Serum leptin, resistin, visfatin and adiponectin levels in tumour necrosis factor receptor-associated periodic syndrome (TRAPS)

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ABSTRACT

Objectives. *The aims of our study were to evaluate serum leptin, resistin, visfatin and adiponectin levels in patients with tumour necrosis factor receptor-associated periodic syndrome (TRAPS), in comparison to healthy controls, and to correlate their levels to parameters of disease activity and/or severity.*

Methods. *Serum leptin, resistin, visfatin and adiponectin levels were obtained from 14 TRAPS patients carrying mutations involving cysteine residues, from 16 TRAPS patients carrying other mutations, and from 16 healthy controls. Demographic, clinical and laboratory parameters, including amyloidosis were entered for each patient. Comparisons between groups as well as reciprocal comparisons have been evaluated.*

Results. *Serum leptin, resistin, visfatin and adiponectin did not significantly differ among the 3 groups. Patients carrying cysteine residues mutations showed lower visfatin serum levels than patients carrying other mutations ($p<0.02$). Serum leptin significantly correlated with the number of attacks/year (multiple $R=0.32$, multiple adjusted $R^2= 0.19$, $p<0.03$). Serum adiponectin levels significantly correlated with the presence of amyloidosis (multiple $R=0.79$, multiple adjusted $R^2=0.57$, $p<0.03$). Adiponectin values were a significant predictor for amyloidosis (AUC 0.75, 95 CI: 0.56–0.94, $p<0.03$), with a predicting cut-off value set at 23.16 pg/ml, the predictive positive value was 53.8%. Visfatin serum levels resulted respectively related to leptin ($r_s=0.42$, $r^2=0.18$, $p<0.02$) and to resistin ($r_s=0.57$, $r^2=0.32$, $p<0.01$) serum levels; whilst leptin and resistin serum levels did not reciprocally correlate.*

Conclusion. *Although a prospective design study and larger cohort are mandatory, adipokines serum levels and their correlations with parameters of disease activity and/or severity seem to show a baseline pattern in TRAPS patients.*

Introduction

The autoinflammatory disorders (AIDs) are a group of diseases of the innate immune system characterised by unprovoked recurrent attacks of fever with localised inflammation that can affect multiple organ systems. AIDs are caused by mutations of genes which are involved in the regulation and/or activation of the inflammatory response (1).

Tumour necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS) is the most common autosomal dominant autoinflammatory disorder and is caused by mutations in the *TNFRSF1A* gene encoding the 55-kD receptor for TNF-α (*TNFRSF1A*), a transmembrane glycoprotein that consists of an extracellular domain comprising 4 tandem repeat cystein-rich domains (CRD1-4), a transmembrane region and an intracellular death domain (2). *TNFRSF1A* extracellular domain mutations negatively affect both *TNFRSF1A* expression and function (2-4).

The majority of mutations is localised in the CRD1 and CRD2 domains, in fact most mutations described involve cysteine residues and are associated with a higher disease penetrance (5).

Characteristic features of TRAPS include recurrent fever, lasting typically more than 1 week, periorbital oedema, a migratory erythaematous plaque with

Table I. Table summarises clinical and demographic data of patients and controls, expressed as median (range) when necessary.

	Group 1 n=14	Group 2 n=16	Group 3 n=16	*p*-value
Age (yrs)	39 (25–42)	34.5 (19–67)	36 (22–65)	
Gender (F/M)	6/8	8/8	7/9	
Disease onset (yrs)	3.0 (1–38)	22 (1–49)	–	**<0.004**
Height (cm)	170 (155–177)	167 (162–185)	169 (158–183)	
Weight (kg)	73 (45–86)	70 (47-90)	71 (46–92)	
BMI (kg/m^2)	24.84 (15.5–31.22)	22.10 (16.30–33.05)	23.34 (17.04–32.28)	
n of attacks/year	6 (2–10)	4 (3–13)	–	**<0.04**
Fever duration (days)	10 (8–15)	7 (5–14)	–	**<0.003**
ESR (mm/hr)	6 (5–116)	4 (7–82)	–	0.17
CRP (mg/dl)	1.81 (0.1–10.10)	1.38 (0.10–17)	–	0.85
SAA (mg/l)	72 (2.07–543.00)	53.8 (1.09–1510.0)	–	0.90
Amyloidosis (present/absent)	6/8	2/14	–	**<0.05**

Group 1: TRAPS patients carrying mutations involving cysteine residues; Group 2: TRAPS patients carrying other mutations; Group 3: healthy controls.

underlying myalgia and arthralgia; serosal membrane inflammation is also possible (6-8). Amyloidosis is the most serious long-term complication of TRAPS, and occurred in about 25% of patients in prebiological era (9). Patients carrying mutations involving cysteine residues may be younger at disease onset and suffer more prolonged and frequent fever attacks (10), thus demonstrating a higher severity of their clinical phenotype; these patients are currently considered to be at higher risk of developing life-threatening AA amyloidosis (9).

White adipose tissue produces more than 50 adipokines and other molecules that participate through endocrine, paracrine, autocrine or juxtacrine mechanisms of action in a wide variety of physiopathological processes, including food intake, insulin sensitivity, vascular sclerotic processes, immunity and inflammation (11-13).

Among the adipokines known to be secreted by adipose tissue, tumour necrosis factor (TNF)-α, interleukin (IL)-6, leptin, resistin and visfatin are considered to be pro-inflammatory, whereas adiponectin has been described to have anti-inflammatory as well as pro-inflammatory properties depending on its molecular form (14). It has been demonstrated that these adipokines can play a fundamental role in inflammatory rheumatological autoimmune diseases (12, 13, 15, 16), and that in several rheumatic diseases, they are often associated with increased cardiovascular risks (17). To date, with regard to AIDs, serum adipokines have been evaluated only in familial Mediterranean fever (FMF), the most common autosomal recessive disorder (18-21). The aims of our study were to evaluate serum leptin, resistin, visfatin and adiponectin levels in patients with TRAPS, in comparison to healthy controls, and also to correlate their serum levels to parameters of disease activity and/or disease severity.

Patients and methods

Patients

In this study, 30 TRAPS patients were recruited from the Rheumatology Unit of the Department of Clinical Medicine and Immunologic Sciences, University of Siena, Italy, and from the Amyloid Research and Treatment Center, Biotechnology Research Laboratories, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

Serum leptin, resistin, visfatin and adiponectin levels were obtained from 14 TRAPS patients carrying mutations involving cysteine residues, known to be associated with a higher disease penetrance (C43Y: 3/14 pts; C88Y: 2/14 pts; C55Y: 1 pts; C114W: 1/14 pts; C52Y: 4/14 pts; C43R: 2/14 pts; C73R: 1/14 pts) (Group 1), and from 16 TRAPS patients carrying other mutations (T50M: 5/16 pts; S59P: 1/16 pts; L167-G175del: 2/16 pts; R92Q: 5/16 pts; delta 103-104del: 1/16 pts; P46L: 1/16 pts; V95M: 1/16 pts) (Group 2) as well as from 16 genetically negative healthy controls attending our outpatient clinic (Rheumatology Unit of Department of Clinical Medicine and Immunologic Sciences, University of Siena, Italy) for arthralgias and/or musculoskeletal pain (fibromyalgia patients, and subjects with tendinitis, bursitis, and primary carpal tunnel syndrome) (Group 3). Healthy controls underwent detailed clinical, laboratory, and instrumental investigations in order to rule out possible rheumatic diseases, infections, endocrine and/or metabolic disorders. Among healthy controls, none presented any sign of inflammation and all of them showed inflammatory markers within normal values. All subjects were Caucasians of Italian origin.

Table I summarises the main clinical and demographic characteristics and laboratory data of Group 1 and Group 2 patients and the main demographic characteristics of healthy controls. For the purposes of this study, we excluded subjects with a history of diabetes mellitus, unstable weight, and those treated with medications known to affect body weight.

Six out of 14 Group 1 patients were receiving corticosteroids (7.5–17.5 mg/daily of prednisone), 2/14 were treated with the recombinant human IL-1 receptor antagonist anakinra (100 mg/daily) and 1/14 was treated with the TNF-α neutralising agent etanercept (50 mg once a week). Seven out of 16 Group 2 patients were receiving corticosteroids (7.5–12.5 mg/daily of prednisone), 2/16 were treated with anakinra (100 mg/daily), 1/16 with methotrexate (10 mg/daily), 1/16 with etanercept (50 mg/weekly), and 3/16 were receiving colchicine (1mg/daily). The remaining patients were not receiving any medication.

Informed consent was obtained both from the patients and from the healthy controls, in accordance with the local ethics committee regulations.

Assessment parameters

Assessment parameters included: gender, BMI, age, age at disease onset, duration of fever episodes, number of fever episodes/year, amyloidosis (presence/absence).

Laboratory assessments

Blood samples from TRAPS patients were collected during fever-free and symptom-free intervals.

Blood samples (6 ml) were drawn from an antecubital vein with the patient in the supine position in the morning after an overnight fast. The blood was immediately centrifuged and serum was stored at -80°C until analysed.

Serum leptin levels were detected with the enzyme-immunoassay method using Leptin (human) EIA Kit (Alexis assay designes/Enzo life Sciences). Sensitivity of samples was 23.4 pg/ml. Inter- and intra-assay coefficients of variation were 3.7–15.2% and 4.4–13.4%, respectively. Serum resistin levels were detected with the enzyme-linked immunosorbent assay method using Resistin (human) Elisa kit (AdipoGen Inc., Korea). Sensitivity of samples was 100 pg/ml. Inter- and intra-assay coefficients of variation were 4.2–7.2% and 2.8–5.2%, respectively. Serum visfatin levels were detected with the enzyme-linked immunosorbent assay method using Nampt(Visfatin/PBEF)(human) Elisa kit (AdipoGen Inc., Korea). Sensitivity of samples was 30 pg/ml. Inter- and intra-assay coefficients of variation were 4.7–7.2% and 2.3–9%, respectively.

Serum adiponectin levels were determined with the enzyme-linked immunosorbent assay method using Adiponectin (human) Elisa kit (AdipoGen Inc., Korea). Sensitivity of samples was 100 pg/ml. Inter- and intra-assay coefficients of variation were 2.8–5.5% and 2.9–3.8%, respectively.

Other laboratory assessment parameters included: a) erythrocyte sedimentation rate (ESR), b) C-reactive protein (CRP), c) serum amyloid A (SAA).

SAA serum concentration was determined with a commercial solid phase sandwich Enzyme linked-immunosorbent assay (ELISA) (Human SAA, BioSource Europe S.A., Belgium). The assay sensitivity was <4 ng/ml. The normal value of SAA was <6.4 mg/l.

ESR was measured using the Westergren method. Values are expressed in mm/hour. An ESR <15 mm/hour was considered to be normal for males and an ESR <20 mm/hour was considered to be normal for females. Serum CRP concentrations were measured using a nephelometric immunoassay. Values are expressed in mg/dl. A CRP <0.5 mg/dl was considered to be normal.

Statistical analysis

All results are expressed as mean ± standard deviation (SD) or median (range). Mann-Whitney U-test, with Fisher's exact test, when appropriate, and analysis of covariance (ANCOVA) with least significant difference (LSD) correction were used to evaluate the mean differences (±SD) between groups, considering the following covariates for ANCOVA: gender, age, age at disease onset, age at the time of collecting sample, weight, height, BMI, SAA levels, ESR, CRP, the presence/absence of amyloidosis, the number of attacks/year, the duration of the fever attacks, entered as days, and treatment (steroids and biological modifier drugs) at the time of serum sample collection. The Spearman rank correlation test was used to determine correlation coefficients between the four adipokines serum levels and the above reported entered variables, including the type of identified mutation. Multiple stepwise regression was performed to determine variables, including demographic variables, that could correlate independently; the predictors used in the final model were those showing a significant correlation in the univariate analysis. A receiver operating characteristic curve (ROC) was constructed for determination of optimal cut-off values of adiponectin for predicting the development of amyloidosis.

Non-parametric tests were used, where necessary, due to the small size of our groups and to the skewness of our data. Levels of $p<0.05$ were considered statistically significant. Analyses were performed on SPSS package for Windows, version 13.0 (SPSS, Inc., Chicago, IL, USA).

Results

The three groups were homogeneous for the following reported demographic variables: gender, age at enrollment, weight, height, and BMI.

Group 1 and Group 2 showed significant differences regarding age at disease onset ($p<0.004$), the number of attacks/year ($p<0.04$), the duration of fever attacks ($p<0.003$), and the presence/absence of amyloidosis ($p<0.05$) (Table I).

Serum leptin, resistin and adiponectin did not significantly differ among the 3 groups. Conversely, visfatin serum levels resulted significantly different between the 3 groups ($p=0.02$). In fact, serum visfatin levels were lower in Group 1 patients in comparison to Group 2 patients (1.48±0.95 pg/ml *vs.* 3.54±2.88 pg/ml, $p<0.008$); no difference was instead detected regarding controls (Table II) (Fig. 1).

Serum leptin was significantly correlated with the number of attacks/year ($r_s=0.48$, $r^2=0.21$, $p<0.001$) and inversely correlated with the duration of fever attacks, entered as days ($r_s=-0.42$, $r^2=0.16$, $p<0.002$). However, there was no correlation between serum leptin levels and SAA levels ($r_s=0.06$, $p=0.7$), nor with the presence of SAA increased values ($r_s=0.19$, $p=0.3$), presence/absence of amyloidosis ($r_s=0.27$, $p=0.1$), ESR ($r_s=0.01$ $p=0.9$), CRP ($r_s=0.02$, $p=0.9$), age at disease onset ($r_s=0.32$ $p=0.1$), steroid treatment ($r_s=0.18$ $p=0.3$), or biologic modifier treatment ($r_s=-0.14$, $p=0.4$). In multivariate analysis, controlled for demographic variables, including gender, age, weight, height and BMI, leptin serum levels maintained their relationship with the number of attacks/year, but not with the duration of the fever attacks (multiple $R=0.32$, multiple adjusted $R^2=0.19$, $p<0.03$) (Fig. 2).

Serum resistin levels did not significantly correlate with number of attacks/year ($r_s=0.02$, $p=0.9$), duration of fever episodes, entered as days ($r_s=0.25$, $p=0.1$), SAA levels ($r_s=0.10$, $p=0.5$), nor with the presence of SAA increased values ($r_s=-0.11$, $p=0.5$), the presence/absence of amyloidosis ($r_s=0.33$, $p=0.07$), ESR ($r_s=0.23$, $p=0.3$) and CRP ($r_s=0.05$, $p=0.7$), age at disease onset ($r_s=0.25$, $p=0.1$), steroid treatment ($r_s=-0.12$, $p=0.4$), or biologic modifier treatment ($r_s=0.01$, $p=0.9$).

Serum visfatin levels significantly correlated with the number of attacks/year ($r_s=0.42$, $r^2=0.19$, $p<0.003$), whilst inversely correlated with the presence of mutations involving cysteine residues

Table II. Mean serum levels (±SD) of adiponectin, leptin, resistin and visfatin in patients and controls.

	Leptin pg/ml	Resistin pg/ml	Visfatin pg/ml	Adiponectin pg/ml
Group 1 n=14	215.91±386.96	6.13±10.89	1.48±0.95	22.80±9.34
Group 2 n=16	208.84±272.49	14.37±18.93	3.54±2.88	19.98±10.0
Group 3 n=16	133.26±142.63	6.02±5.76	2.62±1.49	17.76±6.07
p-value	0.67	0.13	0.02	0.27

Group 1: TRAPS patients carrying mutations involving cysteine residues; Group 2: TRAPS patients carrying other mutations; Group 3: healthy controls.

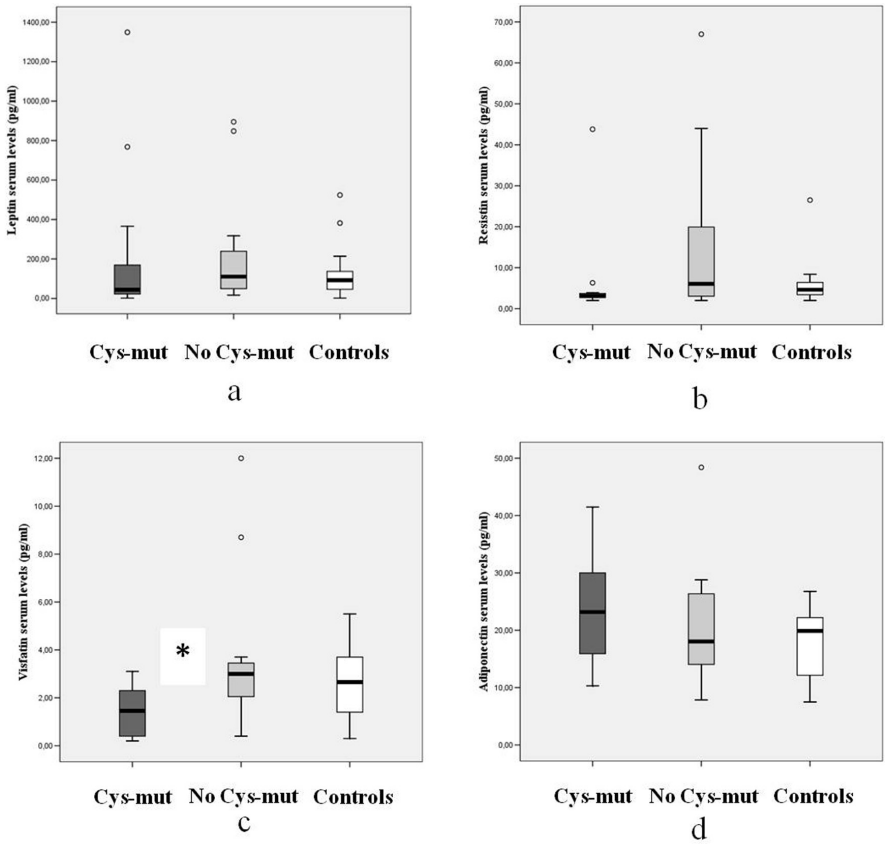

Fig. 1. Figure shows leptin (**a**), resistin (**b**), visfatin (**c**) and adiponectin (**d**) serum levels in TRAPS patients carrying mutations involving cystein residues (Cys-mut), TRAPS patients carrying other mutations (No Cys-mut) and healthy controls. The central line represents the distribution median, boxes span 25th to 75th percentiles, and error bars extend from 10th to 90th percentiles. Dots (°) are outlier values, higher than the 90th percentile. *represents *p*-value 0.008.

(r_s=-0.57, r^2=0.19, p<0.001) and the duration of fever attacks, entered as days (r_s=-0.43, r^2=0.14, p<0.002). Its levels did not, however, correlate with SAA levels (r_s=0.02, p=0.09), nor with its increased values (r_s=0.11, p=0.5), with the presence/absence of amyloidosis (r_s=0.18, p=0.3), age at disease onset (r_s=0.12, p=0.2), ESR (r_s=0.30, p=0.2), CRP (r_s=0.08, p=0.6), steroid treatment (r_s=-0.03, p=0.8), or biologic modifier treatment (r_s=-0.06, p=0.7). However, in multivariate analysis, controlled for demographic variables including gender, age, weight, height and BMI, the presence of mutations involving cysteine residues remained the single predictors of visfatin serum levels (multiple R=-0.43, multiple adjusted R^2=-0.15, p<0.01).

Adiponectin serum levels did not correlate with the number of attacks/year (r_s=0.28, p0.1), the age at disease onset (r_s=0.07, p=0.7), the duration of fever attacks, entered as days (r_s=0.14 p=0.4), ESR (r_s=0.15 p=0.5), CRP (r_s=0.13, p=0.5), steroid treatment (r_s=-0.19, p=0.9) or biologic modifier treatment (r_s=0.01, p=0.5). Serum adiponectin levels were correlated with the presence of amyloidosis (r_s=0.49, r^2=0.29, p=0.008), and inversely correlated with the presence of increased SAA levels (r_s=-0.42, r^2=0.17, p<0.04), although not with SAA levels (r_s=-0.21, p=0.2). In multivariate analysis, controlled for demographic variables including gender, age, weight, height and BMI, serum adiponectin levels significantly correlated with the presence of amyloidosis, but not with an increased SAA (multiple R=0.79, multiple adjusted R^2=0.57, p<0.03). Adiponectin serum levels were significantly higher in patients with amyloidosis compared to patients without amyloidosis (26.92±7.53 *vs*. 19.25±9.63 pg/ml, p<0.05) (Fig.3). ROC analysis was used to examine the diagnostic accuracy of adiponectin serum levels to discriminate patients with and without amyloidosis. The area under the ROC curve (AUC) was 0.75 (95 CI: 0.56–0.94), thus resulting adiponectin values were a significant predictor for amyloidosis (p<0.03). The optimal cut-off value for predicting amyloidosis was set at 23.16 pg/ml; serum adiponectin levels >23.16 had a sensitivity of 87.5% (7/8), and a specificity of 77.3% (5/22), with a predictive positive value of 53.8% (7/13).

Adiponectin serum levels did not show any statistically significant correlation with the other considered adipokines. Visfatin serum levels, corrected for their specific relationships with age, weight and BMI, resulted respectively related to leptin (r_s=0.42, r^2=0.18, p<0.02) and to resistin (r_s=0.57, r^2=0.32, p<0.01) serum levels; leptin and resistin serum levels did not reciprocally correlate (r_s=0.22, p=0.2).

Discussion

In recent years, scientific interest in adipose tissue-derived peptides has increased dramatically since several mediators known as adipokines such as leptin, resistin, visfatin and adiponectin have been shown to play a relevant role in systemic inflammation (11). AIDs are typical systemic inflammatory conditions (1).

In this study, we investigated whether baseline serum levels of leptin, resistin, visfatin and adiponectin are increased in patients with TRAPS *versus* healthy controls and, in addition, we also investigated whether such patient serum levels significantly correlated with parameters of disease activity and/or disease severity.

Serum leptin, resistin and adiponectin levels were not increased in TRAPS patients *versus* healthy controls, nor in patients carrying mutations known to be associated with a higher disease penetrance or in patients carrying other mutations. On the contrary, serum visfatin levels were significantly lower in patients carrying mutations involving cysteine residues in comparison to patients carrying non-cysteine mutations. However, serum leptin levels significantly correlated with the number of fever attacks/year, and patients carrying mutations involving cysteine residues, who showed more frequent attacks of fever, had higher levels of serum leptin compared to the patients carrying other mutations. Resistin and visfatin did not show any significant correlation, while serum adiponectin levels significantly correlated with the presence of amyloidosis. No significant correlation was found between serum adipokines levels and steroid treatment or biologic modifier drugs.

Toy *et al*. demonstrated that, among AIDs, serum leptin levels do not increase during FMF fever attacks, and leptin proved not to be useful for diagnostic purposes and follow-up during treatment (18). However, serum resistin level does significantly increase during FMF fever attacks, while visfatin serum levels provide no information either during attacks or for attack-free periods (19). In addition, recent data have shown that adiponectin serum levels increase during FMF attacks and down-regulate during symptom-free intervals (20, 21). These findings suggest that different chronic autoinflammatory disorders may show different serum adipokines patterns.

FMF is a chronic inflammatory disorder and preliminary studies suggest that its attack-free periods are characterised by subclinical inflammation and associated endothelial dysfunction, increased atherosclerotic burden and platelets activation. However, increased atherosclerosis, than that in the general population, was not observed in patients with FMF (22). In most studies it has been speculated that colchicine therapy is responsible for a less aggressive course of atherogenesis (23).

Visfatin is an insulin-mimetic adipokine that was originally discovered in liver, skeletal muscle and bone marrow as a growth factor for B lymphocyte precursors (whence its alternative name, pre-B-colony enhancing factor, or PBEF) (17).

Li *et al*. have recently shown that tumour necrosis factor (TNF)-α acts directly on adipocytes, thus down regulating visfatin serum levels through activation of *TNFRSF1A* (24). In patients with TRAPS, TNF-α serum levels may not increase (25), however the altered *TNFRSF1A* activity might be responsible for lowering serum visfatin levels as we shown in patients carrying mutations involving cysteine residues.

Leptin is a 16 kDa hormone synthesised by adipocytes which regulates appetite and energy expenditure at the hypothalamic level (26), and is involved in immune modulation in that it influences the innate immune response by promoting activation of monocyte/macrophages, chemotaxis and activation of neutrophils, and activation of natural killer cells (27).

Finck *et al*. have recently demonstrated that tumour necrosis factor (TNF)-α acts directly on adipocytes, thus inducing leptin through activation of *TNFRSF1A* (28). In patients with TRAPS, the altered *TNFRSF1A* activity might be responsible for serum leptin correlation with TRAPS severity (5).

Adiponectin is a 244-residue adipose-specific protein which is produced in much greater quantities than leptin and is abundantly present in human plasma. The gene encoding adiponectin is

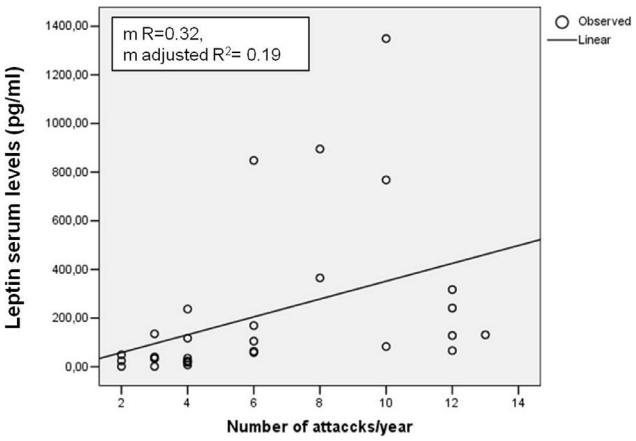

Fig. 2. R correlations (multiple R, and multiple adjusted R^2) in multivariate analysis of leptin serum levels (dependent variable) with the number of fever attacks/year (independent variable) in 30 TRAPS patients. $p<0.03$.

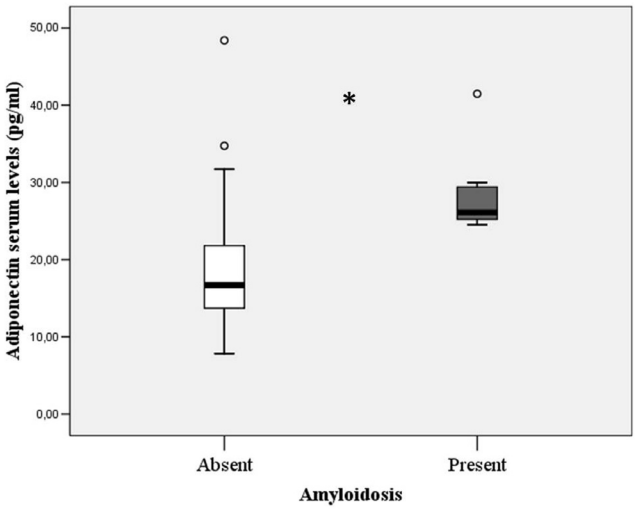

Fig. 3. Figure shows adiponectin serum levels in TRAPS patients (n=8) with amyloidosis and in patients (n=22) without amyloidosis. The central line represents the distribution median, boxes span 25th to 75th percentiles, and error bars extend from 10th to 90th percentiles. Dots (°) are outlier values, higher than the 90th percentile. represents *p*-value 0.05.

located at chromosomal band 3q27, a susceptibility locus for diabetes and cardiovascular disease (29). Although adiponectin was first documented to have anti-inflammatory actions on metabolic pathways and vasculature (30, 31), it is now well-demonstrated that its pro-inflammatory effects are paradoxically more prominent than its anti-atherogenic and anti-inflammatory properties (32).

Its correlation with the presence of amyloidosis may be linked to the deterioration of renal function, and it may represent an adaptive response to the altered metabolic profile associated with high cardiovascular risk in chronic kidney disease patients (33). TRAPS patients have been reported to have an increased risk of cardiovascular diseases such as atherosclerosis and acute myocardial infarction (AMI) (34, 35); our findings suggest that leptin and adiponectin might play a relevant role therein. High leptin is a significant risk factor for AMI, since it exerts many potentially atherogenic effects such as induction of endothelial dysfunction, stimulation of inflammatory reaction, oxidative stress, decrease in paraoxonase activity, platelet aggregation, migration, hypertrophy and proliferation of vascular smooth muscle cells (36). In addition, leptin serum levels have recently been demonstrated to significantly correlate with markers of subclinical atherosclerosis (carotid artery intima-media thickness and coronary artery calcifications) (37). Elevated adiponectin has also been shown to be significantly correlated with a higher risk of cardiovascular disease (fatal and non-fatal myocardial infarction) and coronary artery disease (38).

Further studies are needed in order to evaluate whether the types of adipokine serum level modifications we describe may have concrete repercussions on cardiovascular risk factors in TRAPS patients. Toward this end, it would be interesting to evaluate the presence of carotid artery plaque in these patients, which may be a stronger predictor of atherosclerotic disease (39).

Our study has some limitations. The lack of correlation with additional disease activity parameters might be, at least in part, due to the applied study design. This is, in fact, a cross-sectional study: it is our aim to duplicate the results in a prospective fashion, using paired analysis for each subject at different times of disease activity; repeated measurements of adipokines over time might provide additional information. Recent studies have shown that the biochemical markers of inflammation may remain elevated in TRAPS also during symptom-free intervals. For this reason, the lack of difference in adipokines serum levels, which we demonstrated in our study between TRAPS patients and healthy controls, could be better substantiated taking serum samples both during fever attacks and during fever-free periods. In addition, the numbers of participants may be too small to arrive at more significant associations, and collaborative large-scale studies are needed.

Although many issues still remain hazy, increasing research efforts in the area of adipokines are gradually revealing the intricate adipokine-mediated interplay among white adipose tissue, chronic autoinflammatory disorders and cardiovascular risk. Further insights into the intimate mechanisms regulating the central and peripheral activity of adipokines might in the future generate well-supported therapeutic hypotheses, however, the rate at which their roles are being clarified makes it likely that they will become central to pharmacotherapeutic approaches in immune disorders (40, 41).

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