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Increased plasma CgA levels associated with nonalcoholic fatty liver disease

LIVER

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ABSTRACT

Background/Aims: Chromogranin A (CgA), a major soluble protein released by the neuroendocrine system, functions as a prohormone by giving rise to several biologically active peptides. Our study aimed to evaluate the relationship between CgA levels and nonalcoholic fatty liver disease (NAFLD).

Materials and Methods: In total, 111 NAFLD patients and 120 healthy controls were enrolled in the trial. The levels of plasma CgA were quantified by an available enzyme-linked immunosorbent assay kit. Pearson's correlation analysis was conducted to detect whether CgA levels correlated with oxidative stress, insulin resistance, and inflammation profile. A multiple stepwise regression model was used to explore independent determinants for plasma CgA levels. Multivariate logistic regression analysis was conducted to assess whether CgA levels were independent predictors of NAFLD.

Results: The levels of plasma CgA between the case and control groups were significantly different (70.9±8.1 μg/L vs 47.6±11.3 μg/L). The levels of plasma CgA positively correlated with high-sensitivity C-reactive protein (hs-CRP; p=0.000), fasting blood glucose (FBG; p=0.025), homeostasis model assessment of insulin resistance index (HOMA-IR; p=0.012), and malondialdehyde (MDA; p=0.037) levels, but negatively associated with superoxide dismutase (SOD; p=0.041) levels.

The multiple stepwise regression model indicated that hs-CRP, MDA, and HOMA-IR were independent determinants for plasma CgA levels. The logistic regression analysis showed that plasma levels of CgA were independent predictors of NAFLD.

Conclusion: Increased plasma CgA levels were associated with NAFLD.

Keywords: CgA, enzyme-linked immunosorbent assay, nonalcoholic fatty liver disease

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), one of the chronic liver diseases, is characterized by the accumulation of triglycerides in hepatocytes without excessive alcohol intake. Individuals with NAFLD are more likely to suffer from metabolic disease including type 2 diabetes, cardiovascular diseases, and dyslipidemias (1). It is well established that the fundamental risk factors for NAFLD are oxidative stress, inflammation, and insulin resistance (IR) (2). Nevertheless, the pathogenesis of NAFLD cannot be fully elucidated by these classic risk factors.

Chromogranin A (CgA) is often co-stored and co-released with catecholamine (3). The cleavage of CgA can generate several bioactive peptides by prohormone convertases (4). CgA and its several bioactive fragments exhibit a broad spectrum of bioactive effects on the modulation of inflammatory and oxidative processes and insulin secretion (4). Therefore, our study aimed to evaluate relationship between CgA levels and NAFLD.

MATERIALS AND METHODS

Patients and controls

This study was approved by the Ethics Committee in Rennin Hospital of Wuhan University. Written informed consent was obtained from each subject. In total, 111 controls subjects and 120 NAFLD patients were select-

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ed from the hepatology outpatient unit between January 2012 and August 2014. NAFLD was diagnosed by pathologic classification made in China (10). Briefly, all NAFLD patients had sonographic feature findings and had no history of specific diseases that may result in fatty liver and habit of drinking (male: >20g/day, female: >10 g/day).

The healthy controls were recruited according to normal hepatic sonographic features.

Data collection

General information such as age, sex, alcohol drinking, and self-reported history of disease especially live disease was collected by trained physicians. Anthropometric parameters including height, weight, body mass index (BMI) {BMI=weight (kg)/[height (m) ×height (m)]}, and waist circumference (WC) were collected by a complete physician.

Laboratory measurements

Blood samples were collected at fasting state and were frozen and stored at –80°C for further use. Lipid profile including triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), fasting blood glucose (FBG), alanine transaminase (ALT), and aspartate transaminase (AST) levels were measured by routine laboratory tests. IR was evaluated by a homeostasiv s model assessment of insulin index (HOMA-IR). HOMA-IR was calculated using the following formula: HOMA-IR=fasting plasma insulin fasting level×plasma glucose level/22.5. Those with HOMA-IR >2.5 were regarded to have IR. The oxidative stress parameters including malondialdehyde (MDA) and superoxide dismutase (SOD) were detected by the colorimetric method (Nanjing Bioengineering Co., LTD, Nanjing, China). Plasma levels of CgA and high-sensitivity C-reactive protein (hs-CRP) were evaluated by a commercially available enzyme-linked immunosorbent assay kit (Fushou indusrial Co., LTD, Shanghai, China).

Statistical analysis

Statistical analysis was conducted using SPSS version 11.0 (SPSS Inc., Chicago, Illinois, USA). The independent sample t-test and chi-square test were performed to compare categorical and continuous variables, respectively. Pearson's correlation analysis was performed to detect the relationship between CgA levels and oxidative stress, IR, and inflammation profile. The variables that dependently associated with CgA were identified by multiple linear regression. A backward stepwise multivariate logistic regression model with variables with p<0.10 in univariate analysis was performed to identify independent predictors of NAFLD. The difference was considered to be significant when p<0.05.

RESULTS

Characteristics of subjects

The general characteristics of the case and control groups are described in Table 1. Age, gender, WC, and HC distribution were not statically significant between the case and control groups. As expected, significant differences were detected in the TC, TG, HDL-C, LDL-C, hs-CRP, FBG, HOMA-IR, MDA, SOD, ALT, and AST levels. The NAFLD patients had significantly higher levels of plasma CgA than the controls (70.9±8.1 µg/L vs 47.6±11.3 µg/L). The plasma CgA levels of NAFLD patients with or without IR were significantly different (75.0±7.1 vs 60.1±8.2) (Figure 1).

Table 1. Clinical characteristic of NAFLD and control subjects

Variables	NAFLD	Healthy controls	p
Sex (M/F)	64/47	67/53	0.780
Age (years)	53.7±11.8	49.9±9.5	0.536
BMI (kg/m^2)	30.0±9.1	26.12±5.1	0.000
WC (cm)	89.1±11.7	89.6±7.5	0.747
TG (mmol/L)	3.6±0.8	1.7±1.3	0.000
TC (mmol/L)	5.7±1.6	3.8±0.5	0.000
HDL-c (mmol/L)	1.7±0.9	2.0±0.5	0.002
LDL-c(mmol/L)	3.7±0.4	1.2±1.3	0.000
ALT (U/L)	59.5±20.8	38.6±11.7	0.000
AST (U/L)	56.5±16.7	36.3±7.4	0.000
SOD (U/mL)	158±23.5	100.0±45.4	0.026
MDA (nmol/L)	52.3±13.3	21.3±10.3	0.031
hs-CRP (ng/mL)	3.4±0.9	2.9±1.1	0.000
FBG (mmol/L)	4.7±1.0	3.7±2.0	0.057
HOMA-IR	3.7±0.5	2.7±1.2	0.002

BMI: body mass index; WC: waist circumference; TG: total triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; ALT: alanine transaminase; AST: aspartate transaminase; SOD: superoxide dismutase; MDA: malondialdehyde; hs-CRP: high-sensitivity C-reactive protein; FBG: fasting blood glucose; HOMA-IR: homeostasis model assessment of insulin resistance index

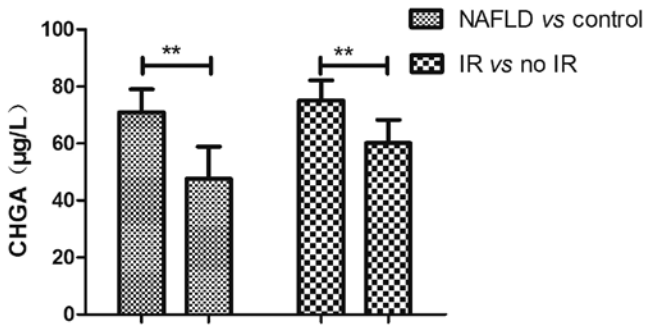

Figure 1. Plasma CgA levels in patients with NAFLD and controls; (B) Plasma CgA levels in patients with NAFLD with or without insulin resistance (IR) (**, p<0.05).

Correlation and multiple linear regression analysis

The results show that plasma CgA levels were positively associated with hs-CRP (r=0.524, p=0.000), FBG (r=0.331, p=0.025), HOMA-IR (r=0.378, p=0.012), and MDA (r=0.412, p=0.037) levels, but negatively correlated with SOD (r=–0.326, p=0.041)

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Table 2. Correlation analysis between CgA and other parameters

Variables	r	p
Sex (M/F)	0.312	0.417
Age (years)	0.123	0.256
BMI (kg/m^2)	0.327	0.436
WC (cm)	0.269	0.817
TG (mmol/L)	0.769	0.298
TC (mmol/L)	0.219	0.589
HDL-c (mmol/L)	–0.613	0.646
LDL-c (mmol/L)	0.429	0.359
hs-CRP (ng/mL)	0.524	0.000
FBG (mmol/L)	0.331	0.025
HOMA-IR	0.378	0.012
ALT (U/L)	0.364	0.059
AST (U/L)	0.498	0.109
SOD (U/mL)	–0.326	0.041
MDA (nmol/L)	0.412	0.037

BMI: body mass index; WC: waist circumference; TG: total triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; ALT: alanine transaminase; AST: aspartate transaminase; SOD: superoxide dismutase; MDA: malondialdehyde; hs-CRP: high-sensitivity C-reactive protein; FBG: fasting blood glucose; HOMA-IR: homeostasis model assessment of insulin resistance index

Table 3. Logistic Regression Analysis for Patients with NAFLD

	Simple regression OR (95%CI)	p	Multiple regression OR (95%CI)	p
Sex (M/F)	0.945 (0.826–1.239)	0.468		
Age (years)	1.469 (0.807–2.256)	0.289		
BMI (kg/m^2)	1.623 (1.003–2.256)	0.056	1.850 (0.974–5.758)	0.091
WC (cm)	0.913 (0.6541–1.282)	0.557		
TG (mmol/L)	1.512 (0.907–2.314)	0.169		
TC (mmol/L)	1.236 (1.003–1.635)	0.047	1.017 (0.450–2.300)	0.964
HDL-c (mmol/L)	1.112 (0.312–4.162)	0.912		
LDL-c (mmol/L)	0.951 (0.626–2.229)	0.689		
hs-CRP (ng/mL)	1.312 (1.127–1.658)	0.025	1.991 (1.582–11.677)	0.036
FBG (mmol/L)	1.236 (0.456–3.236)	0.938		
HOMA-IR	1.572 (1.336–2.113)	0.039	2.572 (1.065–6.963)	0.000
SOD (U/mL)	0.435 (0.217–0.874)	0.019	0.191 (0.140–0.890)	0.014
MDA (nmol/L)	1.426 (0.869–2.113)	0.112		
CgA (ng/mL)	1.412 (1.117–2.113)	0.000	2.659 (1.492–8.720)	0.024

BMI: body mass index; WC: waist circumference; TG: total triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; ALT: alanine transaminase; AST: aspartate transaminase; SOD: superoxide dismutase; MDA: malondialdehyde; hs-CRP: high-sensitivity C-reactive protein; FBG: fasting blood-glucose; HOMA-IR: homeostasis model assessment of insulin resistance index; CgA: chromogranin A

levels. A significant correlation between CgA levels and sex, age, BMI, HC, WC, HDL-C, LDL-C, TC, TG, AST, and ALT levels was not found (Table 2). The variables that significantly correlated with CgA levels were further analyzed by the stepwise multiple linear regression analysis. It was found that hs-CRP (β=2.01, p=0.032), MDA (β=0.654, p=0.001), and HOMA-IR (β=0.574, p=0.000) levels were significant predictors of CgA levels.

Logistic regression analysis

The simple logistic regression analysis revealed that BMI, TC, hs-CRP, HOMA-IR, and CgA levels exhibited a trend p<0.10. All variables mentioned above were included in the multivariate logistic regression analysis. Our results revealed that CgA levels were independent predictors of NAFLD (OR=1.312, 95% CI: 1.131–1.894; p=0.024) (Table 3).

DISCUSSION

To date, there are limited data in literature with regard to changes of plasma CgA levels in NAFLD. In this study, the results obtained indicated that increased plasma CgA levels were detected in patients with NAFLD. Interestingly, there were also striking differences in plasma CgA levels in NAFLD patients with or without IR. Pearson's correlation analysis in our study showed that CgA levels positively correlated with hs-CRP, FBG, HOMA-IR, and MDA levels, but negatively correlated with SOD levels. Moreover, the multiple linear regression analysis indicated that hs-CRP, MDA, and HOMA-IR levels were dependent predictors of CgA, and the multivariate logistic regression analysis indicated that CgA levels were independent predictors for NAFLD. The change of CgA levels in NAFLD is similar to preliminary data from inflammatory disease and metabolic disease (5-7), suggesting that CgA activates the neuroendocrine system to respond to maintain homeostasis. Similar to a previous finding that increased CgA plasma levels often correlate with serum inflammatory markers in inflammatory disease (8,9), it also positively correlated with inflammatory markers in the present study. Considering that CgA and derived peptides exhibit a broad spectrum of bioactive effects on the modulation of oxidative processes, insulin secretion (4) and that Pearson's correlation analysis showed that CgA levels positively correlated with oxidative stress and IR, it can be inferred that circulating CgA may activate the neuroendocrine system in response to oxidative stress and IR in NAFLD (8-10).

CgA is an endogenous peptide that has garnered significant investigative attention during the last decade. The human CgA gene is located on chromosome 14q32.12 and is composed of eight exons and seven introns. Its gene encodes an acidic protein with a molecular weight of 48 kDa; the protein is subsequently cleaved into several biologically active peptides such as pancreastatin, catestatin, and chromostatin (5).

To the best of our knowledge, large numbers of studies previously conducted assessed CgA levels in neuroendocrine tumors (11-13). With further research on CgA and its cleaved peptides, attention was distracted from neuroendocrine tumors to

other diseases. Increased levels of plasma pancreastatin in type 2 diabetes mellitus subjects have been found, and its negative regulator in insulin sensitivity and glucose homeostasis has been suggested (14). Another study reported that pancreastatin inhibits insulin secretion (15).

Increased plasma CgA levels are also involved in inflammatory diseases and metabolic disease (5,6).

Some inevitable limitations warrant consideration. First, the main limitation of this study was that NAFLD was diagnosed by hepatic ultrasound whose sensitivity and specificity was lower compared with those of hepatic biopsy (16). Second, the controls recruited in the present study may not be completely healthy because some of them suffer from other metabolic disease whose pathogenesis was partly similar to NAFLD. Third, the sample size was relatively small and consequently limited the generalizability of our conclusions.

In conclusion, the data in our study showed that increased plasma CgA levels were associated with NAFLD. Given the individual effects of hyperglycemia, hyperlipidemia, and abdominal obesity in NAFLD, larger studies may also aim to detect CgA levels in all phenotypes of NAFLD. The particular mechanisms of increased plasma CgA levels in NAFLD warrant further studies.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - P.B.W., S.Y.T., Y.Z.D.; Design - P.B.W., S.Y.T., Y.Z.D.; Supervision - Y.X.S., M.L., G.F.; Resource - Y.X.S., M.L., G.F.; Materials - Y.X.S., P.B.W., G.F.; Data Collection &/or Processing - Y.X.S., M.L., G.F.; Analysis &/or Interpretation - Y.X.S., Y.Z.D., G.F.; Literature Search - P.B.W., S.Y.T., Y.Z.D.; Writing - P.B.W., S.Y.T., Y.Z.D.; Critical Reviews - S.Y.T.

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