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Serum levels of the adipokine chemerin in relation to renal function

Running Title: Chemerin and renal function

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Objective: To investigate serum levels of the adipokine chemerin in patients on chronic hemodialysis (CD) as compared to controls with a glomerular filtration rate (GFR) above 50 ml/min.

Research Design and Methods: Chemerin was quantified by ELISA in control (n=60) and CD (n=60) patients and correlated to clinical and biochemical measures of renal function, glucose, and lipid metabolism, as well as inflammation, in both groups.

Results: Median serum chemerin levels were more than 2-fold higher in CD patients (542.2 µg/l) as compared to subjects with a GFR above 50 ml/min (254.3 µg/l) ($p < 0.001$). Furthermore, glomerular filtration rate as assessed by the original Modification of Diet in Renal Disease formula independently predicted circulating chemerin concentrations in multiple regression analyses in both control subjects ($p < 0.05$) and patients on CD ($p < 0.01$).

Conclusions: We demonstrate that markers of renal function are independently related to circulating chemerin levels.

Recently, chemerin has been identified as a novel adipocyte-secreted factor playing a crucial role in adipocyte differentiation and insulin signaling (1-4). Several studies have quantified circulating chemerin in humans. Thus, two reports found an independent association between chemerin and markers of inflammation (5,6). Furthermore, correlations between circulating chemerin and metabolic syndrome-related parameters have been described (6-8). In contrast to other adipokines (9-12), no data have been published so far about the relation of chemerin to renal function.

RESEARCH DESIGN AND METHODS

Subjects: The design of the study has been described in detail recently (9-12). Briefly, 120 Caucasian men (n= 62) and women (n= 58) were recruited with 60 patients having a glomerular filtration rate (GFR) above 50 ml/min (controls) as assessed by the original Modification of Diet in Renal Disease formula (13) and 60 patients being on hemodialysis (CD). 30 controls and 32 CD patients had type 2 diabetes mellitus (T2DM). Patients with active inflammatory diseases including pneumonia, urinary tract infection, endocarditis, sinusitis, and cholangitis were excluded from the study. Furthermore, patients with end-stage malignant diseases of any origin were excluded. Inactive systemic lupus erythematodes, stable coronary heart disease, and previous stroke were not exclusion criteria. The study was approved by the local Ethics Committee and all subjects gave written informed consent before taking part in the study.

Assays: Blood samples were taken after an overnight fast. In CD patients, blood was drawn just before hemodialysis started. Chemerin (Biovendor, Modrice, Czech Republic, intra-assay coefficient of variation [CV]: 5.1 % - 7.0 %, inter-assay CV: 6.9 % - 8.3 %), adiponectin (Mediagnost, Reutlingen, Germany, intra-assay CV: <4.7 %, inter-assay CV: <6.7 %), and leptin (Mediagnost, Reutlingen, Germany, intra- and inter-assay CV: <10 %) were determined with ELISAs according to the manufacturers´ instructions. Free fatty acids (FFA), cholesterol, triglycerides (TG), C reactive protein (CRP), insulin, and other routine laboratory parameters were measured in a certified laboratory.

Statistical analysis: SPSS software version 15.0 (SPSS, Chicago, IL) was used for all statistical analyses as further specified in the results section and in the table legend. Distribution was tested for normality using Shapiro-Wilk W test and non-normally distributed parameters were logarithmically transformed before multivariate analyses.

RESULTS

Chemerin serum levels are increased in CD patients. Table 1 summarizes clinical characteristics of the subgroups studied (Control, CD). In table 1 and throughout the text, all continuous variables are given as median ± interquartile range. Median circulating chemerin was more than 2-fold higher in CD patients (542.2 ± 98.1 µg/l) as compared to controls (254.3 ± 88.7 µg/l) ($p < 0.001$) (Table 1). In contrast, a significant difference in chemerin concentrations could not be demonstrated depending on gender (female: 324.4 ± 284.6 µg/l; male: 443.6 ± 315.2 µg/l) and T2DM (T2DM: 388.1 ± 303.7 µg/l; non-T2DM: 331.0 ± 274.6 µg/l). CD patients had a significantly lower body mass index (BMI) as compared to controls ($p < 0.05$) (table 1).

Univariate correlations. Using the Spearman´s rank correlation method, serum chemerin concentrations positively correlated with BMI ($r=0.398$, $p=0.002$), fasting insulin (FI, $r=0.408$, $p=0.001$), leptin ($r=0.516$, $p<0.001$), and CRP ($r=0.256$, $p=0.049$) in controls. In addition, chemerin negatively

correlated with GFR (r=-0.372, p=0.003) in control patients. In CD patients, circulating chemerin levels were negatively associated with GFR (r=-0.413, p=0.001).

Multivariate regression analyses. Multiple linear regression analysis revealed that GFR (logarithmically transformed [log], standardized β-coefficient=-0.337, p=0.013) but not FI (log, standardized β-coefficient=0.186, p=0.128), leptin (log, standardized β-coefficient=0.091, p=0.588), and CRP (log, standardized β-coefficient=0.138, p=0.236) remained independently associated with circulating chemerin (log) levels in controls after adjustment for age (standardized β-coefficient=-0.033, p=0.793) and gender (standardized β-coefficient=0.250, p=0.097). A similar result was obtained when BMI instead of leptin was included in the model (data not shown). In addition, GFR (log, standardized β-coefficient=-0.351, p=0.007) predicted circulating chemerin (log) independent of age (standardized β-coefficient=-0.223, p=0.072) and gender (standardized β-coefficient=-0.076, p=0.546) in CD patients.

DISCUSSION

In the current study, we show for the first time that circulating chemerin levels are more than 2-fold higher in CD patients as compared to controls. Furthermore, CD is a strong independent predictor of chemerin concentrations in multivariate analysis (data not shown). Moreover, GFR remains independently associated with circulating chemerin in multivariate analysis in both control and CD patients. Here, functional studies including urine analyses should be performed to define whether renal elimination influences serum levels of chemerin. Furthermore, renal production of chemerin has been shown (1-4) and it should be determined to which extent this kidney-derived chemerin contributes to circulating levels of the adipokine in control and CD patients. Moreover, since chemerin modulates inflammation (14,15), its contribution to renal disease-associated metabolic and vascular complications should be elucidated in future studies.

Recently, an association of chemerin serum levels with metabolic syndrome-related parameters including BMI (5-7), FI (7), TG (6-8), HDL cholesterol (5-8), leptin (5,6), and CRP (5,6) has been shown. In agreement with these findings, chemerin is positively correlated with BMI, FI, leptin, and CRP in univariate analyses in control patients in our study. However, these associations in controls are all lost in multivariate analyses after controlling for renal function whereas GFR remains independently associated with circulating chemerin. Interestingly, GFR also independently predicts chemerin serum levels in CD patients in our study. These results indicate that renal function is a significant predictor of circulating chemerin not only in subjects with (near) normal glomerular filtration but also in patients with end-stage renal disease.

Some limitations of the study have to be pointed out: First, the study has a cross-sectional design and, therefore, causality cannot be established. Second, the sample size is relatively small and it is well possible that various non-significant associations in multivariate analyses would have become statistically significant if larger samples were studied. Third, differential misclassification of covariates such as T2DM is possible, since T2DM was only excluded in the control but not in the CD patients by 75 g oral glucose tolerance tests due to the necessary fluid restriction in the latter group.

Taken together, our results suggest that renal filtration independently predicts circulating chemerin.

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	Control	**CD**
N	60	60
Chemerin (µg/l)	254.3 ± 88.7	542.2 ± 98.1*
Age (years)	63 ± 17	67 ± 18
Gender (m/f)	27/33	35/25
Diabetics/Nondiab.	30/30	32/28
BMI (kg/m^2)	28.7 ± 5.2	27.0 ± 7.5*
SBP (mmHg)	125 ± 21	120 ± 29
DBP (mmHg)	75 ± 12	70 ± 20
GFR (ml/min)	87 ± 29	7 ± 4*
FG (mmol/l)	5.8 ± 2.6	4.8 ± 1.7*
FI (pmol/l)	47.7 ± 47.7	38.3 ± 61.8
HOMA-IR	1.8 ± 2.2	1.1 ± 2.5
FFA (mmol/l)	0.5 ± 0.2	0.7 ± 0.5
Cholesterol (mmol/l)	5.1 ± 1.1	4.3 ± 1.3*
HDL (mmol/l)	1.3 ± 0.4	1.0 ± 0.5*
LDL (mmol/l)	3.1 ± 1.1	2.4 ± 1.0*
TG (mmol/l)	1.3 ± 0.8	1.6 ± 1.3*
Adiponectin (mg/l)	6.3 ± 4.8	11.9 ± 15.0*
Leptin (µg/l)	17.5 ± 23.9	20.9 ± 45.2
CRP (mg/l)	2.6 ± 4.2	5.0 ± 18.8*
β-blocker (%)	27 (45)	41 (68)†
ACE/AT1-I (%)	27 (45)	40 (67)†
Calcium channel blocker (%)	14 (23)	19 (32)

Table 1. Baseline characteristics of the study population. ACE-I, Angiotensin-converting enzyme inhibitor; AT1-I, Angiotensin AT1 receptor inhibitor; BMI, Body mass index; CD, Chronic hemodialysis; CRP, C reactive protein; DBP, Diastolic blood pressure; FG, Fasting glucose; FI, Fasting insulin; FFA, Free fatty acids; GFR, Glomerular filtration rate; HDL, High-density lipoprotein; HOMA-IR, Homeostasis model assessment of insulin resistance; LDL, Low-density lipoprotein; SBP, Systolic blood pressure; TG, Triglycerides. Values for median ± interquartile range or the total number and percentage of patients taking a medication are shown. *indicates $p < 0.05$ as compared to control as assessed by Mann-Whitney-U test. †indicates $p < 0.05$ as compared to control as assessed by χ^2 test.