ORIGINAL ARTICLE

Serum adropin levels, visceral adiposity index and DNA damage as risk factors associated with non-alcoholic fatty liver disease in obese women

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ABSTRACT

Background: Numerous non-invasive biomarkers have been used to avert liver biopsy in non-alcoholic fatty liver disease (NAFLD). Adropin plays an important role in metabolic homeostasis. Nevertheless, the clinical relevance of adropin to the visceral adiposity and NAFLD is ambiguous. NAFLD is a recurrent disease among overweight and obese individuals; however, some studies have reported prevalence of NAFLD in lean subjects.

Objectives: Our objective was to inspect the relationship between adropin, DNA damage and body characteristics in NAFLD patients and obese healthy controls.

Methods: This study examined 40 healthy obese premenopausal women without NAFLD and 40 age matched obese women with NAFLD. Serum adropin levels, metabolic parameters and anthropometric parameters were investigated. The Visceral Adiposity Index (VAI) was calculated. DNA damage was assessed by comet assay.

Results: NAFLD patients showed significantly lower circulating serum adropin and higher VAI, and waist to hip ratio (WHR) compared to controls. Furthermore, serum adropin concentrations were negatively correlated with obesity-related parameters and serum lipids and DNA damage was observed

Conclusions: NAFLD patients showed significantly lower adropin levels than obese healthy controls and could be a prospective marker for prediction of the development of NAFLD, specially for persons with high visceral fat accumulation and DNA damage.

Keywords: adropin, obesity, DNA damage, non-alcoholic fatty liver disease, visceral adiposity index.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the main prevalent interminable liver disease in urbanised countries, affected about 34–46% of the universal population (1,2). NAFLD pathogenesis has not been distinctly demonstrate until now; nonetheless, few mechanisms have been anticipated NAFLD is considered as the liver display of metabolic syndrome that is related to type 2 diabetes, insulin resistance, obesity, and/or dyslipidemia (3). The Visceral Adiposity Index (VAI) has lately confirmed to be a marker of adipose function and distribution that elucidate indirectly cardiometabolic risk (4).

Adropin is a 76-amino acid peptide encoded by the energy homeostasis-associated (Enho) gene that is expressed in brain and liver. Adropin is a hormone that was first recognised by Kumar *et al.* (5). Diminished levels of adropin could correlate with a higher risk of probably MetS, insulin resistance, and obesity. Its release is mediated by dietary macronutrients. Systemic injections of adropin has shown to ameliorate insulin sensitivity in skeletal muscle and to encourage weight reduction in experimental studies (6).

Adropin has been shown to be diminished in many diseases, such as coronary atherosclerosis, diabetic nephropathies, type 2 diabetes, polycystic ovary disease and hypertension (7-9). The prevailing impacts of adropin on lipid and glucose homeostasis might have a significant role that impair glucose tolerance, dyslipidemia and insulin resistance related to obesity and diabetes (10).

Obesity, an amendable risk factor and prevalent amongst patients with NAFLD, is strongly related with metabolic syndrome (MetS), adipose tissue dysfunction, hepatic steatosis, lipodystrophy and type 2 diabetes Furthermore, MetS is more prevalent among patients with NAFLD than those without it (9). NAFLD is more frequently documented as the hepatic semblance of MetS, concerning an interchange of adipokines liberated from surplus cytokines, visceral adipose tissue (VAT), and inflammatory factors released from the macrophages inhabited in VAT, leading to a decreased hepatic insulin extraction and chronic condition of inflammation, all leading to insulin resistance (11,12). VAI and adropin have not been studied simultaneously in obese patients with NAFLD in comparison to healthy obese patients without NAFLD and the feasible combined roles in relation to disease are unidentified. In the present study we aimed to investigate the hypothesis that serum adropin concentrations and VAI are different among patients with and without NAFLD.

SUBJECTS AND METHODS

All the procedures used in this study were in accordance with the guidelines of the Helsinki Declaration on Human Experimentations. The study was approved by the local ethics committee of the National Research Centre (No: 13176). The aims of the protocol was explained to both the patient and control groups, and written informed consent was obtained from them before beginning the study.

Anthropometric measures

Anthropometric measurements included body weight, height, waist and hip circumferences. All measurements were taken three times on the left side of the body and the mean of the three values was used. Body weight was measured to the nearest 0.1kg and height was measured to the nearest 0.1cm. Height was measured with the patients standing with their backs leaning against the stadiometer of the same scale. BMI was calculated as weight in kilograms divided by height in meters square (kg/m^2). Waist circumference (WC) and hip circumference (HC) were measured in cm using a plastic non-stretchable tailor's tape. WC was measured with light clothing at a level midway between the lower rib margin and the iliac crest standing and breathing normally. HC was measured at the level at the widest circumference over the buttocks (at the greater trochanter). Subsequently the waist hip ratio (WHR) was calculated as WC divided by HC. The diagnosis of NAFLD was based on the presence of a bright liver at ultrasound scanning. VAI was calculated as: VAI = WC ⁄ [36.58 + (1.89 × BMI)] × TG ⁄ 0.81 × 1.52 ⁄ HDL

Plasma lipids

Total cholesterol, triglycerides (TG), HDL-cholesterol and LDL-cholesterol were assessed in serum by the colorimetric assays using the auto analyser Cobas c111 (Roche Diagnostics, Mannheim, Germany).

Adropin

Adropin was measured in serum with an ELISA kit from Sunlong Biotech. Co. LTD. Catalog Number: SL 1934 Hu, with a sensitivity of 0.5 pg/mL and with inter-assay and intra-assay coefficients of variation (CVs) below 12.0% and 10%, respectively.

DNA damage by comet assay

Peripheral blood leukocytes were isolated by centrifugation (30 min at 1300xg) in Ficoll - Pique density gradient (PharmaciaLKB Biotechnology, Piscataway, NJ, USA). After centrifugation, leukocytes in the buffy coat were aspirated and washed twice with phosphate-buffered saline at pH7.4 (PBS). Lysis of cells, DNA unwinding, gel electrophoresis and DNA

staining were done. The slides were examined at 400 x magnification using a fluorescence microscope (Leica Microsystems, CMS GM b H,Wetzlar, Germany).

Statistical analysis

All values are expressed as the mean ± SD. The normality of the data was tested using the Shapiro–Wilk test. NAFLD were compared using an independent samples t-test (two-tailed) or a non-parametric test. Correlation analysis was used to evaluate the relationship between adropin and other laboratory parameters. The correlations between adropin and other parameters were analysed using the Spearman test. Independent relationship between adropin concentrations and obesity–related parameters was done using linear regression analysis.

RESULTS

Levels of adropin in serum were significantly lower in the NAFLD group than the control group (110.56 vs 165.13 pg/ml; $P < 0.001$). In addition, obesity –related parameters, including WHR, VAI and serum lipids, were significantly higher in obese cases having NAFLD than those without (Table 1). Figure 1 shows DNA damage in NAFLD cases.

Correlation analysis in the NAFLD group showed significant negative correlations between serum adropin concentrations and obesity related parameters including VIA, WC and WHR as well as with serum lipids including TC, TG, LDL. Table 2 shows significant negative correlation between serum adropin and obesity related parameters and serum lipids, confirming the potential effect of adropin on the metabolic dysfunction in obese women (13-16).

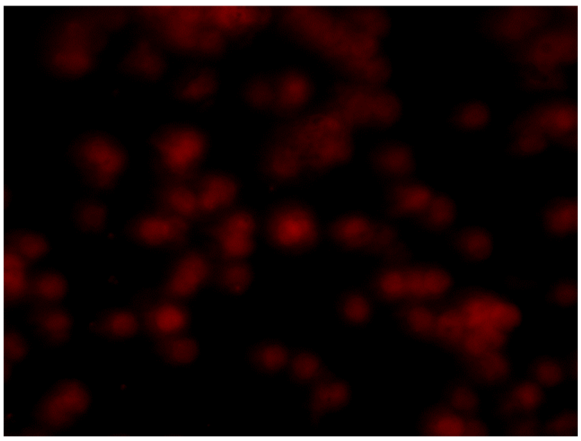

Figure 1. DNA damage in NAFLD with a brightly fluorescent head and a tail to one side.

Table 1. Comparison of adropin, clinical and anthropometric data among obese women with and without NAFLD

Variables	Obese without NAFLD	Obese with NAFLD	P-value
Age (years)	32.7±2.6	33.5±3.4	0.45
Adropin (pg/mL)	165.1 ± 19.9	110.5±13.4*	0.001
BMI (kg/m^2)	32.59±3.56	33.65±4.047	0.06
Waist circumference (cm)	76.90±10.04	105.13±14.83	0.01
Hip circumference (cm)	97.85±9.241	124.77±13.72	0.02
VAI	2.47±.0.9	4.92±1.76	0.001
WHR	0.78±0.073	0.84±0.09	0.01
TC (mmol/L)	3.21± 0.67	4.78±1.34	0.03
TG (mmol/lL	0.78±0.34	2.45±1.02	0.01
HDL (mmol/L)	1.55±1.34	0.98±.03	0.04
LDL (mmol/L)	2.34±1.23	4.89±2.09	0.01

Results are mean ± SD. TG = triglyceride; TC= total cholesterol; LDL= Low-density lipoprotein; HDL = high-density lipoprotein; VAI= Visceral Adiposity Index; WHR= waist to hip ratio; *range of adropin=3pg/mL-180pg/mL.

Table 2. Relationship between adropin levels in serum and obesity-related parameters using correlation Spearman test and linear regression analyses.

Variables	Adropin		Regression analysis	
	r	*p*	Standardized β	*p*
VIA	-0.85	0.001	-0.37	0.01
WC	-0.58	0.02	-0.34	0.05
WHR	-0.53	0.01	-0.33	0.01
TC (mmol/l)	-0.44	0.01	-0.25	0.01
HDL (mmol/l)	0.55	0.02	0.33	0.01
TG (mmol/l)	-0.65	0.01	-0.021	0.05
LDL (mmol/l)	-0.54	0.03	-0.32	0.04

VIA= Visceral Adiposity Index; WC = waist circumference; WHR= WC/HC; TC= total cholesterol; TG = triglyceride; HDL = high-density lipoprotein; LDL= Low-density lipoprotein.

DISCUSSION

Recently, it has been shown that concentrations of adropin in serum in NAFLD and metabolic syndrome were markedly lesser than in patients without NAFLD and healthy persons. It was proposed that a decrease in adropin concentrations could be the only independent factor for fatty liver disease in obese adolescents (17,18). Oxidative stress can have a marked impact on DNA damage (19). Data from animal models suggest that oxidative stress contributes to steatohepatitis and an increase of lipid peroxidation has been documented in human NAFLD (20). Mitochondrial dysfunction is the main source of ROS in fatty liver and is closely related to endoplasmic reticulum stress (21).

Since NAFLD is related with metabolic risk factors, e.g., visceral obesity, insulin resistance, dyslipidemia, and hypertension. It is considered as the hepatic manifestation of the metabolic syndrome (22). NAFLD is a common co-morbidity of obesity. Also hypothyroidism related to obesity might contribute to the dysmetabolic state that influences to NAFLD (23). Adropin is a hormone that was recognised first by Kumar *et al*. (5). It is encoded by the energy homeostasis–related gene that is expressed in brain and liver (5). Decreased adropin concentrations might correlate with insulin resistance, a higher risk of obesity and probably MetS (5). In our study levels of serum adropin were significantly diminished in NAFLD patients, which is consistent with preceding studies (24-26). Adropin over expression in mice leads to decreased weight gain and decrease HOMA-IR values and triglyceride levels (5). As has been reported in many studies, large differences in BMI and abdominal obesity between the groups was observed, particularly among patients with and without NAFLD (27,28). The same variations were found in biochemical variables, specially insulin resistance, that is the mainly prevalent feature of persons with NAFLD (24). Among all the studied anthropometric indexes, VAI is the only one that has demonstrated the maximum number of correlations with lipids and adipocytokines. VAI was capable to express both the state of low-grade inflammation and relative leptin resistance, and the altered endocrine function of adipose tissue that are all variations found in a state of adipose tissue dysfunction. The correlation with visfatin is particularly strong. Adipocytokines are generally excreted by visceral adipose tissue that in accordance with several studies might have adipogenic impacts and is a good candidate to elucidate the visceral adipose tissue accumulation related with insulin resistance (29,30).

CONCLUSIONS

In our study we have shown that VAI and DNA damage are excellent predictors of a distorted adipokine profile and its related NAFLD risk. In conclusion, even though still lacking probable searches that might attribute a predictive function of VAI on cardiovascular risk, given the simplicity of BMI and WC measurements and HDL cholesterol and triglycerides estimation. We recommend that VAI could be a simple tool to assess the metabolic risk in type 2 diabetes or in other subjects without an overt metabolic syndrome. High levels of adropin may be involved in the development of NAFLD in obese women. If these contributory links are true, then adropin administration, or pharmaceutics which encourage its production, may be a novel approach to treat or prevent NAFLD risk.

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