Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences
Tome 77, No 1, 2024

MEDICINE

Clinical medicine

CHEMERIN IN OBESE PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE WITH OR WITHOUT PREDIABETES

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Received on April 28, 2020
Presented by D. Damianov, Member of BAS, on February 26, 2021

Abstract

Chemerin is one of the recently discovered adipokines closely related with white adipose tissue and insulin resistance (IR) in obesity, type 2 diabetes mellitus (T2DM), and nonalcoholic fatty liver disease (NAFLD), but some of these data remain controversial.

This study evaluated the relationship between serum chemerin levels, prediabetes and other biochemical and clinical parameters in obese patients with NAFLD.

A total of 79 obese NAFLD patients without ($n = 41$) and with prediabetes ($n = 38$) were included. Serum chemerin was measured using ELISA method.

Chemerin correlated with body mass index (BMI) ($r = 0.320$, $p < 0.01$), hip circumference ($r = 0.296$, $p < 0.05$) and visceral adiposity index (VAI) ($r = 0.297$, $p < 0.05$). Chemerin strongly correlated with hepatic steatosis index (HSI) ($r = 0.550$, $p < 0.01$).

Chemerin is adipokine, which has a significant, but yet not unequivocal role in the metabolic process related with obesity, insulin resistance and NAFLD. In this study we were not able to find an association of serum chemerin levels and prediabetes. There were correlations with BMI, hip circumference, Visceral adiposity index and Hepatic steatosis index.

The study was funded by Medical University of Sofia and performed in University Hospital "Alexandrovska", Bulgaria, Project No. 8516/12.12.2016, Contract No. D-114/2017.
https://doi.org/10.7546/CRABS.2024.01.15

Key words: chemerin, prediabetes, nonalcoholic fatty liver disease, obesity

Introduction. Nonalcoholic fatty liver disease (NAFLD) is one of the manifestations of the metabolic syndrome (MetS) closely related with T2DM, IR and obesity [1]. NAFLD comprises a broad spectrum of disorders ranging from simple fatty liver to nonalcoholic steatohepatitis (NASH) and cirrhosis and may increase the risks of T2DM and its complications [2]. Vice versa, the prevalence and mortality rates of NAFLD in patients with T2DM are also significantly higher. Both cardiovascular morbidity and liver disease progression have been associated with persistence or worsening of metabolic disorders. Therefore, early diagnosis and correction of metabolic abnormalities is very important for clinical practice.

Chemerin, also known as retinoic acid receptor responder protein 2 (RARRES2) is a new member of the growing adipokine family. It is highly expressed in adipose tissue, liver and lungs, secreted as inactive prochemerin and after activation binds to the chemokine-like receptor 1 (CMKLR1). Binding of chemerin to CMKLR1 in immune cells and adipose tissue stimulates chemotaxis at sites of inflammation. CMKLR1 is highly abundant in liver and is expressed by primary human hepatocytes, hepatic stellate cells, Kupffer cells, and bile duct cells [3]. Chemerin is involved in the regulation of inflammation, glucose and lipid metabolism, stimulates lipolysis by direct activation of hormone-sensitive lipase in white adipocytes and has been proven to have a direct positive correlation with adiposity indices [4]. Serum levels of chemerin have been also associated with NAFLD [5]. In recent studies chemerin in humans is related to BMI, concentration of triglycerides (TG) and total cholesterol (TC), blood pressure, and IR [6].

Furthermore, the data on the association between chemerin and prediabetes in obese NAFLD patients are still insufficient.

The aim of this study was to evaluate the relationship between chemerin, prediabetes and other biochemical and clinical parameters in obese patients with NAFLD.

Materials and methods. ***Study population.*** A total of 79 Caucasian subjects with NAFLD (6 male, 73 female; mean age 50.95 ± 11.11 years, from 25 to 65 years old), recruited in a Clinic of Endocrinology and Metabolic diseases, University Hospital "Alexandrovska", Sofia, participated in the study. Inclusion criteria were: Ultrasound based diagnosis of NAFLD with fibrosis stage 4 score (FIB-4) < 1.30 [1]; obesity (BMI ≥ 30 kg/m^2; normal or impaired fasting glucose and/or normal or impaired glucose tolerance; age between 18–65 years. Study participants were not included if any of the following criteria were present: Secondary cause of hepatic steatosis and absolute alcohol consumption > 20 g daily for women and > 30 g daily for men; diabetes mellitus; proven neoplasia; chronic kidney disease (eGFR calculated by CKD-EPI formula < 60 ml/min/1.73 m^2);

subclinical atherosclerosis or heart failure; hypo/hyperthyroidism and drug treatment for prediabetes and NAFLD. The subjects were divided into two groups. Group 1 included 41 obese NAFLD patients without carbohydrate disturbances. Group 2 included 38 obese NAFLD patients with prediabetes. The project was approved by the University ethics committee for clinical studies and all included patients signed an informed consent for participation in the study.

Methods. Anthropometric parameters as weight (kg), height (m), body mass index (BMI; kg/m^2), waist and hip circumference (cm), and arterial blood pressure (mmHg) were measured by standard criteria. Waist-to-hip ratio (WHR) and waist-to-stature ratio (WSR) were calculated. Visceral adiposity index (VAI) was performed using the following formula: VAI = (WC/(36.85 + (1.89 × BMI)) × (TG/0.81) × (1.52/HDL) for females VAI = (WC/(39.68 + (1.88 × BMI)) × (TG/1.03) × (1.31/HDL) for males. Percentage Body Fat (%) was measured by means of Body Impedance (BIA) by a TANITA™ TBF-215 GS Body Composition Analyzer in fasting state. Hepatic Steatosis Index (HSI) was performed using the following formula: HSI = 8×(ALT/ASTratio)+BMI(+2, if female; +2, if diabetes mellitus). HSI has an area under receiver-operating curve of 0.812 (95% confidence interval, 0.801–0.824). At values of < 30.0 or > 36.0, HSI rules out NAFLD with a sensitivity of 93.1%, or detects NAFLD with a specificity of 92.4%, respectively. Metabolic syndrome was diagnosed following the IDF criteria [7]. A standard oral glucose tolerance test (OGTT) with measurement of glucose and insulin on 0 min (glucose 0, insulin 0), 60 min (glucose 60, insulin 60) and 120 min (glucose 120, insulin 120), was performed. Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated, using the following formula: HOMA-IR = fasting plasma glucose (mmol/l) × fasting serum insulin (μIU/ml)/22.5. Insulin resistance was defined as a value of HOMA-IR > 2.5. The Quantitative Insulin Sensitivity Check Index [QUICKI Index = 1/log (fasting plasma glucose in mg/dl) + log (fasting insulin in μIU/mL)], as well as Stumvoll index of insulin sensitivity [ISI Stumvoll, based on plasma glucose (mmol/l) and insulin (pmol/l) concentrations during OGTT with or without demographic parameters (BMI, age)] were also calculated.

The diagnosis of NAFLD was based on the criteria of EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease [8]. Abdominal ultrasonography with Doppler sonography was performed in all patients. FIB-4 was calculated to exclude advance fibrosis. To assess subclinical atherosclerosis and peripheral artery disease we used the following non-invasive methods: 1) Intima-media thickness (IMT) measurement of the common carotid artery by Cardio Health Station (Panasonic, Japan); 2) Anckle-Brachial Index (ABI, represents the ratio between the systolic blood pressure at the ankle and the upper arm.

To detect distal small fibre neuropathy we measured sweat function, using Sudoscan™ (Impeto Medical, Paris, France).

Measurement of serum chemerin levels was performed by enzyme-linked immunosorbent assay (Human Chemerin ELISA kit, Biovendor, Czech Republic). The patients' samples were analyzed in duplicates. Analytical sensitivity of the test for chemerin detection was 0.1 ng/ml. For statistical analysis the data were processed using the statistical package IBM SPSS Statistics 25.0. The following statistical methods were applied: descriptive analysis, variation analysis, graphic analysis, Kolmogorov–Smirnov's one sample non-parametric test, and Shapiro–Wilk test; One-factor dispersion analysis ANOVA for several independent samples; Kruskal–Wallis non- parametric test for several independent samples; Mann–Whitney's non-parametric test for two independent samples; Correlation analysis for linear dependence between quantitative signs, binary logistics regression for evaluation of impact of the researched factors. The level of significance for rejecting the null hypothesis was $p < 0.05$.

Results. Significantly higher levels of VAI, very low density lipoproteins (VLDL), TG, plasma glucose and insulin in OGTT, HOMA-IR, as well as lower levels of high density lipoproteins (HDL) and QUICKI index was found in obese NAFLD patients with prediabetes (Group 2) than in normal OGTT (Group 1) (Table 1).

There was no statistically significant difference in serum chemerin levels between obese NAFLD patients with prediabetes compared to those patients without carbohydrate disturbances (192.34 ± 47.66 vs. 196.01 ± 47.66 mcg/ml, $p = 0.710$).

Chemerin correlated with BMI ($r = 0.320$, $p < 0.01$), hip circumference($r = 0.296$, $p < 0.05$) and VAI ($r = 0.297$, $p < 0.05$). With the BMI the correlation is moderate and the other two is weak. There was no correlation with the other investigated clinical and insulin resistance parameters (Table 2). Chemerin correlated markedly with HSI ($r = 0.550$, $p < 0.01$), unlike the other parameters linked to subclinical atherosclerosis and peripheral artery disease (Table 3). There were no statistically significant differences between the groups with/without MetS (190.81 ± 40.32 vs. 196.02 ± 45.19, $p = 0.616$) as well as in patients with/without dyslipidemia (199.76 ± 47.68 vs. 188.67 ± 39.40, $p = 0.265$).

Discussion. In this study we evaluated the relationship between serum chemerin levels, prediabetes and other biochemical and clinical parameters in obese patients with NAFLD. Chemerin correlates directly with BMI, hip circumference, surrogate marker for visceral fat (VAI) and HSI. NAFLD is commonly combined with overweight, abdominal obesity, or general obesity (up to 93%). Previous studies showed that NAFLD may increase the risk for T2DM and its complications [2]. The link between NAFLD and T2DM is supported by their common pathogenesis, insulin resistance. In a trial including 2839 patients with T2DM, the level of glycosylated hemoglobin in patients with NAFLD was significantly increased compared with patients with T2DM without NAFLD [9]. Additionally, the development of T2DM significantly increases the risk of cardiovascular and hepatic morbidity and mortality. The risk assessment for the occurrence of T2DM

T a b l e 1

Comparative analysis between groups 1 (without prediabetes) and 2 (with prediabetes) according to age, blood pressure, anthropometric and metabolic parameters

Parameters	Group 1		Group 2		P
	$\bar{X}$	SD	$\bar{X}$	SD	
Age (years)	51.20	12.97	50.68	8.83	0.84
BMI (kg/m2)	36.6	6.00	36.33	5.24	0.741
Waist (cm)	110.07	12.38	107.36	14.65	0.262
Hip (cm)	119.05	11.02	116.89	11.34	0.631
Fat tissue (%)	45.84	5.44	46.63	5.51	0.437
WHR	0.912	0.073	0.908	0.067	0.85
WSR	0.687	0.082	0.664	0.092	0.271
VAI	1.71	0.900	3.04	1.49	< 0.001
SBP (mmHg)	130.24	17.14	131.95	17.89	0.948
DBP (mmHg)	83.54	10.68	84.45	10.16	0.928
TC (mmol/l)	5.06	1.05	5.22	1.17	0.54
HDL (mmol/l)	1.42	0.29	1.10	0.25	< 0.001
LDL (mmol/l)	3.05	1.02	3.13	0.94	0.753
VLDL (mmol/l)	0.51	0.24	0.76	0.30	0.001
TG (mmol/l)	1.143	0.510	1.733	0.753	< 0.001
Glu 0(mmol/l)	5.30	0.42	6.13	0.52	< 0.001
Glu 60 (mmol/l)	7.20	2.16	9.82	2.37	< 0.001
Glu 120 (mmol/l)	5.37	1.17	7.35	1.86	< 0.001
Insulin 0 (µIU/ml)	11.88	5.57	16.87	7.40	0.004
Insulin 60 (µIU/ml)	82.07	60.07	103.57	49.41	0.03
Insulin 120 (µIU/ml)	39.40	23.01	98.13	64.15	< 0.001
HOMA-IR	2.82	1.42	4.36	2.27	0.002
QUICKI Index	2.25	1.00	1.51	0.53	0.001
ISI Stumvoll	0.057	0.023	0.045	0.045	0.322

Abbreviations: BMI, body mass index; WHR, Waist-to-hip ratio; WSR, waist-to-stature ratio; VAI, visceral adiposity index; HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; TG, triglycerides; Glu 0, glucose at the 0 min; Glu 60, glucose at the 60th min; Glu 120, glucose at the 120th min; Insulin 0, insulin at the 0 min; Insulin 60, insulin at the 60th min; Insulin 120, insulin at the 120th min; HOMA-IR, Homeostatic model assessment of insulin resistance; QUICKI, quantitative insulin-sensitivity check index; ISI Stumvoll, Stumvoll index of insulin sensitivity

Table 2

Correlation coefficients between chemerin and anthropometric parameters, blood pressure, lipid profile, blood glucose and insulin from OGTT, HOMA-IR, QUICKI, Stumvoll

Parameters	Chemerin
Age (years)	−0.109
SBP(mmHg)	0.007
DBP(mmHg)	−0.013
BMI (kg/m^2)	0.320**
Waist (cm)	0.187
Hip (cm)	0.296*
WHR	−0.025
WSR	0.232
VAI	0.297*
Fat tissue(%)	0.050
Glu 0 (mmol/l)	−0.072
Glu 60 (mmol/l)	0.039
Glu120 (mmol/l)	0.188
Insulin 0 (µIU/ml)	0.218
Insulin 60 (µIU/ml)	0.057
Insulin120 (µIU/ml)	0.168
HOMA-IR	0.196
QUICKI	−0.203
ISI Stumvoll	−0.205

* – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WHR, Waist-to-hip ratio; WSR, waist-to-stature ratio; VAI, visceral adiposity index; Glu 0, glucose at the 0 min; Glu 60, glucose at the 60th min; Glu 120, glucose at the 120th min; Insulin 0, insulin at the 0 min; Insulin 60, insulin at the 60th min; Insulin 120, insulin at the 120th min; HOMA-IR, homeostatic model assessment of insulin resistance; QUICKI, quantitative insulin-sensitivity check index; ISI Stumvoll, Stumvoll index of insulin sensitivity

Table 3

Correlation coefficients between chemerin and hepatic steatosis index, ankle-brachial index, intima-media thickness and autonomic neuropathy risk

Parameters	Chemerin
Hepatic steatosis index	0.550^{**}
Ankle-brachial Index	-0.189
Intima-media thickness	0.064
Autonomic neuropathy risk	0.147

* – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$

and the early diagnosis of carbohydrate and other metabolic abnormalities in obese subjects with NAFLD is of crucial importance for the prevention, treatment and reduction of complications and mortality. However, no significant differences in chemerin serum concentration were observed in the two groups with/without prediabetes. As expected, in the patients with prediabetes there were more significant abnormalities in HOMA-IR and sensitivity (QUICKI), lipids and VAI compared to the group of patients without carbohydrate disturbances. The absence of any correlation between serum chemerin and carbohydrate abnormalities in the present study could be due to our study population consisting of patients with obesity and NAFLD. Several studies reported that liver disease may affect circulating chemerin levels and demonstrate higher chemerin levels in patients with clinical or biopsy-proven NAFLD [5,10]. Döcke et al. [11] demonstrated that chemerin's mRNA and serum chemerin concentration were significantly elevated in patients with NAFLD compared to healthy individuals. Chemerin has been reported to be associated with NAFLD, obesity and T2DM, certain components of MetS, but many of these data remain controversial.

Some evidence showed that inflammatory cytokines may play a role in chemerin release from adipose tissue. IL-1b and TNF-α induce chemerin mRNA expression and secretion from 3T3-L1 adipocytes in vitro, and TNF-α increases serum total chemerin levels in wild-type mice but not in TNF receptor superfamily 1a/1b-deficient mice in vivo [12]. Muruganandan et al. [13] investigated the relationship between chemerin expression and peroxisome proliferator-activated receptor γ (PPARγ), a nuclear receptor that is a critical regulator of adipogenesis and adipokine expression. Rosiglitazone, a PPARγ agonist, reduces expression of chemerin selectively in mature adipocytes, both in vitro and in vivo. However, further studies are needed regarding the role of PPARγ activation in chemerin induction. Other investigators suggest chemerin to be a marker of MetS as chemerin expression in adipose tissue is increased in obese individuals [14].

We did not find any association between chemerin levels and fasting glucose and insulin, glucose and insulin during OGTT, surrogate markers for insulin resistance (HOMA-IR), and insulin sensitivity (QUICKI Index). There were no correlations between chemerin and components of MetS, WHR and WSR. The increase of chemerin levels in obese patients with NAFLD may be more influential than effects of IR even more that it correlates with hepatic steatosis index. Some of our results also correspond to the data already reported by other authors [16]. The meta-analysis of MetS and obesity markers indicated that TG, TH, CRP, BMI, TBF%, WC, WHR and leptin were positively correlated with chemerin. Other markers like systolic blood pressure, diastolic blood pressure, HbA1c, fasting plasma glucose, LDL-C, ALT and r-GT were not significantly correlated [15]. Other studies reported chemerin to be associated with many components of the MetS, including BMI, TG, HDL-C, and hypertension, fasting insulin, HOMA-IR and also with systemic markers of inflammation, such as high sensitivity C-reactive protein, interleukin-6 [16, 17]. Sell et al. [18] demonstrated that the release of chemerin is clearly increased from adipose tissue explants of obese patients compared with lean control subjects. Furthermore, chemerin release from adipose tissue correlated with WHR and fat cell volume, whereas no correlation could be found with blood pressure and HOMA. The same authors demonstrate that chemerin is necessary for normal adipogenesis but also induces insulin resistance in peripheral tissues such as skeletal muscle and chemerin might be involved in the negative cross talk between skeletal muscle and adipose tissue. Moreover, another work revealed that chemerin secretion is increased by insulin in adipose tissue explants, whereas metformin is able to reduce chemerin secretion [19].

This study has some limitations. The sample size was small and we included more women than men because the participation rate was higher among women. Some strengths of this study are that all subjects were with NAFLD and obesity with or without prediabetes and did not receive any glucose-lowering medications. The whole group was homogeneous by age and BMI.

Conclusions. Chemerin is adipokine, which has a significant, but yet not unequivocal role in the metabolic process related with obesity, insulin resistance and NAFLD. In this study we were not able to find an association of serum chemerin levels and prediabetes. There were correlations with BMI, hip circumference, visceral adiposity index and hepatic steatosis index. The lack of association between serum chemerin and carbohydrate disturbances in obese patients with NAFLD may suggest that chemerin cannot be a predictor of carbohydrate disorders. Chemerin is more related to obesity and NAFLD than other components of MetS. Further experimental and clinical studies are necessary to confirm these hypotheses.

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