Visfatin is not associated with inflammation or metabolic syndrome in patients with severe rheumatoid arthritis undergoing anti-TNF-α therapy

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Abstract

Background and Objective

Visfatin is an insulin-mimetic adipokine. In non-rheumatoid arthritis (RA) patients circulating levels of visfatin are correlated with the amount of visceral fat. Recent studies have disclosed an implication of visfatin in inflammation. Chronic systemic inflammation is of major importance in the development of atherosclerosis in RA. In the present study we investigated whether inflammation, obesity or metabolic syndrome are potential determinants of circulating visfatin concentrations in a group of RA patients on periodical treatment with the TNF-α blocker infliximab due to severe disease. We also assessed whether the infusion of infliximab may alter circulating visfatin concentrations in patients with severe RA.

Methods

We investigated 33 non-diabetic patients with RA on periodical treatment with infliximab. Serum visfatin levels were determined immediately prior to and after infliximab infusion.

Results

There was no correlation between body mass index of RA patients and baseline serum level of visfatin. Also, no significant correlations between baseline visfatin levels and the age at the time of the study or at the onset of the disease, disease duration, ESR and CRP levels, DAS28, lipids, insulin sensitivity, resistin or the cumulative prednisone dose at the time of the study were found. Visfatin levels did not change upon infliximab infusion.

Conclusions

In RA patients on TNF-α blocker treatment, circulating visfatin levels are unrelated to disease activity, adiposity or metabolic syndrome. The beneficial effect of anti-TNF-α therapy on cardiovascular mortality in RA does not seem to be mediated by changes in serum levels of visfatin.

Key words

Rheumatoid arthritis, inflammation, circulating visfatin, anti-TNF-α antibody infliximab, cardiovascular risk.

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This study was supported by a grant from Fondo de Investigaciones Sanitarias PI06-0024 (Spain).

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Received on June 16, 2009; accepted in revised form on October 12, 2009.

Competing interests: none declared.

Introduction

Accelerated atherosclerosis (1) and increased incidence of cardiovascular (CV) events (2) are implicated in the elevated mortality observed in patients with rheumatoid arthritis (RA). Besides traditional CV risk factors (3, 4) and genetic predisposition (5, 6), chronic systemic inflammation is of major importance in the progression of atherosclerosis observed in patients with this chronic autoimmune disease (6, 7).

The adipose tissue is now recognised to be a multifunctional organ. In addition to the central role of lipid storage, it has a major endocrine function secreting several hormones (7). These various protein signals have been given the collective name of adipokines. These bioactive molecules influence metabolic processes like insulin resistance, glucose and lipid metabolism, and immunologic and inflammatory reactions (8-10).

Circulating levels of visfatin, also called pre-B cell colony-enhancing factor, were found to be correlated with the amount of visceral fat (11). Visfatin is an insulin-mimetic adipokine (12) that has also been associated with inflammation (13-16). In this regard, Moschen *et al*. showed that recombinant visfatin activates human leukocytes and induces cytokine production (15). These authors showed that visfatin activates human leukocytes and induces the production of IL-1β, TNF-α, and IL-6 (15). Interestingly, Otero *et al*. disclosed higher circulating visfatin levels in patients with RA compared to healthy subjects (17). Moreover, Brentano *et al*. confirmed the role of visfatin as a proinflammatory and matrix-degrading mediator of joint inflammation in RA (14). It is now evident that chronic increase of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 causes deleterious effects including a proatherogenic lipid profile, insulin resistance and endothelial dysfunction in RA (18). TNF-α blockers are highly effective in the treatment of RA and they have proved to reduce CV mortality more than traditional disease modifying anti-rheumatic drugs (DMARD) (19). Marked improvement of endothelial function following anti-TNF-α blockade using the chimeric anti-TNF-α monoclonal antibody infliximab was observed in RA with severe disease on periodical treatment with this drug (20). Moreover, infliximab infusion yielded an immediate effect decreasing circulating nitric oxide concentration (21) and increasing circulating ghrelin concentrations (22) in patients with RA refractory to conventional DMARDs. Also, following infliximab infusion, RA patients with severe disease experienced a rapid improvement in insulin sensitivity (23) and also a reduction in the levels of adhesion molecules associated with atherogenesis (24).

In assessing a series of RA patients with severe disease, refractory to conventional DMARD therapy, on periodical treatment with infliximab, we found that high-grade inflammation was independently and negatively correlated with circulating adiponectin concentrations whereas low adiponectin levels clustered with metabolic syndrome features that reportedly contribute to atherogenesis in RA (25). Also, we found a strong positive correlation between serum leptin levels and body mass index (BMI) in these patients (26). However, no changes on adiponectin or leptin levels were found upon infliximab administration (25, 26). In the same cohort of RA patients, we found a significant positive correlation between laboratory markers of inflammation, particularly C-reactive protein (CRP), with the serum levels of the adipocyte-derived mediator resistin (27). Moreover, anti-TNF-α infliximab therapy induced a rapid and significant reduction of serum resistin levels in RA patients with severe disease (27).

Taking into account all these considerations, in the present study, we investigated whether inflammation, adiposity, insulin resistance or some other characteristics associated with the development of metabolic syndrome are potential determinants of circulating visfatin concentrations in a group of RA patients on periodical treatment with the TNF-α blocker infliximab due to severe disease. We also assessed whether the intravenous administration of this anti-TNF-α monoclonal antibody might alter circulating visfatin concentrations in patients with severe RA.

Patients and methods

Patients

We investigated 33 consecutive patients that met the 1987 American College of Rheumatology criteria for RA (28) and that were recruited from Hospital Xeral-Calde, Lugo, northwest Spain. Information on the characteristics of this Caucasian population was reported elsewhere (6, 29). They formed part of an ongoing study on CV disease in RA (20, 30).

As previously described, each of the RA patients had been switched from traditional DMARD to anti-TNF-α infliximab treatment because of severe and active disease (Disease Activity Score-28 (DAS28) >5.1) (24, 31). Treatment with a DMARD had been initiated in all patients when a diagnosis of RA was made. Prior to anti-TNF-α therapy, patients were required to have been treated with at least two DMARDs including chloroquine or hydroxychloroquine, sulphasalazine, gold, methotrexate (at least 15 mg/week), leflunomide, and cyclosporine A (3 mg/kg/day). Infliximab therapy (initial dose of 3 mg/kg) was administered intravenously at 0, 2, 6 weeks and subsequently every 8 weeks. However, in some patients, because of disease severity, the dose was increased to 5 mg/kg and, if deemed necessary, the interval between infliximab infusions was shortened to 6 weeks.

All patients had received treatment with both non-steroidal antiinflammatory agents and low doses of prednisone (generally 5 mg bid) immediately after disease diagnosis. At the time of the study, each patient was on infliximab 3 or 5 mg/kg given at 6 or 8 weekly intervals and methotrexate 15-25 mg weekly with or without chloroquine 250 mg day or hydroxychloroquine 200 mg/day, prednisone 2.5-7.5 mg daily and a non-steroidal antiinflammatory agent (naproxen 500–1000 mg or diclofenac 50–100 mg daily). The blood pressure was below 140/90 mmHg in each patient at the time of the study. However, 8 were taking antihypertensive agents (enalapril (n=3); losartan (n=3); valsartan (n=1), enalapril and hydrochlorothiazide (n=1)). Six patients were using a statin (simvastatin 20-40 mg/day or atorvastatin 20 mg/day). Patients with diabetes were excluded. For ethical reasons, patients included in the present study were not randomised to a placebo group. The same procedure has been found acceptable and followed in a recent study on the effect of infliximab therapy on lipid profiles in patients with RA (32). The local institutional committee approved anti-TNF-α therapy and each patient gave informed consent to participate in the study.

Neither this study nor previous studies on RA patients receiving periodical treatment with infliximab (20-27) were supported by any pharmaceutical drug company.

Study protocol

As described before (21-27), in each patient a DAS28 (31) was recorded prior to infliximab infusion (the same day). In all cases, the drug was given at 8 am as an intravenous infusion in a saline solution. Blood samples were taken immediately before and after the intravenous administration of infliximab. This anti-TNF-α monoclonal antibody was given intravenously over a period of 120 minutes (21-27). The first sample test was considered time 0. The second sample test after the end of the infliximab administration was considered time 120 minutes. None of the patients received any nutrient before and during infusion (all of them were fasting patients).

All measurements were made in the fasting state. Blood samples were taken at 0800 hours for determination of the erythrocyte sedimentation rate-ESR-(Westergren), CRP (latex immuno-turbidimetry, Nephelometer Analyzer II, Dade Behring, Marburg, Germany), lipids (enzymatic colorimetry), plasma glucose and serum insulin (DPC, Dipesa, Los Angeles, CA, USA). We measured serum concentrations of visfatin, immediately prior to (at time 0) and immediately after infliximab infusion (just at the time that the infusion of the drug ended-at time 120 minutes) by Visfatin C-terminal (Human) enzyme immunoassay kit- EK-003–80- (minimum detectable concentration: 1.85 ng/ml; range: 0.1–1000 ng/ml; linear range: 1.85–19.5 ng/ml; intra-interassay coefficients: 5%–12%) (Phoenix Pharmaceuticals, Inc. Burlingame, California, USA). Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula = (insulin (μU/ml) x glucose (mmol/l)÷22.57. Also, as previously described (27), resistin values were also measured prior to infliximab infusion.

Subsequently, final blood sampling was performed for determination of insulin, glucose, visfatin and resistin after the end of infliximab infusion, which was administered over 120 minutes.

Statistical analyses

Results were expressed as mean ± standard deviation (SD), median and interquartile (IQ) range, or number (n) (%). The associations between baseline characteristics and serum visfatin concentrations (expressed as mean ± SD, median and IQ range) were assessed by estimating the Spearman correlation coefficient (rho) for continuous variables, as visfatin concentrations were not normally distributed. Also, correlation between changes in serum levels of visfatin at time 120 (immediately after the end of infliximab infusion) with changes in other laboratory data following infusion of this drug were analyzed by the Spearman correlation coefficient.

An association between visfatin concentrations and gender was assessed by the Mann-Whitney U-test. Differences in basal (baseline) visfatin concentrations by quartiles of CRP concentration or by quartiles of body mass index (BMI) were analysed by Kruskal-Wallis test. The changes in serum visfatin concentrations upon infliximab therapy (just prior to infusion at time 0 and immediately after the end of infliximab infusion at time 120 minutes) were evaluated using the paired Student's *t*-test. Statistical significance was accepted at $p<0.05$.

Results

Descriptive data

The baseline-recorded variables in this series of 33 RA patients on periodical treatment with infliximab are shown in Table I. Despite clinical improve-

Table I. Baseline characteristics in 33 rheumatoid arthritis patients on treatment with anti-TNF-α therapy. Results are expressed as n (%), mean± standard deviation (SD); median (interquartile range-IQ).

	n (%)	Mean± SD;	Median (IQ)
Age, years			
At disease onset		46.8 ± 10.7	45 (39-55)
At the time of the study		60.9 ± 12.2	59 (53-72)
Women,	27 (83)	---	---
Disease duration, years		14.1 ± 9.3	12 (7-19)
Time from the onset of RA to the beginning of infliximab therapy, years		10.2 ± 9.3	8 (3-14)
Rheumatoid factor positive,	29 (88)	---	---
Disease activity			
DAS28		3.5 ± 1.0	3.5 (2.8-4.3)
Swollen joint count, n		2.2 ± 3.1	1 (0-3)
Tender joint count, n		2.6 ± 3.6	1 (0-4)
VAS patient disease activity		28.1 ± 20.7	20 (10-40)
CRP at the time of the study, mg/l		6.5 ± 7.7	4.2 (1.1-8.4)
Mean CRP from disease diagnosis, mg/l		9.1 ± 7.2	7.4 (3.7-12.0)
ESR at the time of the study, mm/hr		28.6 ± 18.3	25 (20-37)
Mean ESR from disease diagnosis, mm/hr		27.5 ± 11.4	25 (19-38)
Platelet count at the time of the study, x10^9/l		283 ± 121	292 (231-324)
Cumulative prednisone dose, gr		14.7 ± 9.2	13.4 (6.4-19.9)
Years of treatment with infliximab		3.9 ± 2.2	3 (2-5)
Metabolic syndrome features			
Body mass index, kg/m^2		26.7 ± 3.6	26.0 (24.0-28.0)
Hypertension,	8 (24)	---	---
Systolic blood pressure, mmHg		117 ± 18	120 (100-130)
Diastolic blood pressure, mmHg		70 ± 7	70 (70-70)
Glucose, mg/dl		94.2 ± 24.1	90 (85-97)
Insulin, pmol/l		15.8 ± 14.0	13.0 (4.5-18.1)
HOMA-IR, μU.mmol/ml.l		3.7 ± 3.5	3.0 (0.9-3.9)
Total cholesterol, mg/dl		188 ± 31	191 (160-214)
HDL cholesterol, mg/dl		59 ± 10	60 (51-68)
LDL cholesterol, mg/dl		111 ± 28	110 (88-131)
Triglycerides, mg/dl		104 ± 36	92 (73-134)
Resistin, ng/ml		12.5 ± 6.0	11.7 (9.1-14.7)
Visfatin, ng/ml		7.9 ± 2.2	7.8 (6.5-9.4)

DAS: disease activity score; VAS: visual analogue scale; BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

ment, as reflected by a reduction in the DAS28 score compared to that found prior to the onset of anti-TNF-α therapy, most patients still had active disease (DAS28>2.6) (33).

Correlations between the basal recorded characteristics and serum visfatin concentrations

Although visfatin concentrations (ng/ml) were higher in women with RA (8.0±2.1) than in men (7.4±2.5), this difference was not statistically significant (p=0.89).

No significant correlations between visfatin concentrations obtained before infliximab administration and the age at the time of the study or at the onset of the disease and disease duration were found. Likewise, no significant correlations between tender and swollen joints, VAS patient's disease activity, DAS28, the mean ESR and CRP from disease diagnosis and the ESR, CRP and platelet count at the time of the study or the cumulative prednisone dose and baseline visfatin concentrations were observed (Table II).

Relationships of visfatin concentrations with metabolic syndrome features

Visfatin concentrations did not show a significant correlation with BMI (rho= -0.201, p=0.26). Also, in this series of RA with severe disease and persistently elevated inflammatory response, visfatin concentrations did not show a significant correlation with the HOMA-IR (rho = - 0.118, p=0.52) and with basal insulin (rho = -0.200, p=0.27) (Table II).

Moreover, visfatin concentrations were not significantly correlated with total cholesterol, HDL and LDL cholesterol, triglycerides and plasma glucose levels (Table II).

Relationships of baseline visfatin concentrations with resistin

Since we recently reported a potential role of resistin in the inflammatory cascade in RA (27), in the present study we aimed to establish whether a correlation between resistin and visfatin might exist in RA patients with severe disease and persistent elevation of inflammation markers. However, as shown in Table II, there was no significant correlation between basal visfatin and resistin concentrations.

Differences in baseline visfatin concentrations according to CRP or BMI

To establish if patients with severe disease had greater concentrations of serum visfatin than those with mild or moderate disease, RA patients were split in quartiles of CRP levels prior to infliximab infusion. Again, visfatin concentrations did not differ significantly amongst groups when patients were stratified by basal CRP concentrations (1st quartile (CRP <1.1mg/l): 8.4± 1.4 ng/ml; 2nd quartile (CRP within 1.2 and 4.7 mg/l): 7.0±1.2 ng/ml; 3rd quartile (CRP within 4.8 and 9.0 mg/l): 7.6±1.6 ng/ml; 4th quartile (CRP >9.0 mg/l): 8.3±3.1 ng/ml; p=0.61).

Also, to determine whether BMI might influence the levels of visfatin in this series of patients with severe chronic inflammatory response, individuals were stratified in four groups according to BMI. However, no significant differences between subgroups were found (BMI <25: 8.2±2.2 ng/ml; BMI 25-27: 7.7±1.8; BMI 27-30: 8.6±3.0; BMI >30: 7.0±2.2; p=0.59).

Changes in visfatin concentrations upon infliximab therapy

Visfatin concentrations (ng/ml) did not change significantly upon administration of an infliximab infusion (before:

Table II. Association between baseline patient characteristics and serum visfatin levels in 33 patients with severe rheumatoid arthritis.

Patient characteristic	isfatin (ng/ml) rho (*p*-value)
Age, years	
At disease onset	0.018 (0.92)
At the time of the study	0.176 (0.33)
Disease duration, years	0.210 (0.24)
Time from the onset of RA to the beginning of infliximab therapy, years	0.261 (0.14)
Disease activity	
DAS28	0.128 (0.48)
Swollen joints	0.165 (0.36)
Tender joints	0.150 (0.40)
VAS patient disease activity	0.198 (0.27)
CRP protein at the time of the study, mg/l	0.303 (0.17)
Mean CRP from disease diagnosis, mg/l	0.017 (0.94)
ESR at the time of the study, mm/hr	0.114 (0.53)
Mean ESR from disease diagnosis, mm/hr	0.034 (0.85)
Platelet at the time of the study, hundred/mm^3	0.217 (0.23)
Metabolic syndrome	
BMI, kg/m^2	-0.201 (0.26)
Basal glucose, mg/dl	0.178 (0.32)
Basal Insulin, μU/ml	-0.200 (0.27)
Basal HOMA-IR, μU.mmol/ml.l	-0.118 (0.52)
Triglycerides, mg/dl	-0.050 (0.78)
Total cholesterol, mg/dl	-0.027 (0.88)
HDL cholesterol, mg/dl	-0.122 (0.50)
LDL cholesterol mg/dl	0.014 (0.94)
Systolic blood pressure, mmHg	-0.015 (0.93)
Diastolic blood pressure, mmHg	-0.026 (0.89)
Cumulative prednisone dosage, gr	0.054 (0.82)
Resistin, ng/ml	0.079 (0.62)

DAS: disease activity score; VAS: visual analogue scale; BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

mean±SD: 7.91±2.16; median: 7.8; IQ range: 6.5- 9.4; after: 7.66±2.45; 7.0; 6.0–9.6; p=0.09) and baseline visfatin concentrations were strongly correlated with visfatin concentrations after an infliximab infusion (rho= 0.922, p<0.001).

The correlations of post infliximab circulating visfatin concentrations with the baseline recorded characteristics (Table II) did not differ from the correlations of baseline circulating visfatin concentrations with the baseline recorded characteristics (as described above) in both univariate and multivariable analysis (data not shown).

Discussion

The present study shows that in patients with severe RA, refractory to conventional DMARD therapy, on periodical treatment with the anti-TNF-α blocker infliximab and ongoing disease activity, there is no correlation between serum visfatin levels and most clinical and laboratory parameters of disease activity and inflammation. Also, in this series of patients the serum levels of visfatin did not correlate with metabolic syndrome characteristics. Furthermore, this study shows for first time that there is no significant change in serum visfatin levels upon anti-TNF-α infliximab infusion.

Metabolic syndrome features are independently associated with atherosclerosis in RA (34, 35). Visfatin promotes adipogenesis (12, 36). However, it is still unclear what would be its physiological role and clinical relevance in the setting of RA. In this regard, in contrast to what was reported in non-RA subjects with a wide range of obesity (37), levels of visfatin did not correlate with BMI in our series of patients with severe RA.

Human obesity-related diabetes and the accompanying metabolic disorders have been specifically linked to increased visceral adipose tissue mass. Visfatin is produced by visceral adipose tissue and also has insulin-mimetic actions (13, 36). However, the relationship between visfatin levels and insulin resistance and other features associated to metabolic syndrome needs to be clarified. With respect to this, in our study we did not observe association between circulating visfatin and insulin or insulin resistance determined by HOMA. This was in contradiction with data from a series of 21 obese women, which showed a significant correlation between serum concentrations of visfatin and insulin in these women (38). Moreover, the serum concentration of visfatin was significantly higher in obese women than in controls (38). In contrast, in our series most patients were not obese and the stratification of patients according to BMI did not disclose significant differences.

In our study we did not observe significant difference in RA patients stratified by gender. In assessing metabolic parameters including fasting serum visfatin, fasting serum insulin, fasting plasma glucose and lipid profile in 500 individuals, Chen *et al*. found no significant differences in serum visfatin levels between men and women (39). Also, although visfatin correlated negatively with BMI in men, this adipokine did correlate with any other anthropometric or any metabolic parameters in men from that study (39). Unlike to our findings, Chen *et al*. observed a positive correlation with HDL-cholesterol levels and a negative correlation with LDL-cholesterol levels in the subgroup of women (39).

Chronic and persistent inflammatory response in patients with RA, in particular in those with severe and active disease, may lead to alteration in the lipid profile and BMI index (1, 18), which may explain metabolic differences between RA patients and non-RA individuals, obese or not. Due to this, it is possible that the presence of a severe inflammatory response rather than the

direct effect of visfatin might account for the development of metabolic syndrome features in patients with severe RA.

Visfatin levels have also been found to be increased in individuals with hyperglycemia (40). However, this situation could not be explored in our study as patients with diabetes were excluded during the period of recruitment.

It is known that visfatin activates human leukocytes inducing cytokine production (15). Also, this adipokine has inflammatory and destructive functions promoting joint damage in patients with RA (14). However, in our series of individuals with severe RA no correlation between visfatin levels and routine markers of inflammation or disease activity, measured by number of tender or swollen joints, VAS scale or DAS28, was observed. The reason for that finding in patients with RA is somehow unexpected. However, it is possible that in patients with severe RA the secretion of adipokines might be disturbed as a result of a chronic and persistent inflammatory response. Thus, the situation observed in RA patients with refractory disease may be completely different from that found in non-RA obese o diabetic patients. In this regard, it is possible that in patients with RA and high inflammatory burden visfatin secretion might be negatively influenced by other adipokines or other factors or by some compensatory mechanism that try to maintain a normal homeostasis in these patients. With respect to this, a recent report of our group disclosed that in RA patients undergoing infliximab therapy due to the severe disease refractory to conventional DMARDs there is a significant association between the mean ESR and CRP from the time of disease diagnosis, ESR and CRP and platelet count at the time of the study and resistin levels obtained before infliximab infusion (27). As observed for visfatin, no significant correlation between serum resistin levels and BMI was found (27). However, in the present study we could not find a significant correlation between visfatin and resistin levels determined immediately prior to and after infliximab infusion.

In conclusion, in RA patients with severe disease on periodical treatment with anti-TNF-α blocker infliximab, there is no correlation between circulating visfatin levels and clinical and laboratory parameters of disease activity, adiposity or metabolic syndrome. In addition, serum visfatin concentrations do not show significant changes upon infliximab administration. Therefore, the beneficial effect of anti-TNF-α therapy on CV mortality in RA does not seem to be mediated by changes in serum levels of visfatin. However, due to the potential role of the adipose tissue in the mechanisms associated with inflammation and metabolic syndrome in patients with RA, the search for the potential implication of other adipokines in the accelerated atherosclerosis observed in patients with this chronic inflammatory rheumatic disease is warranted.

Acknowledgements

The authors thank Mrs Susana Escandon and Isabel Castro-Fernandez, nurses from the Rheumatology Outpatient Clinic, and Ms Pilar Ruiz, a nurse from the Hematology Division (Hospital Xeral-Calde, Lugo, Spain) for their valuable help in undertaking this study.

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