

## Plasma levels of leptin and visfatin in rheumatoid arthritis patients; is there any relationship with joint damage?

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### ARTICLE INFO

**Article type:**  
Original article

**Article history:**  
Received: Nov 2, 2013  
Accepted: Jan 26, 2014

**Keywords:**  
Adipocytokine  
Rheumatoid arthritis  
Leptin  
Larsen score  
Visfatin

### ABSTRACT

**Objective (s):** Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder, primarily targeting the synovium and articular cartilage that leads to joint damage. Recent reports have suggested the role of adipocytokines in mediating joint damage; however it still is a matter of debate. The purpose of this study was to evaluate the association between serum values of adipocytokines (leptin, visfatin) and radiographic joint damage in patients with RA.

**Materials and Methods:** Fifty-four patients diagnosed with RA, based on Revised ACR Criteria 2010, with 1-5 year disease duration since diagnosis, were enrolled. Twenty-nine of patients had erosion in radiographic studies and 25 patients had no erosion. Radiographic joint damages were defined according to Larsen Score. Additionally, serum levels of adipocytokines were measured and cross-sectional associations with radiographic damage were explored, adjusting for pertinent confounders.

**Results:** The serum level of visfatin were significantly higher in patients with radiographic joint damage compared with patients with no joint damage ( $P=0.013$ ). This difference remained significant after adjustment for C-reactive protein levels ( $P=0.008$ ), but not after adjustment for disease duration ( $P=0.247$ ). The mean leptin serum levels were not different between these two groups ( $P=0.903$ ). There was a positive correlation between leptin levels and BMI ( $r=0.494$ ,  $P<0.001$ ). However, after adjustment for BMI, leptin levels had no difference between two groups ( $P=0.508$ ).

**Conclusion:** This study revealed that visfatin levels were significantly higher in patients with radiographic joint damage dependently to disease duration. Therefore, it seems that adipocytokine may be a valuable factor in therapeutic targets in the future.

### ► Please cite this paper as:

Mirfeizi Z, Noubakht Z, Etemad Rezaie A, Jokar MH, Shariati Sarabi Zh. Plasma levels of leptin and visfatin in rheumatoid arthritis patients; is there any relationship with joint damage? Iran J Basic Med Sci 2014; 17:662-666.

### Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that primarily targets the synovium and articular cartilage, leading to synovitis and progressive joint damage. However the pathophysiology of RA is poorly understood, the pro-inflammatory adipocytokines such as leptin, resistin and visfatin have been suggested to have an important role in the pathogenesis of the disease (1).

Leptin, which is mainly produced by white adipose tissue (WAT) cells, is a hormone encoded by the obese gene (*ob*), the murine homologue of the human *LEP* (2). In addition to its well-known ability to regulate body weight by inhibiting food intake and increasing energy consumption, it also has inflammatory and immunomodulatory effects (3). Serum leptin levels were increased in RA patients

compared to healthy controls (4-7); it was significantly higher in women than in men (8).

Visfatin, a growth factor for B lymphocyte precursors, is another pro-inflammatory adipocytokine, seen in liver, skeletal muscles and bone marrow and produced by visceral WAT, which mimics the effects of insulin (9, 10). Serum visfatin levels were increased in patients with RA (4, 7, 11-13). Recently, reports have suggested the significant role of adipocytokines such as visfatin in mediating joint damage (4, 14, 15). Nevertheless, it is still a matter of debate whether infliximab significantly changes the visfatin levels (16). The purpose of this study was to examine the association between serum levels of adipocytokines (leptin and visfatin) and radiographic joint damage in patients with RA.

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**Table 1.** Demographics parameters and laboratory findings in studied patients

Variables	Erosive RA	Non-erosive RA	P-value
Age (year)	44.6±6.72	43.5±8.32	0.605
Sex (female %)	75.9%	96.0%	0.056
BMI	25.2±3.38	26.4±2.82	0.158
Disease duration-year			
Median (IQR)	5 (3-5)	2 (1-4.5)	0.003
Elevated ESR (%)	39.1%	52.2%	0.375
Normal CRP (%)	58.6%	40.0%	0.870
Leptin (ng/ml)			
Mean±SD	32.4±31.78	33.4±26.15	0.903
Visfatin (ng/ml)			
Median (IQR)	34.6 (29.1-100)	28.4 (22.1-34.5)	0.013

## Materials and Methods

This study was conducted on 29 patients with erosive rheumatoid arthritis (RA) and 25 patients with non-erosive RA. All patients fulfilled the American College of Rheumatology classification criteria for RA (17). Eligible patients were those who met all of the following criteria: having RA for at least 1 year, but no more than 5 years; aged between 20 and 50 at the disease onset; and BMI ≤ 30. Patients were excluded if they had any of the following concomitant diseases: erosive joint diseases such as gout, seronegative spondyloarthropathies; osteoarthritis; blood pressure ≥ 140/90 mmHg; cardiovascular diseases such as congestive heart failure or coronary artery diseases.

The protocol was approved by the Ethical Committee of Mashhad University of Medical Sciences. Each subject signed a written informed consent after explaining the objective of the study and verification of the inclusion and exclusion criteria. X-rays of the hands and feet were obtained from each of the subjects and 20 joints (the wrists, 1<sup>st</sup> to 5<sup>th</sup> metacarpophalangeal joints, and 2<sup>nd</sup> to 5<sup>th</sup> metatarsophalangeal joints) were evaluated by a particular radiologist who was blinded to the study. The amount of damage was quantified by using the Larsen score, with ranging from 0 to 100 for all 20 joints (18).

After overnight fasting, blood samples were drawn from all patients and stored at -20°C until the time of analysis by Leptin Kit, (Visfatin kit: Rav Bio Human Visfatin E1 America; Me Diagnose Company, code: Ref-E07 Germany) through ELISA. Descriptive statistics were calculated as mean ± standard deviation (SD) or median [interquartile range (IQR)] according to distributions of continuous variables.

### Statistical analysis

Statistical analysis was performed with SPSS for

windows software version 11.5 (SPSS Inc., Chicago, IL, USA). Mean values and standard deviations were used for continuous variables.

For comparing means, Student's t-test was used. The Mann-Whitney test was used to determine the mean differences.

For evaluating relationships among normally distributed variables, the Pearson correlation coefficient was calculated, whereas the Spearman's rho was calculated in case of nonparametric data.

Values of  $P < 0.05$  were considered significant.

## Results

In our study, age, sex and BMI did not show any statistically significant differences between the two groups of patients (erosive and non-erosive RA) ( $P$ -values > 0.05). The median disease durations were 5 years (IQR (3–5)) and 2 years (IQR (1–4.5)) in patients with erosive and non-erosive RA, respectively; which showed statistically significant differences between the two groups of patients ( $P=0.003$ ). Leptin concentrations did not show any statistically significant differences between the two groups of patients ( $P=0.903$ ). Visfatin concentrations were significantly higher in patients with erosive RA (34.6 [29.1– 100] ng/ml) compared with non-erosive RA (28.4 [22.1–34.5] ng/ml) ( $P=0.013$ ) (Table 1).

Leptin and visfatin correlations with BMI, disease duration and Larsen score are shown in Table 2. BMI correlated positively with leptin ( $r=0.494$ ,  $P<0.001$ ), Figure 1 while disease duration correlated positively with visfatin ( $r=0.488$ ,  $P<0.001$ ) Figure 2. No correlations were seen between Larsen score and leptin and visfatin ( $r= -0.318$ ,  $P=0.093$  and  $r=0.254$ ,  $P=0.184$  respectively).

By using the linear regression coefficient model, leptin showed correlation with BMI ( $t=4.096$ ,  $P<0.001$ ) and visfatin showed positive correlations with C-reactive protein (CRP) and disease duration

**Table 2.** Leptin and visfatin correlations with BMI, disease duration and Larsen score

	Leptin		Visfatin	
	Coefficient of variation	P-value	Coefficient of variation	P-value
BMI	0.494	<0.001	-0.018	0.898
Disease duration	-0.119	0.392	0.488	<0.001
Larsen score	-0.318	0.093	0.254	0.184

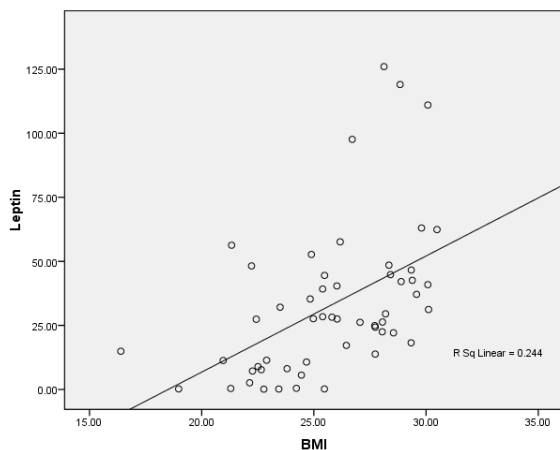


Figure 1. Relationship between BMI and leptin

( $t=2.165$ ,  $P=0.035$  and  $t=2.719$ ,  $P=0.009$  respectively).

Nevertheless, using univariate variance analysis, there was no correlation between leptin and BMI in the two groups of RA ( $P=0.508$ ), and leptin serum levels in the two study groups were not significantly different, not considering the BMI ( $P=0.522$ ).

While there was a positive correlation between visfatin and CRP ( $P=0.008$ ), no correlation was seen between visfatin and disease duration in the two erosive and non-erosive RA groups ( $P=0.247$ ).

## Discussion

There are a few studies about the effect of adipokines like leptin and visfatin on radiographic changes of involved joints in rheumatoid arthritis. In Giles *et al* study, in 2009, there was no association between the level of leptin and radiographic joint damage (14). However, in Rho *et al* studies, there was a positive correlation between these adipokines with radiographic changes, and higher levels of leptin were associated with less radiographic damage (13).

In this case-control study, we evaluated the association between the serum levels of leptin and visfatin with radiographic damage in two groups of patients with rheumatoid arthritis: with and without radiographic damage, to better understand the physiology of radiologic joint damage in rheumatoid arthritis patients which is the most important cause of morbidity in rheumatoid arthritis.

We found that serum levels of visfatin in our rheumatoid arthritis patients with joint erosion were much higher than those in patients without joint erosion. There was no correlation between serum levels of visfatin with either sedimentation rate or CRP. Additionally, BMI and Larsen score were not associated with serum visfatin.

There was a statistically significant positive correlation between visfatin serum levels with disease duration ( $P<0.001$ ,  $r=0.538$ ). C-reactive

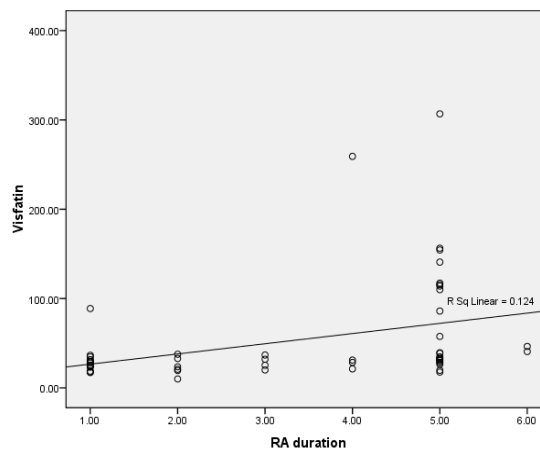


Figure 2. Scattered graph showing relationship between visfatin and disease duration

protein level and disease duration were correlated to serum visfatin by linear regression method.

Univariate analysis showed that serum visfatin in our two study groups had a statistically significant positive correlation ( $P=0.008$ ) with radiographic damage disregarding CRP as co-founder ( $P=0.008$ ).

However, dispensing with the disease duration, visfatin serum levels in the two groups had no statistical significance ( $P=0.695$ ).

These findings represent that serum visfatin level, independently, with serum CRP as an inflammatory marker, is associated with joint damage in radiographic studies, but this correlation (visfatin level and radiographic damage) depends on the disease duration.

Our study was similar to the Rho *et al* study in 2009, which revealed that visfatin serum levels were positively correlated with radiographic joint damage considering age, gender, disease duration, BMI, and inflammation, with statistical significance (13).

Although visfatin is an adipokine, based on previous studies, there is no correlation between visfatin and obesity (17).

However, some studies have shown that there is an association between visfatin and inflammatory markers (18, 19).

In the Rho *et al* study there was a positive relationship between visfatin serum levels and inflammatory mediators like CRP, TNF $\alpha$  and IL6 with statistical significance, but no correlation between visfatin and BMI. In our study, we could not find a correlation between BMI and visfatin levels. Additionally, no association existed between ESR and CRP with visfatin levels. This finding is probably due to the difference between inflammatory markers and also qualitative use of data in our study.

Brentano and colleagues reported that visfatin was accumulated in the invasive synovial tissue in rheumatoid arthritis patients and its levels were increased in synovial fibroblasts as well (20).

Visfatin is able to induce IL6, Matrix metalloproteinase (MMP1 and MMP3) in synovial fibroblasts in RA patients and induce TNF  $\alpha$  and IL6 in monocytes too (20).

In our study, we found that visfatin level is associated with radiographic damage and has a catabolic effect on joints; therefore it has an important role in pathophysiology in rheumatoid arthritis.

Busso *et al*, revealed that in arthritic rats, visfatin inhibitors induce remission exactly like TNF  $\alpha$  inhibitors (21). Interestingly, pharmacologic inhibition in visfatin, decreases intracellular NAD levels (intracellular nucleotide adenine deaminase) in inflammatory cells and reduces production of IL6 and TNF  $\alpha$  in the involved joints of RA patients (21).

With respect to above findings, we can come to the conclusion that visfatin is an independent mediator in the pathogenesis of rheumatoid arthritis and is related to radiographic joint damage. Therefore, it may be considered a new treatment goal in RA.

Visfatin level was not related to ESR, CRP, Larsen score or disease duration, but had a meaningful, positive statistical relation with BMI ( $P=0.004$ ,  $r=0.385$ ).

Our study showed that leptin levels in RA patients with or without joint erosion were not any different. BMI is related to serum leptin considering its role in linear regression model.

These findings show that leptin level does not play a role in joint destruction.

Rho *et al* (13) showed that serum leptin level associated with less joint space narrowing after suppression of inflammatory process. Also there is a positive relation among leptin, DAS28, CRP, IL6, and BMI which is statistically significant in RA patients. In our study, we found a correlation between leptin and BMI and no association between leptin with disease activity, CRP and IL6. This discrepancy might be due to the design difference between our study and that of Rho *et al*. We did not evaluate disease activity score in our patients; all patients had had rheumatoid arthritis for less than 5 years. According to the studies of Westhoff *et al* (22), Kaufmann *et al* (23) and Van Der Helmm *et al* (24) obesity has a protective effect in joint damage in RA patients (22–24). Therefore, the important roles of adipocytokines like leptin and adiponectin in obesity should be considered in RA patients.

## Conclusion

We found that visfatin level as an inflammatory marker, independently of CRP level, is associated with joint radiographic damage and is related to disease duration. However, leptin does not have any effect on the joint damage process. The mechanism of visfatin as a proinflammatory and its catabolic role is not known and visfatin biology seems to be complex; the key roles of visfatin as a treatment are

still obscure. Additionally, our study is one of the earliest in the field of association of adipocytokine levels and radiographic joint damage.

We evaluated adipocytokines regardless of DAS28 and types of treatment. Therefore, we suggest evaluating levels of adipocytokines before and after treatment in longitudinal studies to make clear the relationship between adipocytokines and joint damage in human models in the future.

In future research, it would be reasonable to measure leptin and visfatin simultaneously, in addition to the other adipocytokines like adipocytokines of resistin.

## Acknowledgment

This article was extracted from the thesis prepared by Dr Zohreh Nobakht to fulfill the requirements needed for earning the rheumatology fellowship degree. The Research Council of Mashhad University of Medical Sciences, Mashhad, Iran, is appreciated for financially supporting this study (grant #900232). We are also grateful to Rheumatic Diseases Research Center and also patients for their sincere support.

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