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• *BRIEF REPORTS* •

Is adiponectin level a predictor of nonalcoholic fatty liver disease in nondiabetic male patients?

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Abstract

AIM: To study the levels of adiponectin in nondiabetic patients with nonalcoholic fatty liver disease (NAFLD) in comparison with control group.

METHODS: Thirty-five patients who had elevated serum aminotransferase levels with bright liver and 34 healthy volunteers without liver disease were evaluated. Age, gender and body mass index (BMI) were recorded. Fasting plasma glucose, insulin, adiponectin, proinsulin and lipid profile were measured. A standard oral glucose tolerance test (OGTT) with insulin response was performed and the index of insulin resistance was calculated according to the homeostasis model assessment (HOMA) method.

RESULTS: According to the OGTT results, none of the participants had diabetes. Serum adiponectin levels were statistically significantly lower in patients with NAFLD than in control group (8.14±3.4 μg/mL *vs* 12.4±9.4 μg/mL, respectively, $P<0.01$). A statistically significant correlation was found between adiponectin and BMI (r: -0.33, $P<0.01$), HOMA (r: -0.26, $P<0.05$), proinsulin (r: -0.32, $P<0.01$), AST (r: -0.25, $P<0.05$), ALT (r: -0.26, $P<0.05$) or GGT (r: -0.22, $P<0.05$). In multiple regression analysis models, adiponectin levels were the only predictor of NAFLD in males, whereas in female group it was the BMI.

CONCLUSION: Low adiponectin level might be a predictor of NAFLD especially in male nondiabetics.

Key words: Nonalcoholic fatty liver disease; Adiponectin; Gender

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INTRODUCTION

The growing epidemic of obesity has led to many studies on the role of adipose tissue as an endocrine organ that secretes many factors termed as adipokines, which mediate many of the vascular and metabolic complications of adiposity. These products, namely free fatty acids, TNF-α, interleukins, resistin, and leptin reduce insulin sensitivity[1-3].

Adiponectin is a plasma protein, which is secreted from adipose tissue, and serum levels are markedly reduced in obesity. Adiponectin levels are negatively correlated with body fat percent, central fat distribution, fasting plasma insulin, oral glucose tolerance and positively with glucose disposal during euglycemic insulin clamp[3-7]. It has two receptors; adipoR1 is abundantly expressed in skeletal muscle and at moderate levels in other tissues, whereas adipoR2 is predominantly expressed in the liver[8-10].

Adiponectin is a hepatic insulin sensitizer and also an inhibitor of tumor necrosis factor and therefore we studied its levels in nondiabetic patients with nonalcoholic fatty liver disease (NAFLD) and compared the results with healthy volunteers and looked for the effect of gender on adiponectin levels.

MATERIALS AND METHODS

Thirty-five patients admitted to the Department of Gastroenterology at Kartal Education and Research Hospital with elevated serum aminotransferase levels for longer than 6 mo and bright liver on ultrasound scan were included in this study. Thirty-four healthy volunteers without liver disease, who were gender and age matched with study group, were enrolled as the control group. Patients were excluded from this cohort if one of the following criteria was present: hepatitis B (hepatitis B surface antigen, antibody to hepatitis surface antigen, antibody to hepatitis B core antigen), hepatitis C (antibody to hepatitis C virus) and Epstein-Barr virus infection, non-organ specific autoantibodies (antimitochondrial antibody, antinuclear antibody, antismooth muscle antibody, and anti-liver/kidney microsomal antibody), hereditary defects (fasting serum iron, transferrin saturation, ferritin, ceruloplasmin, alpha-1-antitripsin levels), alcohol consumption (ethanol ingestion >20 g/d), use of amiodarone, corticosteroids, tamoxifen, methotrexate, or oral contraceptives, jejunoileal bypass or extensive small bowel resection, total parenteral nutrition, malignancy, hypo-hyperthyroid disease, pregnancy, other known liver diseases like cirrhosis and diabetes. The diagnosis of cirrhosis was based on the clinical and laboratory findings (hypoalbuminemia, prolongation of prothrombin time, hyperbilirubinemia, presence of ascites or other findings of portal hypertension). The absence of

diabetes was confirmed with oral glucose tolerance test (OGTT) in both groups.

The absence of liver disease in the control group was established based on clinical, laboratory and imaging criteria. All controls had normal liver biochemistries and lack of any evidence from physical examination of chronical liver disease. However, an abdominal ultrasound was performed to exclude bright liver in all of the controls. All subjects gave informed consent to take part in the study.

Age, gender, height, weight, and body mass index (BMI) were recorded. Fasting plasma glucose, insulin, proinsulin, lipid profile, uric acid, and serum proteins were measured. Adiponectin levels were measured by Adiponectin Human ELISA Kit. A standard 75 g OGTT with insulin response according to WHO criteria was performed on all patients.

The index of insulin resistance (IR) was calculated on the basis of fasting values of plasma glucose and insulin, according to the homeostasis model assessment (HOMA) method.

Ultrasound liver studies were carried out by the same experienced radiologist who was blinded to laboratory values. The diagnosis of bright liver was based on abnormally intense, high-level echoes arising from the hepatic parenchyma, with amplitude similar to that of echoes arising from the diaphragm.

Statistical analysis

Statistical analysis was performed with SPSS 11.5 program. Any *P* value less than 0.05 was considered statistically significant. Results are expressed as mean±SD. Comparison between the two groups was made with Student's *t*-test and Mann-Whitney *U* test. Pearson's and Spearman's rank correlations were used to detect the associations between serum adiponectin and various demographic, anthropometric, and metabolic variables. When necessary, multiple regression analysis was performed.

RESULTS

Demographic, anthropometric, metabolic, and laboratory characteristics of patients with NAFLD and their matched controls are shown in Table 1.

According to the OGTT results, none of the participants had diabetes. However, six patients in NAFLD group and three patients in control group had impaired glucose tolerance ($P>0.05$). The remaining had normal glucose tolerance.

Insulin (fasting, 60 and 120 min), HOMA, proinsulin and c-peptide levels were statistically significantly higher in NAFLD group than in control group (Table 2).

Serum adiponectin levels were statistically significantly lower in patients with NAFLD than in the control group (8.14±3.4 μg/mL *vs* 12.41±9.4 μg/mL, respectively, $P<0.01$; Table 2). A statistically significant correlation was found between adiponectin and BMI (r: -0.33, $P<0.01$), HOMA (r: -0.26, $P<0.05$), proinsulin (r: -0.32, $P<0.01$), AST (r: -0.25, $P<0.05$), ALT (r: -0.26, $P<0.05$) or GGT (r: -0.22, $P<0.05$). In multiple regressions analysis, gender was found to be a predictor of adiponectin but not the age and BMI (Table 3).

When we subclassified the patient and the control group according to gender, the adiponectin levels were found to be low in males compared to females ($P<0.05$). But there was no statistically significant difference in age, BMI, HOMA, and proinsulin between the subgroups of gender ($P>0.05$, Table 4). In multiple regression analysis, adiponectin levels were the only predictor of NAFLD in males (Table 5), whereas in female group it was the BMI (Table 6).

Table 1 Demographic, anthropometric, and biochemical measurements in NAFLD and control groups

	NAFLD (*n*: 35)	Controls (*n*: 34)	*P*
Age (yr)	39.1±7.8	36.1±6.0	NS
Gender (female/male)	20/15	20/14	NS
BMI (kg/m^2)	30.5±4.1	23.7±3.8	<0.001
Fasting glucose (mg/dL)	93±9	84±11	<0.01
Total cholesterol (mg/dL)	209±35	186±36	<0.01
HDL cholesterol (mg/dL)	50±12	51±12	NS
LDL cholesterol (mg/dL)	125±28	118±37	NS
Triglycerides (mg/dL)	177±100	103±57	<0.01
AST (IU/L)	36±10	18±5	<0.001
ALT (IU/L)	49±17	18±9	<0.001
GGT (IU/L)	43±16	16±8	<0.001

NS, nonsignificant.

Table 2 Comparison of IR parameters in NAFLD and control groups

	NAFLD (*n*: 35)	Controls (*n*: 34)	*P*
Fasting insulin (IU/L)	14.6±6.7	9.9±4.5	<0.01
Insulin 60 min (IU/L)	99.6±63.1	62.4±43.2	<0.01
Insulin 120 min (IU/L)	65.3±49	40.2±31.9	<0.01
HOMA index	3.3±1.5	2.1±1.1	<0.001
Proinsulin (pmol/L)	10.6±6.4	6.2±4.5	<0.001
C-peptide (ng/mL)	2.7±0.7	2.0±0.6	<0.001
Adiponectin (μg/mL)	8.14±3.4	12.41±9.4	<0.01

Table 3 Regression model of adiponectin as a dependent variable

Independent variables	*T*	Significance
Gender (male)	–2.791	0.009
Age (yr)	0.774	NS
BMI (kg/m^2)	–0.523	NS

NS, nonsignificant.

Table 4 Comparison of male and female patients in both NAFLD and control groups

	Female (*n:* 40)	Male (*n*: 29)	*P*
Age (yr)	37.0±7.1	38.6±7.1	NS
BMI (kg/m^2)	27.5±5.8	26.9±4.5	NS
HOMA	2.6±1.4	2.8±1.4	NS
Adiponectin (μg/mL)	11.7±8.9	8.2±3.3	0.025

NS, nonsignificant.

Table 5 Regression model of NAFLD as a dependent variable in

males

Independent variables	*B*	Exp (*B*)	Significance
Adiponectin (μg/mL)	0.466	1.593	0.045
Age (yr)	–0.086	0.918	NS
BMI (kg/m^2)	–0.237	0.789	NS
HOMA	–0.068	0.934	NS

NS, nonsignificant.

Table 6 Regression model of NAFLD as a dependent variable in females

Independent variables	*B*	Exp (*B*)	Significance
BMI (kg/m^2)	–0.505	0.972	0.007
Age (yr)	–0.028	0.604	NS
HOMA	–0.097	0.908	NS
Adiponectin (μg/mL)	0.003	1.003	NS

NS, nonsignificant.

DISCUSSION

Cytokines are mediators of cellular communication produced by multiple liver cell types such as Kupffer cells, stellate cells, hepatocytes and endothelial cells. Cytokines can directly induce necrosis or apoptosis. Increased levels of hepatotoxic cytokines such as tumor necrosis factor-α are documented in alcoholic liver disease and nonalcoholic steatohepatitis and have been shown to play a mechanistic role in both of these processes. There are also beneficial cytokines, such as interleukin (IL-10 and IL-6). Adiponectin, one of the beneficial cytokines, is made outside the liver and appear to protect against liver damage[11-13]. Adiponectin is an important adipokine specifically secreted by adipocytes that circulates at relatively high levels in the bloodstream[1]. Plasma levels of adiponectin are reduced in obese rodents and humans, as well as in humans with type 2 diabetes. It has been suggested that adiponectin might function as an adipostat in regulating energy balance and that its deficiency might contribute to the development of obesity and type 2 diabetes[14,15].

It has been shown that increased hepatic fat content is associated with hepatic IR in type 2 diabetic patients and IR was found in 85% of these patients by Willner *et al*.[16]. Insulin resistance and systemic hypertension features of the metabolic syndrome are also independently associated with advanced forms of NAFLD[17,18]. In our study, hyperinsulinemia was present in all the patients and a high value of HOMA index in NAFLD patients is present in comparison to control group which is an index of IR.

The euglycemic-hyperinsulinemic clamp is accepted as the gold standard in defining the IR, but it is difficult to perform and time consuming and can be used only in studies with limited number of patients. Therefore, we preferred to use HOMA, which is easy to perform and it has been shown that it is in correlation with the euglycemic-hyperinsulinemic clamp (*R*: 0.88, *P*<0.0001; *R*: 0.85, *P*<0.0001 and *R*: 0.73, *P*<0.0001). A literature search using Medline found that the use of the HOMA model has been reported in 572 published works. In >50% of reports, the model is used in nondiabetic populations[19-21].

For the diagnosis of NAFLD, we used the exclusion of known etiological factors, which are responsible for the liver disease and ultrasound examination. Liver biopsy was not done because the stage and grade of the NAFLD was not of importance in this study and according to Saverymuttu *et al*., ultrasound examinations can accurately identify steatosis with a sensitivity of 94% and a specificity of 84%[22]. Ricci *et al*., also demonstrated that standard ultrasonography may be used for the diagnosis of NAFLD[23].

In NAFLD, most of the liver damage in insulin-resistant and dyslipidemic patients is thought to be caused by accumulation of hepatic triglycerides, and adiponectin might be able to preserve liver function by preventing lipid accumulation in hepatocytes. Adiponectin is also a potent insulin sensitizer and modulates the inflammatory response[2,17,24-26]. In our study, low adiponectin levels in NAFLD patients are compatible with previous studies.

Adiponectin was found to circulate in inverse proportion to IR syndrome such as BMI, fasting glucose and triglycerides[15,25,27,28]. In our study, we also found an inverse correlation between adiponectin levels with BMI, insulin, HOMA, proinsulin and triglycerides.

A recent study showed that adiponectin levels are correlated in healthy humans with various liver function tests such as ALT and GGT[2]. We also found a statistically significant correlation between adiponectin and liver function tests like AST, ALT, and GGT.

This is the first study looking for adiponectin levels in nondiabetic NAFLD patients. Bajaj *et al*., demonstrated a relationship between plasma adiponectin levels with hepatic insulin sensitivity and hepatic fat content in patients with type 2 diabetes, for the first time[14]. Yamamoto *et al*., have reported that adiponectin predicts future IR in a Japanese population in a 2-year follow-up study[4].

In our study, it is remarkable that in males, NAFLD is definitely correlated with low adiponectin levels but the female gender did not show such a correlation. This gender predilection might be due to the correlation of low adiponectin with visceral adiposity in females.

As a summary, adiponectin level is lower in nondiabetic patients with NAFLD in comparison to healthy volunteers. Low adiponectin level might be a predictor of NAFLD especially in male nondiabetics.

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