Original Article

Serum Adipokines in Non-alcoholic Fatty Liver Disease

Pulsombat A[1]
Warodomwichit D[2]
Sornmayura P[3]
Angkathunyakul N[3]
Rattanasiri S[4]
Chaiyaratana W[5]
Kaewdoung P[1]
Petraksa S[1]
Sobhonslidsuk A[1]

ABSTRACT

Objectives: Background: Non-alcoholic fatty liver disease (NAFLD) is a hepatic manifestation of metabolic syndrome that is associated with obesity and insulin resistance. Non-alcoholic steatohepatitis (NASH) can progress to cirrhosis and advanced fibrosis. Chemerin, vaspin and omentin-1 are new adipokines releasing from adipose tissue. Adipokines may play a role in the pathogenesis of NAFLD.

Objectives: We aimed to assess chemerin, vaspin and omentin-1 levels in patients with NAFLD, and to identify predictive markers for NASH and advanced fibrosis.

Methods: A cross-sectional study was performed from January to December 2012. Data on anthropometric measurements, blood chemistry, index of insulin resistance (HOMA-IR) and adipokines (chemerin, vaspin and omentin-1) were collected in patients with NAFLD along with age- and sex-matched controls. NAFLD patients needed to have NAFLD-confirmed liver biopsy within 2 years before enrollment. NAFLD activity score (NAS) and fibrosis staging were assessed according to Kleiner *et al.* NAS ≥5 was defined as NASH. Fibrosis stage ≥3 was defined as advanced fibrosis. Adipokine levels were determined by enzyme-linked immunosorbent assay.

Results: A total of 60 NAFLD patients and 55 controls were enrolled. Mean (SD) ages of NAFLD patients and controls were 54.7 (8.7) and 46.9 (8.1) years. The anthropometric data, liver chemistry, lipid profiles and HOMA-IR in NAFLD group were significantly different from control group. Chemerin, vaspin and omentin-1 in NAFLD group were higher than in control group [194.58 vs. 120.51 (p<0.001), 0.163 vs. 0.156 (p<0.292), 452.25 vs. 372.1 ng/mL (p<0.006)]. There were 22 (36.7%) patients with NASH, and 38.3 (63.3%) patients with simple steatosis. Chemerin, vaspin and omentin-1 levels in NASH and simple steatosis were not different. Twenty-one patients (35%) had advanced fibrosis. From univariate analysis age, NAS, hemoglobin A1C, omentin-1 were significantly elevated in advanced fibrosis patients. Age and NAS were independently associated with advanced fibrosis.

Conclusions: Chemerin and omentin-1 are elevated in NAFLD patients. Chemerin, omentin-1 and vaspin are not associated with advanced fibrosis and NASH. Age and NAS are related to advanced fibrosis. The role of adipokines in the pathogenesis of NAFLD requires further study.

Key words : Non-alcoholic fatty liver disease, NASH, chemerin, vaspin, omentin-1, adipokines

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[1]*Division of Gastroenterology and Hepatology,* [2]*Division of Nutrition and Medical Biochemistry, Department of Medicine,* [3]*Department of Pathology,* [4]*Section for Clinical Epidemiology and Biostatistic,* [5]*Clinical Research center, Faculty of Medicine Ramathibodi Hospital, Mahidol University.*

Address for Correspondence: *Abhasnee Sobhonslidsuk, M.D., Division of Gastroenterology and Hepatology, Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University.*

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a well-established hepatic manifestation of the metabolic syndrome and is one of the most common causes of chronic liver disease. It can progress to advanced liver fibrosis, cirrhosis and hepatocellular carcinoma. The prevalence of NAFLD has been increasing worldwide[1]. The spectrum of the disease ranges from simple steatosis, non-alcoholic steatohepatitis (NASH), advanced fibrosis and cirrhosis[2-3]. Liver biopsy is the gold standard for diagnosis, and can be used for grading severity of NAFLD and degree of liver fibrosis, but it is an invasive procedure with high risk for complication[4-6].

Adipokines are the fat-derived hormones released from adipose tissue. Many are associated with insulin resistance, obesity and metabolic syndrome. Adipokines can be both pro-inflammation and anti-inflammation factors[7-8]. Many reports have found that adipokines have a role in the pathogenesis of NAFLD and are linked to insulin resistance. Levels of adipokines in NAFLD patients was found to be different compared to controls[7-9,12-13].

Chemerin is a new adipokine mainly released from fat cells that is associated with high body mass index (BMI), insulin resistance and the metabolic syndrome[10-11]. Kukla *et al*[12] and Yusuf *et al*[13] found that serum chemerin was significantly higher in NAFLD than in normal volunteer.

Vaspin is another new adipokine from adipose tissue that is associated with high BMI, body fat percentage and insulin resistance, especially in patients with type 2 diabetes mellitus[14]. However, the level of vaspin in patients with NAFLD in comparison to healthy controls is still controversial[12,15].

Omentin-1 is another adipokine secreted from fat cells that has an inverse relationship with BMI, waist circumference and insulin resistance. The level of omentin-1 in the NAFLD patients and normal volunteer is still inconsistent[13,16].

The aims of the study were to assess the level of chemerin, vaspin and omentin-1 in patients with NAFLD, and to identify new predictive markers for NASH and advanced fibrosis.

Materials and Methods

Study patients

We conducted a cross-sectional study enrolling patients from the Liver Clinic, Ramathibodi Hospital, Thailand, from January 2012 to December 2012. We recruited patients aged 18-75 years with biopsy-proven NAFLD. The main reasons for liver biopsy were abnormal liver function test and high transient elastography score. The exclusion criteria were alcohol consumption more than 20 g/day, other chronic liver diseases (chronic viral hepatitis B, chronic viral hepatitis C, autoimmune hepatitis, hemochromatosis, Wilson's disease, drug-induced and herbal-induced hepatitis), uncontrolled medical conditions (autoimmune diseases, cancer, thyroid diseases, kidney diseases and HIV infection), pregnancy or lactation, or refusal to participate in the study.

Healthy controls were age- and sex-matched to the NAFLD patients. Normal anthropometric data, blood tests for liver function, metabolic parameters (including fasting glucose, hemoglobin A1C and lipid profile) and normal ultrasonographic findings of the liver were required before enrollment. A physical examination was performed on both NAFLD patients and controls. Anthropometric data including weight and height for calculating body mass index (BMI) and waist circumference were recorded. Blood tests for biochemistry were taken; complete blood count, liver biochemistry, index of insulin resistance (fasting glucose, hemoglobin A1C and fasting insulin) and lipid profile (triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C)).

The insulin resistance level was calculated via homeostasis model assessment of insulin resistance (HOMA-IR) by using the formula: Fasting insulin (μIU/mL) × Fasting glucose (mg/dL)/405.

The Ethics Committee of the Faculty of Medicine Ramathibodi Hospital, Mahidol University approved this study, and all participants provided written informed consent prior to participation.

Serum adipokines assays

We assessed the serum concentration of chemerin, vaspin and omentin-1 by enzyme-linked immuno-sorbent assay (ELISA) with commercial kits: HUMAN Chemerin ELISA kit (catalogue No.RD191136200R; BioVendor Laboratory Medicine, Czech Republic), HUMAN Vaspin ELISA kit (catalogue No. RD191097200R; BioVendor Laboratory Medicine, Czech Republic) and HUMAN Omentin-1 ELISA kit (catalogue No.RD191100200R; BioVendor Laboratory

Medicine, Czech Republic). All blood samples were taken at least 8 hours after fasting and stored at temperature -80°C. The remaining laboratory parameters were assessed by routine hospital laboratory methods.

Liver histology assessment

A liver biopsy was performed in all NAFLD patients using 16-guage Hepafix needle. Liver tissue containing at least 6 portal tracts was sent to an experienced pathologist to confirm NAFLD diagnosis, grading and staging of fibrosis. The grading and staging of NAFLD were assessed using NAFLD activity score (NAS) by Kleiner *et al*[17]. NAS scoring was calculated. Separate scoring was made for of steatosis (score 0-3), lobular inflammation (score 0-3) and hepatocyte ballooning (score 0-2). Combined scores NAS ranged from 0 to 8. The degree of liver fibrosis was graded from 0 to 4: grade 0 = no fibrosis, stage 1 = perisinusoidal or periportal fibrosis, stage 2 = perisinusoidal and periportal fibrosis, stage 3 = bridging fibrosis and stage 4 = cirrhosis. Patients with NAS score of less than 3 were defined as not NASH, NAS scores of 3-4 were defined as simple steatosis, and NAS scores of ≥5 were defined as NASH. Patients with fibrosis stage 3-4 were defined as advanced fibrosis.

Statistical analysis

Sample size was calculated based on the previous study of Kukla M. *et al*[12]. We estimated that 60 patients were needed in each group. The results were reported in mean ± standard deviation (SD), or median with interquartile range (IQR) as appropriate. Continuous variables were analyzed using student t-test or Mann-Whitney U-test, while categorical variables were analyzed using χ^2 test or Fisher exact test. The correlation between serum adipokines and degree of liver fibrosis was tested by Spearman's correlation coefficient. Multivariable stepwise linear regression analyses were performed to identify independent factors of NAS and fibrosis staging. *P*-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 18.0 for Windows

Table 1. Baseline characteristics of the NAFLD and the control groups.

Parameter	NAFLD group n = 60	Control group n = 55	*p*-value
Age (years)	54.78±8.7	46.92±8.1	<0.001
Male sex (%)	45.00	36.63	0.448
BMI (kg/m^2)	26.96±2.87	21.55±2.10	<0.001
Waist circumference (cm)	89.43±7.19	77.48±8.2	<0.001
SBP (mmHg)	137.05±14.4	121.02±10.7	<0.001
DBP (mmHg)	81.46±7.9	76.25±7.1	<0.001
AST (U/L)	60.68±21.8	22.81±5.7	<0.001
ALT (U/L)	100.08±35.8	26.05±10.6	<0.001
ALP (U/L)	80 (43-297)	56 (31-126)	<0.001
GGT (U/L)	106 (27-553)	26 (9-117)	<0.001
Triglyceride (mg/dL)	169.78±66.9	107.41±36.0	<0.001
Cholesterol (mg/dL)	210.45±42.8	184.8±24.8	<0.001
HDL (mg/dL)	47.38±10.9	57.89±13.9	<0.001
LDL (mg/dL)	135.43±37.2	122.92±26.9	0.04
Fasting glucose (mg/dL)	132.95±47.9	88.33±7.2	<0.001
Hemoglobin A1C (%)	7.11±1.5	5.71±0.3	<0.001
Fasting insulin (μiu/mL)	13 (2-75.7)	4.3 (2-7)	<0.001
HOMA-IR	3.86 (0.86-27.6)	0.94 (0.4-1.4)	<0.001
Underlying diseases			
Diabetes mellitus (%)	60.0	0	<0.001
Hypertension (%)	90.0	0	<0.001
Dyslipidemia (%)	95.0	0	<0.001

Table 2. Comparison of serum adipokines in the NAFLD and the control groups.

Adipokines	NAFLD group n = 60	Control group n = 55	*p*-value
Chemerin (ng/mL)	194.58±47.4	120.51±56.4	<0.001
Vaspin (ng/mL)	0.163 (0.018-2.1)	0.156 (0.006-1.865)	0.292
Omentin-1 (ng/mL)	452.25 (249.7-1482.4)	372.1 (151.4-1106.6)	0.006

(SPSS, Inc., Chicago, IL, USA).

Results

Baseline characteristics of study patients

A total of 60 patients in the NAFLD group and 55 healthy controls were enrolled. Baseline characteristics of the two groups were shown in Table 1. Patients in the NAFLD group had significantly higher BMI, waist circumference, blood pressure, liver biochemistry (AST, ALT, ALP and GGT), index of insulin resistance (fasting glucose, hemoglobin A1C, fasting insulin and HOMA-IR) and lipid profile (triglyceride, total cholesterol, HDL-C and LDL-C) compared to the control group. All NAFLD patients had metabolic disease (diabetes mellitus 60%, hypertension 90% and dyslipidemia 95%), and the percentages were higher than in the control group.

Serum adipokine levels

The serum levels of chemerin and omentin-1 were significantly higher in the NAFLD group compared to the control group. The mean level ± SD of serum chemerin in the NAFLD group and the control group was 194.58±47.4 and 120.51±56.4 ng/mL, (p<0.001). The median level of serum omentin-1 in the NAFLD group and the control group were 452.25 (IQR, 249.7-1482.4) and 372.1 ng/mL (IQR, 151.4-1106.6), respectively (p = 0.006). On the other hand, the serum vaspin was not significantly higher in the NAFLD group compared to the control group (p=0.292) (Table 2). The AUROC using serum level of chemerin, vaspin and omentin-1 for diagnosis of NAFLD were 0.843, 0.557 and 0.647, respectively (Figure 1).

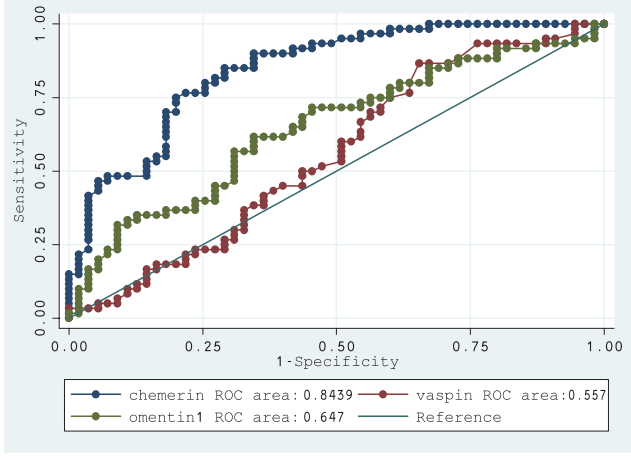

Figure 1. The AUROC of serum adipokines in the diagnosis of NAFLD.

Liver histology evaluation

NAS in NAFLD patients in this study ranged from 2 to 6 (NAS 3 = 22, NAS 4 = 15, NAS 5 = 17 and NAS 6 = 6). We identified 37 patients (61.7%) with simple steatosis and 23 patients (38.3%) with NASH. The baseline characteristics, anthropometric data, liver biochemistry, lipid profile and index of insulin resistance were not significantly different between patients with simple steatosis and NASH. The mean waist circumference, however, was significantly higher in patients with NAFLD (p = 0.049) (Table 3). The serum levels of chemerin and vaspin in patients with simple steatosis were non-significantly higher compared to patients with NASH (p = 0.386 and 0.673). On the other hand, the serum level of omentin-1 in patients with simple steatosis was non-significantly lower compared to patients with NASH (p = 0.807). Therefore, in our study, the serum adipokines; chemerin, vaspin and omentin-1 were not significantly different between patients with simple steatosis and patients with NASH.

In the NAFLD group, the degree of liver fibrosis ranged from 0 to 4 (F0 = 8, F1 = 24, F2 = 7, F3 = 15, F4 = 6). There were 21 patients (35%) with advanced fibrosis. Only age, hemoglobinA1C and serum omentin-1 were found to be significant higher in patients with advanced fibrosis compared to those without advanced fibrosis (p=0.002, 0.024 and 0.047, re-

spectively) (Table 4). We evaluated the relation coefficient of serum adipokines with NAS and fibrosis staging. Serum chemerin, vaspin and omentin-1 had no significant correlation with NAS. Fibrosis staging in NAFLD had positive correlation with only serum omentin-1 (r=0.303, p=0.018). Additional adjustment for covariate factors (age, NAS, hemoglobin A1C, BMI, WC and HOMA-IR) did not change the significant association between serum omentin-1 level and the degree of fibrosis (*p*=0.02). Multivariate logistic

Table 3. Characteristics of patients with simple steatosis compared to NASH.

Parameters	Simple Steatosis group n = 37	NASH group n = 23	*p*-value
Age (years)	55.08±8.2	54.30±9.5	0.741
BMI (kg/m^2)	26.51±3.0	27.69±2.4	0.123
Waist circumference (cm)	88.00±7.0	91.74±6.9	0.049
AST (U/L)	58.08±21.2	64.87±22.4	0.244
ALT (U/L)	100.81±36.0	98.91±36.1	0.844
Triglyceride (mg/dL)	174.68±71.6	161.91±59.1	0.477
Cholesterol (mg/dL)	217.32±47.0	199.39±32.8	0.116
HDL (mg/dL)	47.03±10.6	47.96±11.7	0.753
LDL (mg/dL)	136.92±38.8	133.04±35.3	0.699
Fasting glucose (mg/dL)	129.68±46.6	138.22±50.5	0.507
Hemoglobin A1C (%)	7.03±1.6	7.24±1.4	0.626
Fasting insulin (μiu/mL)	15.64±15.9	14.27±5.7	0.694
HOMA-IR	5.02±5.2	4.80±2.4	0.854
Chemerin (ng/mL)	198.8±48.5	187.7±45.8	0.386
Vaspin (ng/mL)	0.38±0.47	0.33±0.46	0.674
Omentin-1 (ng/mL)	498.2±192	514.4±277	0.790

Table 4. Characteristics of patients with advanced fibrosis and patients with non-advanced fibrosis.

Parameters	Non-advanced fibrosis n = 39	Advanced Fibrosis n = 21	*p*-value
Age (years)	52.28±8.2	59.43±7.8	0.002
BMI (kg/m^2)	26.49±2.9	27.85±2.5	0.070
Waist circumference (cm)	88.38±7.2	91.38±6.9	0.122
AST (U/L)	59.03±22.2	63.76±21.0	0.420
ALT (U/L)	105.15±37.2	90.67±31.7	0.120
Triglyceride (mg/dL)	174.69±69.9	160.67±61.5	0.427
Cholesterol (mg/dL)	215.92±43.1	200.29±41.3	0.176
HDL (mg/dL)	47.59±11.5	47.00±10.1	0.838
LDL (mg/dL)	139.82±41.9	127.29±25.6	0.157
Fasting glucose (mg/dL)	130.21±55.3	138.05±30.4	0.482
Hemoglobin A1C (%)	6.77±1.4	7.74±1.5	0.024
Fasting insulin (μiu/mL)	12.68±7.9	19.64±18.4	0.112
HOMA-IR	4.2±3.1	6.46±5.6	0.093
Chemerin (ng/mL)	200.50±43.6	183.59±53.0	0.219
Vaspin (ng/mL)	0.34±0.38	0.41±0.58	0.634
Omentin-1 (ng/mL)	453.99±163	598.27±294	0.047

regression analysis showed that only age and NAS were associated with the degree of fibrosis. Serum adipokines had no significant correlation with any component of liver histology (degree of steatosis, lobular inflammation and hepatocyte ballooning).

Discussion

As far as we are aware, our study is the first on serum adipokines and NAFLD in an Asian population. We found that serum chemerin and omentin-1 levels in the patients with NAFLD were significantly higher than those in normal controls. On the other hand, serum vaspin level was not significantly different between the two groups. These findings were in agreement with previous studies (Kukla M, *et al*[12] and Yusuf Y, *et al*[13]). The AUROC of serum chemerin level for diagnosis NAFLD was found to be 0.843, and a cut-off level for chemerin of 165.4 ng/mL would give a good sensitivity (76.67%), specificity (74.55%), positive likelyhood ratio (3.01) and negative likelihood ratio (0.31) for the diagnosis of NAFLD. The AUROC of omentin-1 was only 0.647, which was too low to be used as a diagnostic test. The use of serum chemerin for diagnosing NAFLD in patients with abnormal liver function test should be further evaluated for accuracy and cost-effectiveness.

In the NAFLD group, serum adipokines levels were tested in patients with simple steatosis and NASH, and tested for correlation with the degree of fibrosis. Only serum omentin-1 level was found to also correlate with the degree of liver fibrosis. However, the small sample size in this study might have made it difficult to achieve significant findings. The differentiation between patients with simple steatosis and NASH, and the degree of fibrosis prediction using serum adipokines requires further study.

Conclusion

Chemerin and omentin-1 were elevated in NAFLD patients and may be of diagnostic value. Chemerin, omentin-1 and vaspin were not associated with advanced fibrosis and NASH. Only age and NAS correlated with the degree of liver fibrosis. The role of adipokines in the pathogenesis of NAFLD requires further study.

REFERENCES

1. Bellentani S, Marino M. Epidemiology and natural history of non-alcoholic fatty liver disease (NAFLD). Ann Hepatol 2009; 8 suppl 1:S4-8.
2. Marchesini G, Bugianesi E, Foriani, *et al.* Non-alcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917-23.
3. Chitturi S, Farrell GC, George J. Non-alcoholic steatohepatitis in the Asia-Pacific region: future shock. J Gastroenterol Hepatol 2004;19:368-74.
4. Brunt EM. Nonalcoholic steatohepatitis. Semin Liver Dis 2004;24:3-20.
5. Cheng O, Sanyal AJ. Recent advances in non-alcoholic fatty liver disease. Curr Opin Gastroenterol 2010;26:202-8.
6. Strassburg CP, Manns MP. Approaches to liver biopsies techniques-revisited. Semin Liver Dis 2006;26:318-27.
7. Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Mol Cell Endocrinol 2010;314:1-16.
8. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allegy Clin Immunol 2005;115:911-9.
9. Tilg H. Adipocytokines in Nonalcoholic Fatty Liver Disease: Key Players Regulating Steatosis, Inflammation and Fibrosis. Curr Pharma Des 2010;16:1893-5.
10. Kloting N, Fasshauer M, Dietrich A, *et al.* Insulin-sensitive obesity. Am J Physiol Endocrino Metab 2010 Sep;299(3):506-15.
11. Bozaoglu K, *et al.* Chemerin is a novel adipokine associated with obesity and metabolic syndrome. Endocrinology 2007; 148:4687-94.
12. Kukla M, Zwirska-Korczala K, Hartleb M. Serum chemerin and vaspin in non-alcoholic fatty liver disease. Scandi J Gastroenterol 2010;45:235-42.
13. Yilmaz Y, Yonal O, Krut R, *et al.* Serum levels of omentin, chemerin and adipsin in patients with biopsy-proven nonalcoholic fatty liver disease. Scandi J Gastroenterol 2011;46:91-7.
14. Wada J. Vaspin: a novel serpin with insulin-sensitizing effects. Expert Opin Investig Drugs 2008;17:327-33.
15. Aktas B, Yilmaz Y. Serum levels of vaspin, obestatin, and apelin-36 in patients with nonalcoholic fatty liver disease. Metabolism 2011;60:544-9.
16. Auguet T, Quintero Y, Riesco D, *et al.* New adipokines vaspin and omentin. Circulating levels and gene expression in adipose tissue from morbidly obese women. BMC Medical Genetics 2011;12:60.
17. Kleiner DE, Brunt EM, Van Natta M, *et al.* Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-21.