ORIGINAL ARTICLE

The relationship between resistin and ghrelin levels with fibrosis in nonalcoholic fatty liver disease

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Background: Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease. It is generally accepted that insulin resistance is a pathophysiological factor in the development of NAFLD. In the present study, the aim was to determine the relationship between resistin and ghrelin levels, which were found to be closely related to insulin resistance and fibrosis scores in NAFLD. **Materials and Methods**: A total of 40 (21 male, 19 female) NAFLD patients whose diagnosis was confirmed with biopsy and 40 (18 male, 22 female) healthy controls were included in the study. **Results:** In the comparison of resistin and ghrelin levels, only resistin values were found to be significantly higher in NAFLD group while there was no significant difference in ghrelin values (respectively $P < 0.05$; $P = 0.078$). In according to the fibrosis groups there was no difference about fasting plasma glucose, insulin values, Homeostatic Measurement Assessment-Insulin Resistance measurements and also resistin and ghrelin levels. **Conclusion:** It has been understood that insulin resistance plays an important part in NAFLD. Larger studies are required that investigate the gene expression of hormones influencing insulin resistance, particularly resistin and ghrelin in order to determine their role in NAFLD.

Key words: Ghrelin, insulin resistance, nonalcoholic fatty liver disease, resistin

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease. The disease is mostly silent and is often discovered through incidentally elevated liver enzyme levels. NAFLD is a clinical presentation characterized mainly by macrovesicular fat accumulation in cases without alcohol use at doses which are not thought to affect the liver (<20 g/day). The histology of liver may vary from simple fat to steatohepatitis.[1] For NAFLD; obesity, diabetes and hyperlipidemia are the most important risk factors. It is generally accepted that insulin resistance is a pathophysiological factor in the development of NAFLD.[2] In addition, other risk factors are as follows; rapid weight loss (especially after surgical procedure), the presence of gallstones, total parenteral nutrition, protein-calorie malnutrition and the employment of various drugs.[3]

Resistin contains 108 amino acids with a molecular weight of 12.5 kDa. It is considered to be adipocyte-derived mediator of hepatic insulin resistance.[4] Ghrelin is an endogenous ligand for growth hormone secretogog receptor with peptide structure that contains 28 amino acids. Recently, authors suggest that hyperinsulinemia or insulin resistance lead to a reduction in ghrelin concentration.[5]

In the present study, the aim was to determine the relationship between resistin and ghrelin levels, which were found to be closely related to insulin resistance and fibrosis scores in NAFLD.

Study design

This study is a cross-sectional, observational study, and we used simple random sampling method. We studied 40 (21 male) biopsy-proven NAFLD patients and 40 (18 male) healthy control patients at the Gastroenterology Department of the Kocaeli University Hospital between January 2005 and October 2006. Patients with NAFLD included in the patient group who referred with high liver enzyme levels and in whom fatty liver was detected by ultrasonography, and the diagnosis was confirmed with liver biopsy. In both groups, there was no alcohol use or it was at negligible levels (<20 g/day). Patients with

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etiological causes that can lead to high levels of liver enzymes, including (viral and autoimmune hepatitis, Wilson's disease, α-1 antitrypsin deficiency, storage diseases or drug use) and those with malignancy, adrenal or hypophysis disease or undergoing gastrointestinal operation were excluded from the study. After 10 h of fasting; alanine transaminase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total cholesterol, triglyceride (TG), low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, glucose and insulin values were measured. Resistin levels measured by ELISA kit (Phoenix Pharmaceuticals, Inc., CA, USA) (Intra-assay variation: <10%; inter-assay variation: <15%; sensitivity: 0.016 ng/ml). Serum ghrelin levels also measured by EIA kit (Phoenix Pharmaceuticals, Inc.) (Intra-assay variation: <5%; inter-assay variation: <14%; sensitivity: 0.07 ng/ml); Insulin resistance in all patients was calculated using Homeostatic Measurement Assessment-Insulin Resistance (HOMA-IR) value with the formula below:

HOMA-IR: Fasting insulin (μU/ml) × Fasting plasma glucose (mg/dl) /405.

Written informed was obtained from all patients and study protocol was approved by ethics committee of the hospital (2007-6/4).

Liver biopsy was stained with Hematoxylin-Eosin and Mason-Trichrome stain and were examined by an experienced pathologist unaware of the biochemical parameters of the patient. All patients displayed findings of macrovesicular steatosis influencing at least 5% of hepatocytes. Histopathologically, the degree of activity (grade) and fibrosis (stage) was determined using 4 parameters published by Brunt *et al*. [Table 1].[6] NAFLD group was divided into two groups in itself in order to reveal the factors that may be associated with the degree of fibrosis. Those in Stage 0-1 formed one group (Group-1) and in Stage-2 and more another group (Group-2).

Table 1: Activity (grade) and fibrosis (stage) scoring in NASH

Macrovascular fat
Grade 0: Absent
Grade 1: <33%
Grade 2: 33–66%
Grade 3: >66%
Fibrosis (stage)
Stage 1: Zone III perisinusoidal/pericellular fibrosis; focal
Stage 2: Zone III perisinusoidal/pericellular fibrosis; focal or widespread periportal fibrosis
Stage 3: Zone III bridging fibrosis together with perisinusoidal/pericellular fibrosis and portal fibrosis
Stage 4: Cirrhosis

NASH = Nonalcoholic steatohepatitis

Statistics

All analyses were transferred to a computer-based statistical program SPSS 13.0 software (IBM Corp., NY, USA). Results were expressed as mean ± standard error of the mean. For comparing sex we used Chi-square test. Data were divided into parametric and nonparametric ones using Kolmogorov-Smirnov test. In a comparison between two groups, in parametric ones Student's *t*-test and in nonparametric ones Mann-Whitney U-test was used. Correlation between resistin and ghrelin and other data were evaluated using Pearson coefficient of correlation separately. A $P < 0.05$ was considered to be statistically significant.

RESULTS

There was no significant difference between two groups in terms of age and sex (respectively $P = 0.095$ and $P = 0.502$). ALT, AST, and GGT values were found to be significantly higher in NAFLD group ($P < 0.001$). TG, total cholesterol and LDL-cholesterol values were found to be significantly higher in NAFLD group ($P < 0.001$) whereas there was no significant difference in HDL-cholesterol levels ($P = 0.544$). In addition, fasting plasma glucose values and insulin levels were also significantly higher in NAFLD group ($P < 0.001$). Accordingly, HOMA-IR values used to measure insulin resistance were significantly higher in NAFLD group as well ($P < 0.001$).

Because of the possible correlation with insülin resistance, we investigate the comparison of resistin and ghrelin levels with NAFLD. Resistin values were found to be significantly higher in NAFLD group while there was no significant difference in ghrelin values (respectively $P < 0.05$; $P = 0.078$) [Table 2]. Although, in according to the fibrosis groups there was no difference with regard to sex, fasting plasma glucose, insulin values, HOMA-IR measurements and also resistin and ghrelin levels [Table 3].

We found a statistically significant positive correlation between resistin and HOMA-IR ($r = 0.334$, $P < 0.05$) as a result of Pearson's coefficient of correlation test; although, we found no significant correlation between ghrelin and any other biochemical parameter.

DISCUSSION

Insulin resistance associated with obesity is marked by increased hepatic glucose output and decreased glucose utilization in peripheric tissues. On the discovery of leptin, which is a peptide hormone produced from adipocytes and whose lack is established to lead to obesity in rats and humans, endocrine function of fat tissue was acknowledged beyond debate. It is the hormone which regulates adipocyte

Table 2: Sex distribution and biochemical characteristics of the NAFLD and control groups

Test	Group		*P*
	NAFLD (*n* = 40)	Control (*n* = 40)	
Sex (female/male)*	19/21	22/18	0.502
ALT (IU/L)**	92.2±8.12	21.3±2.13	<0.001
AST (IU/L)**	51.5±7.54	20.2±1.23	<0.001
GGT (IU/L)***	73.6±11.34	27.4±3.54	<0.001
Glucose (mg/dl)**	112.4±5.78	91.3±2.56	<0.001
Total cholesterol (mg/dl)***	211.6±6.45	185.4±6.34	<0.05
TG (mg/dl)***	157.5±9.43	108.5±8.67	<0.001
LDL - cholesterol (mg/dl)**	135.7±5.68	113.5±6.65	<0.05
HDL - cholesterol (mg/dl)**	46.5±2.76	47.6±2.87	0.544
İnsulin (μU/L)***	20.4±2.9	9.0±0.8	<0.001
HOMA-IR**	5.81±0.88	2.04±0.21	<0.001
Resistin (ng/ml)**	0.55±0.04	0.41±0.02	<0.05
Ghrelin (ng/ml)**	8.29±1.51	5.35±0.43	0.207

*Chi-square test; **Student's *t*-test; ***Mann–Whitney U-test; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; GGT = Gamma glutamyltransferase; NAFLD = Nonalcoholic fatty liver disease; TG = Triglyceride; LDL = Low-density lipoprotein; HDL = High-density lipoprotein; HOMA-IR = Homeostatic model assessment-insulin resistance

Table 3: Sex distribution and biochemical characteristics of the groups according to degree of fibrosis

Test	Stage		*P*
	Group-1 (stage 0-1) *n* = 17	Group-2 (≥stage 2) *n* = 23	
Sex (female/male)*	11/6	8/15	0.061
ALT (IU/L)***	75.3±4.23	105.4±12.52	0.101
AST (IU/L)**	39.5±4.56	61.5±11.75	0.232
GGT (IU/L)***	58.6±6.75	83.8±18.45	0.277
Glucose (mg/dl)**	109.6±5.86	115.7±8.45	0.808
Total cholesterol (mg/dl)***	212.5±11.67	210.8±8.65	0.878
TG (mg/dl)***	165.8±14.95	151.8±12.35	0.401
LDL - cholesterol (mg/dl)**	135.8±9.76	134.3±6.58	0.914
HDL - cholesterol (mg/dl)***	48.5±3.75	45.6±2.45	0.314
Insulin (μU/L)***	16.8±2.0	23.1±4.8	0.665
HOMA-IR**	4.72±0.78	6.62±1.41	0.645
Resistin (ng/ml)**	0.58±0.05	0.53±0.05	0.191
Ghrelin (ng/ml)**	7.74±1.07	8.69±2.53	0.191

*Chi-square test; **Student's *t*-test; ***Mann–Whitney U-test; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; GGT = Gamma glutamyltransferase; NAFLD = Nonalcoholic fatty liver disease; TG = Triglyceride; LDL = Low-density lipoprotein; HDL = High-density lipoprotein; HOMA-IR = Homeostatic model assessment-insulin resistance

differentiation by suppressing fat tissue formation in response to increased energy intake, via negative feedback mechanism.

Serum resistin levels are directly proportional to body fat mass. It has been established that intraperitoneal injections decrease glucose tolerance in target cells in mice and pressurize sensitivity to insulin. In this context, it is thought that it acts as an insulin antagonist affecting glucose metabolism. In human studies, serum resistin levels were found to be high in obesity or other diseases associated with insulin resistance, yet no definite relation could be established between serum resistin levels and insulin resistance. High resistin levels detected in patients with NAFLD, were not found to be related to insulin resistance or body mass index (BMI), but when the expression of resistin mRNA was examined, it was found to be related to the degree of histological damage.[7] In a study that included 97 NAFLD patients, they suggested that resistin levels are elevated in patients with NAFLD and could discriminate simple steatosis from definite steatohepatitis.[8] High resistin levels have been reported in Prader–Willi syndrome characterized by varying degrees of obesity and high rates of NAFLD.[9] However, a little study showed no significant difference between the levels of resistin with NAFLD group compared by control group.[10] In our study, resistin levels were found to be significantly higher in NAFLD group than in the control group. However, no relationship was found between resistin levels and fibrosis score in NAFLD.

Ghrelin is the first circulating hormone associated with food intake and energy balance which is established to increase nutrition following systemic administration.[11] It is basically released from the stomach with a different endocrine cell type and at some degree from pancreas, hypothalamus and other organs. In fasting condition ghrelin level is increased. Its levels were found to be high in anorexia nervosa and low in obesity. It has attracted attention that through which mechanisms a hormone released from geographic information system is sensitive to body energy stores and can respond to changes. In this context, it was thought that a fat tissue signal in circulation may be responsible for ghrelin production. Insulin was revealed to be a strong candidate to play the role of this signal (especially in obese individuals insulin resistance is concurrent with hyperinsulinemia). The stimulation of ghrelin production in the stomach is associated with glucose and insulin metabolism. Due to the relation between weight gain and hyperinsulinemia and insulin resistance, it was thought that ghrelin may influence these two conditions. In a study of 40 patients with equal BMI divided into two groups, namely insulin resistant and insulin sensitive, the relation between insulin resistance, hyperinsulinemia and ghrelin was investigated and mean ghrelin level was found to be lower in the insulin resistant group, which was independent of BMI.[5] Marchesini *et al*. found lower ghrelin levels in their NAFLD patients than control group and attributed this to insulin resistance.[12] In another study comparing 37 patients with NAFLD with control patients, ghrelin levels were found to be lower in NAFLD group (independent of BMI and glucose levels). However, no relation could be found between ghrelin levels and histopathological activity and fibrosis. They concluded that ghrelin could play a part in the pathogenesis of NAFLD but

was not related to the severity of damage.[13] Furthermore, a little study showed a nonsignificant trend toward higher ghrelin expression in patients with steatohepatitis, simple steatosis and normal liver.[14] In another study, the authors suggested that an imbalance in adiponectin, leptin, and ghrelin seems to be associated with more severe NAFLD.[15] In our study, no significant difference was found between two groups in terms of ghrelin levels. Similarly, there was not any statistically significant difference between groups divided according to the degree of fibrosis.

The lack of the number of the patients is the main limitation of our study. It may be able to achieve a more powerful and valuable results with more number of patients.

CONCLUSION

It has been understood that insulin resistance plays an important part in NAFLD. Larger studies are required that investigate the gene expression of hormones influencing insulin resistance, particularly resistin and ghrelin in order to determine their role in NAFLD. At present, the relation between NAFLD, whose pathogenesis, prognosis and treatment are intensively investigate, and proinflammatory molecules in fat tissue and other regions is a very popular issue for investigators. Increasing information on this subject and probable discovery of molecules controlling the production of proinflammatory cytokines, are promising for the management approaches in the future.

AUTHOR'S CONTRIBUTION

ATE contributed in the conception of the work, design of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. OS contributed in the conception of the work, design of the work, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. CA contributed in the conception of the work, conducting the study, approval of the final version of the manuscript, and agreed for all aspects of the work. TK contributed in the conception of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. OK contributed in the conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AC contributed in the conducting the study, approval of the final version of the manuscript, and agreed for all aspects of the work. YG contributed in the conception of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. SH contributed in the design of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

REFERENCES

1. Ramesh S, Sanyal AJ. Evaluation and management of non-alcoholic steatohepatitis. J Hepatol 2005;42 Suppl:S2-12.
2. McCullough AJ. Update on nonalcoholic fatty liver disease. J Clin Gastroenterol 2002;34:255-62.
3. Feldman M, Friedman LS, Sleisenger MH. Gastrointestinal and Liver Disease. 8th ed., Vol. 2. Philadelphia: WB Saunders Company; 2006. p. 1793.
4. Adeghate E. An update on the biology and physiology of resistin. Cell Mol Life Sci 2004;61:2485-96.
5. McLaughlin T, Abbasi F, Lamendola C, Frayo RS, Cummings DE. Plasma ghrelin concentrations are decreased in insulin-resistant obese adults relative to equally obese insulin-sensitive controls. J Clin Endocrinol Metab 2004;89:1630-5.
6. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: A proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467-74.
7. Pagano C, Soardo G, Pilon C, Milocco C, Basan L, Milan G, *et al.* Increased serum resistin in nonalcoholic fatty liver disease is related to liver disease severity and not to insulin resistance. J Clin Endocrinol Metab 2006;91:1081-6.
8. Senates E, Colak Y, Yesil A, Coskunpinar E, Sahin O, Kahraman OT, *et al.* Circulating resistin is elevated in patients with non-alcoholic fatty liver disease and is associated with steatosis, portal inflammation, insulin resistance and nonalcoholic steatohepatitis scores. Minerva Med 2012;103:369-76.
9. Pagano C, Marin O, Calcagno A, Schiappelli P, Pilon C, Milan G, *et al.* Increased serum resistin in adults with Prader-Willi syndrome is related to obesity and not to insulin resistance. J Clin Endocrinol Metab 2005;90:4335-40.
10. Krawczyk K, Szczesniak P, Kumor A, Jasinska A, Omulecka A, Pietruczuk M, *et al.* Adipohormones as prognostric markers in patients with nonalcoholic steatohepatitis (NASH). J Physiol Pharmacol 2009;60 Suppl 3:71-5.
11. Hosoda H, Kojima M, Kangawa K. Biological, physiological, and pharmacological aspects of ghrelin. J Pharmacol Sci 2006;100:398-410.
12. Marchesini G, Pagotto U, Bugianesi E, De Iasio R, Manini R, Vanni E, *et al.* Low ghrelin concentrations in nonalcoholic fatty liver disease are related to insulin resistance. J Clin Endocrinol Metab 2003;88:5674-9.
13. Yalniz M, Bahcecioglu IH, Ataseven H, Ustundag B, Ilhan F, Poyrazoglu OK, *et al.* Serum adipokine and ghrelin levels in nonalcoholic steatohepatitis. Mediators Inflamm 2006;2006:34295.
14. Uribe M, Zamora-Valdés D, Moreno-Portillo M, Bermejo-Martínez L, Pichardo-Bahena R, Baptista-González HA, *et al.* Hepatic expression of ghrelin and adiponectin and their receptors in patients with nonalcoholic fatty liver disease. Ann Hepatol 2008;7:67-71.
15. Machado MV, Coutinho J, Carepa F, Costa A, Proença H, Cortez-Pinto H. How adiponectin, leptin, and ghrelin orchestrate together and correlate with the severity of nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol 2012;24:1166-72.

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