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 R²= 0.19, p<0.03). Serum adiponectin levels significantly correlated with the presence of amyloidosis (multiple R=0.79, multiple adjusted R²=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=0.42, r²=0.18, p<0.02) and to resistin (r=0.57, r²=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 domain comprising 4 tandem repeat cystein-rich domains (CRD1-4).

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
underlying myalgia and arthralgia; sero-

csal membrane inflammation is also pos-
sible (6-8). Amyloidosis is the most seri-
ous 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 pheno-
type; these patients are currently consid-
ered to be at higher risk of developing
life-threatening AA amyloidosis (9).
White adipose tissue produces more than 50 adipokines and other mole-
cules that participate through endo-
ctrine, paracrine, autocrine or juxtacrine
mechanisms of action in a wide vari-
ety of physiopathological processes, in-
cluding food intake, insulin sensitivity,
vascular sclerotic processes, immunity
and inflammation (11-13).
Among the adipokines known to be se-
creted by adipose tissue, tumour necro-
sis factor (TNF)-α, interleukin (IL)-6,
leptin, resistin and visfatin are consid-
ered to be pro-inflammatory, whereas
adiponectin has been described to have
anti-inflammatory properties depending on
its molecular form (14). It has been
demonstrated that these adipokines can play a fundamental role in inflam-
matory rheumatological autoimmune
conditions (12, 13, 15, 16), and that in
several rheumatic diseases, they are
often associated with increased car-
diovascular 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 con-
trols, 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, Bio-
technology Research Laboratories,
Fondazione IRCCS Policlinico San
Matteo, Pavia, Italy.
Serum leptin, resistin, visfatin and adi-
oponectin levels were obtained from 14
TRAPS patients carrying mutations in-
volving cysteine residues, known to be
associated with a higher disease penetr-
ance (C43Y: 3/14 pts; C88Y: 2/14 pts;
C55Y: 1 pt; 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 sub-
jects with tendinitis, bursitis, and pri-
mary carpal tunnel syndrome) (Group
3). Healthy controls underwent detailed
clinical, laboratory, and instrumental
investigations in order to rule out pos-
tible rheumatic diseases, infections,
endocrine and/or metabolic disorders.
Among healthy controls, none pre-
anted 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 mel-
itus, 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 etaner-
cept (50 mg once a week). Seven out of
16 Group 2 patients were receiving
corticosteroids (7.5–12.5 mg/daily of
prednisone), 2/14 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 re-
ceiving 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: gen-
der, BMI, age, age at disease onset,
duration of fever episodes, number of
fever episodes/year, amyloidosis (pres-
ence/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 designs/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 Namp(T/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, 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 statistically 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=0.48, r²=0.21, p<0.001) and inversely correlated with the duration of fever attacks, entered as days (r=-0.42, r²=0.16, p<0.002). However, there was no correlation between serum leptin levels and SAA levels (r=0.06, p=0.7), nor with the presence of SAA increased values (r=0.19, p=0.3), presence/absence of amyloidosis (r=0.27, p=0.1). ESR (r=0.01 p=0.9), CRP (r=0.02, p=0.9), age at disease onset (r=0.32 p=0.1), steroid treatment (r=0.18 p=0.3), or biologic modifier treatment (r=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 R²=0.19, p<0.03) (Fig. 2).

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

Serum visfatin levels significantly correlated with the number of attacks/year (r=0.42, r²=0.19, p<0.003), whilst inversely correlated with the presence of mutations involving cysteine residues.
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Table II. Mean serum levels (±SD) of adiponectin, leptin, resistin and visfatin in patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>Leptin pg/ml</th>
<th>Resistin pg/ml</th>
<th>Visfatin pg/ml</th>
<th>Adiponectin pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>215.9±386.96</td>
<td>6.13±10.89</td>
<td>1.48±0.95</td>
<td>22.8±9.34</td>
</tr>
<tr>
<td>Group 2</td>
<td>208.8±272.49</td>
<td>14.37±18.93</td>
<td>3.54±2.88</td>
<td>19.9±10.0</td>
</tr>
<tr>
<td>Group 3</td>
<td>133.2±142.63</td>
<td>6.02±5.76</td>
<td>2.62±1.49</td>
<td>17.76±6.07</td>
</tr>
</tbody>
</table>

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.

![Figure 1](image1)

Fig. 1. Figure shows leptin (a), resistin (b), visfatin (c) and adiponectin (d) serum levels in TRAPS patients carrying mutations involving cysteine 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.

Adipokine serum levels did not, however, correlate with SAA values, higher than the 90th percentile. *represents span 25th to 75th percentiles, and error bars extend from 10th to 90th percentiles. Dots (°) are outlier observations (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.

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 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
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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).

References
4. Lobito AA, Kimberley FC, Muppidi JR et al.: Abnormal disulfide-linked oligomerization results in ER retention and altered signal-

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