA(IR) = fasting insulin (mIU/L) × fasting glucose (mmol/L)/22.5.

Resistin was measured using a commercially available sandwich ELISA kit (MBL International Corporation, Woburn, MA, USA) within the range of 16–20 000 pg/ml, according to the manufacturer's instructions. The absorbance of each well was read using a microplate reader, stat fax 2100 (Awareness Technology, Inc, Palm City, FL, USA).

Liver histology

Liver biopsy specimens were at least 15 mm in length, had been fixed in neutral-buffered formalin, routinely processed and stained by haematoxylin and eosin, and Masson's trichrome stain, for assessment of fibrosis. All biopsies had the appropriate number of portal tracts (a minimum of four portal tracts) to enable a confident evaluation of histological features and diagnosis.[18] Liver biopsies were read by a single liver pathologist who was unaware of the patients' clinical and laboratory data. All biopsies were interpreted, graded and staged by the Nonalcoholic Steatohepatitis Clinical Research Network scoring system (Nonalcoholic Steatohepatitis Activity Score – NAS), Kleiner et al, 2005, Tables I and II.

Table I: Activity score

Histologic feature	Grade	Description
Steatosis	0	< 5%
	1	5–33%
	2	33–66%
	3	> 66%
Lobular inflammation	0	None
	1	< 2 foci/200 x field
	2	2–4 foci/200 x field
	3	> 4 foci/200 x field
Ballooning	0	None
	1	Few balloon cells
	2	Many cells/prominent ballooning

Table II: Fibrosis staging

Stage	Histologic criteria
1	Zone 3 perivenular perisinusoidal/pericellular fibrosis, focal or extensive · 1A – delicate perisinusoidal fibrosis · 1B – dense perisinusoidal fibrosis · 1C – portal-only fibrosis
2	As above with focal or extensive periportal fibrosis
3	Bridging fibrosis, focal or extensive
4	Cirrhosis

Degree of steatosis was assessed on a scale of 0–3: 1, mild (5%–33% of hepatocytes affected); 2, moderate (33%–66% of hepatocytes affected); and 3, severe (> 66% of hepatocytes affected). The grades of inflammation and stages of fibrosis were modified to two categories as follows: mild inflammation (Kleiner grade 1) and moderate-to-severe inflammation (Kleiner grades 2 and 3), mild fibrosis (stages 1 and 2), and advanced fibrosis (stages 3 and 4).

Statistical analysis

Hepatic steatosis was assessed as categorical (absent or present) or a continuous variable from 0%–100%. Comparisons between the NAFLD group and controls were made using Student's *t* test for continuous variables and the $\chi 2$ or Fisher's exact probability test for categorical data. All values are presented, as mean ± SD. $P < 0.05$ was considered to be statistically significant. Multiple linear regression has been used in multivariate analysis of factors associated with resistin levels, including age, sex, degree of hepatic steatosis, BMI, C-peptide and IR.

Results

Comparison of patients and controls

Clinical and biochemical characteristics of the patients with NAFLD and controls are compared in Table III. Serum resistin levels were found to be significantly higher in patients with NAFLD as compared with the controls ($P < 0.001$), and higher in levels of insulin and HOMA than the controls. There was no significant difference in serum total cholesterol,

Table III: Clinical and laboratory data of patients with NAFLD and controls

	NAFLD	Control	P-value
n	30	30	
Gender (female/male)	15/15	16/14	0.8
Age (years)	41.96 ± 8.36	43.94 ± 8.35	0.375
BMI (kg/m^2)	26.48 ± 3.11	23.98 ± 1.88	< 0.001
Total cholesterol (mg/dl)	159 ± 32.1	153 ± 25	0.446
Triglyceride (mg/dl)	137 ± 54	116 ± 34	0.075
C-peptide (nmol/L)	1.78 ± 0.29	1.8 ± 0.34	0.505
Glucose (mg/L)	98 ± 17	90 ± 12	0.072
Insulin (mU/L)	14.22 ± 2.66	8.64 ± 1.88	< 0.001
HOMA(IR)	3.46 ± 1.04	1.92 ± 0.45	< 0.001
< 0.001	2.72 ± 0.97	4.5 ± 1.52	Resistin (ng/ml)

triglyceride, C-peptide or glucose levels in patients with NAFLD when compared with the controls.

Table IV: Resistin results according to gender

	Male			Female		
	NAFLD	Control	P	NAFLD	Control	P
n	15	14		15	16	
Resistin (ng/ml)	4.3 ± 1.42	2.3 ± 0.94	< 0.001	5.5 ± 1.57	3.2 ± 0.99	< 0.001

Gender difference in serum resistin

As expected, serum resistin levels were significantly higher in women than in men in the NAFLD group (P = 0.002).

Both men and women in the NAFLD group had higher mean serum resistin levels than did the men and women in the control group (all P < 0.001, Table IV).

Table V: Resistin results in relation to degree of steatosis

	Steatosis (n = 30)		
	Mild	Moderate to severe	P
n	17	13	
Gender (female/male)	9/8	6/7	0.7
HOMA(IR)	2.98 ± 0.86	4.20 ± 0.83	0.001
Resistin (ng/ml)	.72 ± 1.76	6.42 ± 1.64	0.004

Histological evaluation

Upon histological staging, steatosis was found to be mild in 17 patients and moderate to severe in 13; inflammation was mild in 16 and moderate to severe in 14; fibrosis was absent in 9, mild in 20, and advanced in one patient. As summarised in Table V, patients with moderate to severe steatosis had significantly higher levels of resistin than those with mild steatosis. Similarly, patients with moderate to severe steatosis were more insulin resistant, as indicated by higher values of HOMA(IR).

Table VI: The results of BMI and resistin levels in NASH patients with normal weight and those that are overweight

	Overweight	Normal weight	P
n	12	18	
Female/male	8/4	9/9	0.5
HOMA(IR)	4.07 ± 1.05	3.13 ± 0.88	0.01
Resistin (ng/ml)	6.83 ± 1.46	4.52 ± 1.14	< 0.001

Relationship of serum resistin levels with BMI

To determine the relationship between obesity and resistin more clearly, the NAFLD patients were divided into two groups according to mean BMI (26.5) as a cut-off point. Thus, patients with a BMI of 26.5 were defined as being overweight. There were 12 patients with a BMI ≥ 26.5, and there were 18 patients with a BMI < 26.5. The serum resistin levels and HOMA(IR) values were significantly higher in the overweight group than in the normal weight group (P < 0.001 and P = 0.01, respectively) (Table VI).

Discussion

Our data shows that NAFLD patients have increased circulating resistin and that increased levels are correlated with insulin resistance, body mass index and histological severity of the disease. We found higher resistin in patients with histological diagnosis of NASH compared to pure fatty liver. Based on this finding, the present study is in agreement with the hypothesis that resistin may play a role in the progression of the natural history of NAFLD. In other studies, the relationship in humans between resistin and BMI and insulin resistance is under debate. Serum resistin was found by some authors to be increased in cases of obesity and to be positively correlated with BMI or body fat.[19–23] Our hypothesis is partially supported by data reported by Bajaj et al 2004, who showed that serum resistin correlated with hepatic fat content and hepatic insulin resistance, but not with peripheral insulin resistance in type 2 diabetic patients.[24–27] A major target organ of resistin is the liver, where resistin induces insulin resistance and increases glucose production. This concept was supported by experiments in rodents that clearly showed a major role of resistin in hepatic glucose metabolism and in the pathogenesis of diet-induced hepatic insulin resistance.[28,29] The findings of the present study show that resistin is involved in metabolic abnormalities that lead to steatosis in NAFLD patients. The serum resistin level was significantly higher in women. The difference is, therefore, in part, due to the higher percentage of body fat in women. In addition, our study showed that serum resistin levels were strongly correlated with BMI or fat mass, consistent with a number of previous studies.[30,31,32] Also, the above results suggest a significant difference between normal-weight and overweight NAFLD patients with regard to serum resistin levels.

One of the important results of the study was that higher serum resistin levels were detected in patients with moderate to severe steatosis, compared with mild steatosis. There are two most likely explanations for this finding. Firstly, resistin is related to IR. It has been suggested that resistin may contribute to hepatic steatosis by promoting IR and

by altering insulin signalling in hepatocytes, so as to promote increased intracellular levels of fatty acids.[33] Moreover, at a later stage, resistin may cause hepatic steatosis to turn into steatohepatitis by amplifying selected pro-inflammatory responses.[33] This explanation is based mainly on the effect of insulin on hepatic fat metabolism. In this study, an elevation of serum insulin level and HOMA (IR) was detected in NAFLD patients, by comparison with the controls, which supports the latter suggestion. Both IL-6 and TNFα may have a role in NAFLD patients, as many studies prove that their levels improve with lifestyle modification, together with the improvement of liver damage.[34] Therefore it is possible that resistin may participate in the pathways underlying liver damage and the progression of pure fatty liver to NASH and fibrosis.[35] Furthermore, we cannot rule out the possibility that increased resistin in NAFLD patients may come also from increased production in immune and inflammatory cells. As the hepatic stellate cells produce a variety of cytokines, it may be that elevated circulating resistin reflects increased resistin production by these cells inside the liver.[36] This requires further study in order to be proved. The inconsistent link between serum resistin, obesity and insulin resistance may also be explained by genetic factors. It has been reported that a genetic polymorphism in the promoter region of the resistin gene may be an independent predictor of circulating resistin in humans.[37,38,39] Hence it is not possible to exclude that this gene polymorphism may be responsible for high resistin levels in NAFLD patients.

Conclusion

Our study reported higher resistin levels in the serum of NAFLD patients, which were correlated to insulin resistance, adiposity and histological effect on the liver. The precise molecular mechanisms of these findings still need to be clarified. Further prospective studies are needed to prove the predictive value of the serum resistin levels for progression of NAFLD, change in the body fat, and change in insulin resistance.

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