Original Article

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Levels of circulating adipokines and their relation with glycemic control and insulin resistance in Saudi patients with non-alcoholic fatty liver disease

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a prevalent condition associated with obesity and insulin resistance (IR). Adipokines include fat-secreted proteins such as leptin or adiponectin and fat- or liver-derived cytokines such as tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) are considered to play an important role in the pathogenesis of the metabolic syndrome, including NAFLD.

Objective: The present study aimed to explore the role of adipokines in the pathogenesis of NAFLD and correlate them with glucose control.

Material and Methods: One hundred Saudi patients with NAFLD (45 males and 55 females) with NAFLD diagnosed by ultrasonographic findings, our group includes one gender, age and body mass index (BMI) matched hundred healthy volunteers. Adiopkines and parameters of glucose control of all participants were detected.

Results: Serum glucose, insulin, aspartate aminotransferase(AST), alanine aminotransferase (ALT), aspartate aminotransferase /alanine aminotransferase ratio (AST/ALT), serum levels of total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), serum TNF-α and L-6 levels were significantly higher in patients with NAFLD when compared to control group. However, serum high density lipoprotein cholesterol (HDL-C) and adiponectin levels were significantly lower in patients with NAFLD when compared to control group. Moreover, serum levels of adipokines showed an association with insulin resistance.

Conclusion: Within the limit of this study non-alcoholic fatty liver disease is associated with adipokines alteration that is correlated with abnormal glucose control and insulin resistance.

Keywords: adipokines, insulin resistance, non-alcoholic fatty liver disease

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease, affecting 20-40% of the general population in the Western countries (1). The increasing prevalence of NAFLD parallels the rise of obesity and its complications as NAFLD occurs in 60%~95% of people with obesity (2). However, obesity, insulin resistance (IR) and metabolic syndrome are the major risk factors associated with NAFLD pathophysiology (3). IR appears plays the key role in the genesis of NAFLD, suggesting a possible interplay among IR, atherosclerosis and NAFLD (4).

Adiponectin is an adipose tissue derived cytokine playing an important role in the regulation of glucose homeostasis and insulin sensitivity (5). Previous studies have shown that adiponectin improves insulin sensitivity and possesses anti-inflammatory and anti-atherosclerotic properties (6). Lower levels of adiponectin might represent an independent risk factor for NAFLD and has been associated with IR and NAFLD (7).

Obesity is associated with high circulating serum leptin levels which adversely affects glucose homeostasis (8). Initial studies demonstrated an association of leptin levels with NASH and the degree of liver steatosis and inflammation (9). Therefore, the role of leptin in NAFLD is still unclear

Resistin is primarily secreted by monocytes or macrophages in humans (10). The link between resistin and NAFLD remains under debate in humans. It is accepted that obesity, particularly central obesity is a risk factor strongly associated with NAFLD (11). Moreover, accumulating evidence indicates that resistin has pro-inflammatory properties. Resistin enhances interleukin 6 (IL-6) and TNF-α production, both are increased in NAFLD patients (12).

The aim of the present study is to explore the role of adipokines in the pathogenesis of NAFLD and correlate them with glucose metabolism.

SUBJECTS AND METHODS

Subjects

One hundred patients with NAFLD with body mass index (BMI) ranged from 30 to 35 Kg/m^2, their age ranged from 35 to 55 years were identified from a large number of patients attending the Liver Clinic in King Abdulaziz University Teaching Hospital. Control group included one hundred healthy volunteers, who were gender, age and BMI matched with study group, were enrolled. NAFLD diagnosis was based according to the standard criteria accepted by the American Gastroenterology Association (13). The diagnosis was based on ultrasonograhpic finding of bright liver (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); In all patients, liver biopsy could not be done because the stage and grade of the NAFLD was not of importance in this study. Otherwise, according to Saverymuttu *et al*., ultrasound examinations can accurately identify steatosis with a sensitivity of 94% and a specificity of 84% (14).

We excluded patients with; hepatitis B and hepatitis C infection and other known liver diseases like cirrhosis. We also excluded patients with diabetes, hypertension, malignancy, hypohyperthyroid disease, coronary artery disease, pregnancy,

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Table 1: *Demographic and anthropometric characteristics of NAFLD patients and control subjects*

	NAFLD (no= 100)	Healthy control (no= 100)	P- value
Age (year)	43.83 ± 6.12	45.16 ± 5.97	0.54
Gender (F/M)	55/45	57/43	0.62
BMI (kg/m^2)	31.5 ± 5.16	30.94 ± 4.87	0.31
Hip circumference (cm)	121.31 ± 13.94	119.21 ± 13.52	0.17
Waist circumference (cm)	106.46 ± 11.27	104.83 ± 10.78	0.28
waist hip ratio	0.933 ± 0.036	0.915 ± 0.029	0.54

BMI: Body Mass Index

alcohol consumption, cigarette smoking, use of amiodarone, corticosteroids, tamoxifen, methotrexate, or oral contraceptives.

This study was approved by the Scientific Research Ethical Committee, Faculty of Applied Sciences, King Abdulaziz University. Informed consent was obtained from all participants. All participants were free to withdraw from the study at any time.

All persons included in the study were subjected to detailed history taking, complete clinical examination, anthropometric evaluation (height, weight, and body mass index "BMI" were recorded. Overweight and obesity were defined as BMI between 25 and 30 kg/m^2 (<30) and ≥ 30 kg/m^2, respectively (15).

Laboratory Investigation

After a 10 hours overnight fast, venous blood samples were drawn to determine levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, glucose, cholesterol, low-density lipoprotein, high-density lipoprotein (HDL), triglycerides, insulin, leptin, adiponectin, TNF-α and IL-6. Glucose was measured on the Hitachi 912 Chemistry Analyzer using the hexokinase reagent from Boehringer Mannheim (Indianapolis, IN 46256). Serum levels of leptin were measured with DRG leptin ELISA Catalog number EIA-2395, supplied by DRG instruments GmbH, Germany. Serum levels of adiponectin were determined using AviBion human adiponectin (Acrp 30) ELISA kit ref. no. ADIPO 25 (Orgenium Laboratories, Finland). Serum levels of resistin was measured by ELISA using commercially available kits (resistin: Rapidbio, West Hills, CA, USA; CK-18: PEVIVA, Alexis, Grunwald, Germany) according to the manufacturer's instructions. Human insulin was measured with an insulin kit (Roche Diagnostics, Indianapolis, IN, USA) using a cobas immunoassay analyzer (Roche Diagnostics). Insulin resistance was assessed by homeostasis model assessment (HOMA-IR), it was computed with the formula:

fasting plasma glucose (mmol/l) times fasting serum insulin (mU/l) divided by 22.5 (16). However, insulin sensitivity was assessed by The quantitative insulin-sensitivity check index (QUICKI) using the formula: QUICKI=1/[log(insulin) + log(glucose)] (17). All serum samples were analyzed in duplicates.

Statistical Analysis

Independent t-test was used to compare mean differences between both groups. Statistical analysis of data was performed using SPSS (Chicago, IL, USA) version 17. The degree of correlation between adiopkines and parameters of glucose control in NAFLD patients was detected by Pearson's product moment correlation coefficients (r). All data were expressed as the mean ± SD. P<0.05 indicated statistical significance.

RESULTS

One hundred NAFLD Saudi subjects were enrolled including 55 women and 45 men, had age ranged from 30 to 56 years and one hundred healthy subjects had age ranged from 31 to 58 years, there was no significant differences in demographic and anthropometric characteristics between both groups (Table 1).

Table 2: *Mean value and significance of biochemical parameters of NAFLD and control subjects*

	NAFLD	Healthy control	P- value
Fasting glucose(mg/dl)	145.43 ± 26.14 *	97.61 ± 18.56	0.015
Insulin (mU/l)	16.52 ± 4.17*	8.31 ± 2.98	0.001
QUICKI	0.121 ± 0.016*	0.185 ± 0.024	0.025
HOMA-IR	5.28 ± 1.95*	2.73 ± 1.13	0.003
AST (IU)	68.22 ± 11.26*	34.75 ± 7.25	0.007
ALT (IU)	57.17 ± 9.53*	38.11 ± 5.42	0.024
AST/ALT	1.19 ± 0.98*	0.92 ± 0.67	0.018
Total cholesterol (mg/dl)	193.35 ± 42.22*	116.24 ± 31.46	0.006
HDL-C (mg/dl)	34.23 ± 8.41*	53.28 ± 10.17	0.015
LDL-C (mg/dl)	127.15 ± 26.18*	92.91 ± 18.43	0.023
Triglycerides (mg/dl)	161.27 ± 31.15*	95.16 ± 20.31	0.017
Leptin (ng/ml)	22.16 ± 3.93	15.82 ± 3.16	0.084
Adiponectin (µg/ml)	4.18 ± 1.79*	8.12 ± 2.53	0.005
Leptin/adiponectin ratio	5.21 ± 1.98*	1.93 ± 1.21	0.012
Resistin (ng/mL)	16.83 ± 4.52*	13.17 ± 4.11	0.025
TNF-α (pg/mL)	6.12 ± 1.91*	3.64 ± 1.17	0.016
IL-6 (pg/mL)	3.46 ± 1.52*	1.81 ± 1.14	0.004

HDL-c: High density lipoprotein cholesterol , **LDL-c:** Low density lipoprotein cholesterol, **AST:** Aspartate aminotransferase ALT: alanine aminotransferase, **AST/ALT:** Aspartate aminotransferase /alanine aminotransferase ratio, **HOMA-IR :** Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index, **QUICKI :** The quantitative insulin-sensitivity check index, **TNF- α :** tumor necrosis factor – alpha, **IL-6:** Interleukin-6, (*) indicates a significant difference between the two groups, P < 0.05.

Table 3: *Correlation coefficient (r) of adiopkines and parameters of glucose control in NAFLD patients*

	Insulin (mU/l)	**QUICKI** (%)	**HOMA-IR** (%)
Leptin (ng/ml)	0.611*	-0.725**	0.512*
Adiponectin (µg/ml)	0.742 **-	0.661**	0.843**-
Resistin (ng/mL)	0.526*	0.672*-	0.721**
TNF-α (pg/mL)	0.728 **	-0.593 *	0.621*
IL-6 (pg/mL)	0.531*	-0.684**	0.513 *

Spearman's correlation was used *: P < 0.05 **: P < 0.01

NAFLD patients were more insulin resistant as indicated by significantly higher values of fasting glucose, insulin and HOMA-IR and lower values of QUICKI, with no significant differences in leptin concentration between patients and controls. Also, NAFLD patients showed significantly higher resistin level, aspartate aminotransferase(AST), alanine aminotransferase (ALT), aspartate aminotransferase /alanine aminotransferase ratio (AST/ALT), serum levels of total cholesterol, triglycerides, serum high density lipoprotein cholesterol (HDL-C) and adiponectin levels in comparison to controls (Table 3).

Table 3 summarizes the relationship between adipokines and parameters of glucose control in NAFLD patients. Serum levels of leptin, resistin, TNF- α and IL-6 showed an inverse relationship with QUICKI and a direct relationship with serum insulin, HOM-IR. However, levels of adiponectin showed a direct relationship with QUICKI and an inverse relationship with serum insulin, HOM-IR (Table 3)

DISCUSSION

It is believed that IR is related to adipose tissue plays a key role in the development of NAFLD (18). Our study underscores that NAFLD is associated with IR and adipokines alterations. Though NAFLD patients had raised ALT suggestive of some hepatocellular injury. In our study, NAFLD patients showed significantly higher serum glucose, insulin, HOMA-IR, resistin level, AST, ALT, AST/ALT ratio, lipid profile, serum TNF-α levels, serum IL-6 levels and significantly lower values of QUICKI, serum HDL-C and adiponectin levels in comparison to controls.

A recent study conducted by Sanches and colleagues proved that patients with IR have 65% greater risk of developing NAFLD. Because they found that obese patients with NAFLD presented greater baseline HOMA-IR values and insulin concentration than their peers without NAFLD (19). Willner et al. reported a strong relation between NAFLD and IR, showing that about 80% of patients with NAFLD have IR (20).

The present study revealed that serum levels of adiponectin were significantly lower in NAFLD patients in comparison to

those of the controls. Also, our study showed that adiponectin levels were also associated with increased IR in NAFLD patients. A number of studies have demonstrated that reduced systemic levels of adiponectin have clinically significant effects and associate closely with obesity-related diseases, including NAFLD (21, 22). Also, in the study by Hu et al., these authors found that adiponectin expression decreases both in obese patients and genetically obese mice (23).

Our study confirmed a previous study found that leptin higher in NAFLD children as well as in obese control subjects (24) and an earlier clinical trial reported significantly higher serum leptin levels in patients with NASH as compared to controls (25). The increases in leptin levels in NASH is not explained by obesity alone but is also due to peripheral leptin resistance. In NASH leptin receptors become resistant to its effect leading to hyperleptinemia which alters insulin signaling and promotes accumulation of intracellular fatty acids in hepatocytes thereby increasing hepatic steatosis and steatohepatitis (26).

Our results revealed higher levels of resistin among the NAFLD patient than control, previous studies have demonstrated that obese individuals display higher serum resistin values than lean subjects and a positive correlation may exist between BMI and resistin (27). Shen et al. showed that resistin was increased in liver of NASH patients, and there was a positive correlation between resistin and inflammatory severity in the liver of NASH patients (28).

CONCLUSION

Within the limit of this study non-alcoholic fatty liver disease is associated with adipokines alteration that is correlated with abnormal glucose control and insulin resistance.

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REFERENCES

1. Bellentani S, Marino M. Epidemiology and natural history of non-alcoholic fatty liver disease (NAFLD). Ann Hepatol 2009;8 (Suppl 1):4-8.
2. Koppe SW. Obesity and the liver: nonalcoholic fatty liver disease. Transl Res. 2014 Jun 25;pii:S1931-5244(14)00215-1.
3. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology. 2006;43,S99–S112.
4. de Lima Sanches P, de Mello MT, Elias N, et al. Improvement in HOMA-IR is an independent predictor of reduced carotid intimamedia thickness in obese adolescents participating in an interdisciplinary weight-loss program. Hypertens Res. 2011;34:232–8.
5. Wolf G. Adiponectin: a regulator of energy homeostasis. Nutr. Rev. 2003;61:290–292.
6. Yamauchi T, et al. Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. J. Biol. Chem. 2003;278:2461–8.
7. Polyzos SA, Kountouras J, Zavos C, et al. The role of adiponectin in the pathogenesis and treatment of nonalcoholic fatty liver disease. Diabetes. Obes Metab 2010;12:365-83.
8. Pelleymounter MA, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 1995;269:540–3.
9. Wong VW, et al. Metabolic and adipokine profile of Chinese patients with nonalcoholic fatty liver disease. Clin. Gastroenterol. Hepatol. 2006;4:1154–61.
10. Curat CA, Wegner V, Sengenès C, Miranville A, Tonus C, Busse R, Bouloumie A. Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. Diabetologia. 2006;49:744–7.
11. Zhou L, Sell H, Eckardt K, Yang Z, Eckel J. Conditioned medium obtained from in vitro differentiated adipocytes and resistin induce insulin resistance in human hepatocytes. FEBS Lett. 2007;581:4303–8.
12. Wong VW, Hui AY, Tsang SW, Chan JL, Tse AM, Chan KF, So WY, Cheng AY, Ng WF, Wong GL, Sung JJ, Chan HL. Metabolic and adipokine profile of Chinese patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2006;4:1154–61.
13. Sanyal AJ. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology. 2002;123:1705-25.
14. Saverymuttu SH, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. BMJ. 1986;292:13-5
15. Balaban YH, Sumer H, Simsek H, Us D, Tatar G. Metabolic syndrome, non-alcoholic steatohepatitis (NASH), and hepatocyte growth factor (HGF). Ann Hepatol. 2006;5(2):109-14.
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta cell function from plasma FBS and insulin concentrations in man. Diabetologia. 1985;28:412–9.
17. Katz A, Nambi SS, Mather K, Baron DA, Follman DA, Sullivan F, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85:2402–10.
18. Lomonaco R, Ortiz-Lopez C, Orsak B, et al. Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. Hepatology. 2012;55: 1389-97.
19. Sanches PL, de Piano A, Campos RM, Carnier J, de Mello MT, Elias N, Fonseca FA, Masquio DC, da Silva PL, Corgosinho FC, Tock L, Oyama LM, Tufik S, Dâmaso AR. Association of nonalcoholic fatty liver disease with cardiovascular risk factors in obese adolescents: the role of interdisciplinary therapy. J Clin Lipidol. 2014 May-Jun;8(3):265-72.
20. Willner IR, Waters B, Patil SR, et al. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency and severity of disease. Am J Gastroenterol. 2001;96:2813e2814.
21. Weyer C, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J. Clin. Endocrinol. Metab. 2001;86:1930–5.
22. Louthan MV, et al. Decreased serum adiponectin: an early event in pediatric nonalcoholic fatty liver disease. J. Pediatr. 2005;147:835–8.

23. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996;271:10697e10703.
24. Schwimmer JR, Deutsch R, Rauch JB, Behling C, Newbury R & Lavine JE. Obesity, insulin resistance, and other clinicopathological correlates of pediatric nonalcoholic fatty liver disease. Journal of Pediatrics. 2003;143:500–5.
25. Uygun A, Kadayifci A, Yesilova Z, Erdil A, Yaman H, Saka M, Deveci MS, Bagci S, Gulsen M, Karaeren N & Dagalp K. Serum leptin levels in patients with nonalcoholic steatohepatitis. American Journal of Gastroenterology. 2000;95:3584–9.
26. Chalasani N, Crabb DW, Cummings OW, Kwo PY, Asghar A, Pandya PK, et al. Does leptin play a role in the pathogenesis of human nonalcoholic steatohepatitis? Am J Gastroenterol. 2003;98:2771-6.
27. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem. 2004;50:1511-25.
28. Shen C, Zhao CY, Wang W, Wang YD, Sun H, Cao W, Yu WY, Zhang L, Ji R, Li M, Gao J. The relationship between hepatic resistin overexpression and inflammation in patients with nonalcoholic steatohepatitis. BMC Gastroenterol. 2014 Feb 23;14:39.

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