Adipocytokines: Adiponectin, Resistin and Visfatin in Serum and Synovial Fluid of Rheumatoid Arthritis Patients and Their Relation to Disease Activity

EMAN A.M. AL-KADY, M.D.  ; HANAN M. AHMED, M.D.**; LUBNA TAG, M.D. * * and MADLEN ADEL, M.D. * * *

The Departments of Rheumatology & Rehabilitation*, Internal Medicine** and Clinical Pathology***, Faculty of Medicine, Assuit University, Assuit, Egypt

Abstract

Background: Adipocytokines; adiponectin, visfatin and resistin are mainly adipocyte-derived cytokines regulating metabolism and as such are key regulators of insulin resistance. Recent data provided evidence on the implication of these adipocytokines in the inflammation, immune response and tissue destruction and revealed several links between them and arthritis. These data suggest a possible role of adipocytokines in the modulation of the inflammatory environment in rheumatoid arthritis (RA).

Aim of the Study: The purpose of this study was to assess the level of adiponectin, visfatin and resistin in serum and synovial fluid from patients with rheumatoid arthritis and their relation to the disease activity.

Subjects and Methods: Seventy female patients with RA and 30 age and sex matched healthy controls were enrolled. The clinical activity of RA patients was assessed according to the 28 joint count Disease Activity Score and patients were classified into two groups; 39 patients with active disease (group A) and 31 patients in remission (group B). Synovial fluid was obtained by arthrocentesis of the affected knee joints from 39 patients with active disease. Serum adiponectin, visfatin and resistin concentrations were measured in RA patients, controls and their synovial concentrations in active group by using specific enzyme-linked immunosorbent assays.

Results: Serum levels of adiponectin and visfatin were significantly higher in all studied RA patients and patients with active disease compared with control group and patients in remission. No significant difference was observed in resistin level between patients and controls. Serum adiponectin, visfatin and resistin levels were significantly higher than their synovial fluid concentrations in patients with active RA.

Conclusion: Our data support the hypothesis that adiponectin and visfatin are involved in the inflammatory process of rheumatoid arthritis.

Key Words: A dipocytokines – Adiponectin – Visfatin – Resistin – RA.

Introduction

RHEUMATOID arthritis (RA) is a chronic inflammatory disease that ultimately leads to progressive destruction of the joints [1]. The major secretory compartment of adipose tissue, adipocytes, has the ability to synthesize and release proinflammatory molecules, complement factors, macrophage colony-stimulating factor, growth factors, and adhesion molecules [2,3] suggesting an integrated function of adipocytes in tissue inflammation. Adiponectin [4], resistin [5] and visfatin [6] are examples of these molecules which named adipocytokines [2]. This name reflects the novel function of adipose tissue as an immunological, endocrine, and paracrine organ. Several studies have revealed numerous links between adipose tissue, adipocytokines, and inflammatory joint disease [7-12].

Adiponectin, the most abundant adipocyte protein, has potent anti-inflammatory properties by inhibiting pro-inflammatory TNF- a [6] and macrophage phagocytic activity production, also inhibiting myelomonocytic cell proliferation by inducing apoptosis [13] and inducing the production of the anti-inflammatory mediators IL-10 and IL-1 receptor antagonist (IL-1RA) [14]. In contrast, Ehling, et al. [15] suggested that adiponectin is a potent driving force of arthritis by stimulating the production of pro-inflammatory and key mediators of destructive arthritis, IL-6 and pro-matrix metallo-proteinase-1 (MMP-1) from RA synovial fibroblasts in vitro. Previous clinical studies showed that serum adiponectin concentrations are higher in RA patients than in healthy control [13,16].
Resistin, a protein produced by adipocytes, considered to be a hormone that potentially links obesity to diabetes [5]. Resistin is engaged in inflammatory conditions in humans by means of its secretion in substantial quantities by mononuclear cells. It has been found in plasma and synovial fluid (SF) of RA patients [17].

Visfatin is an adipocytokine secreted mainly by visceral fat [18]. Visfatin is considered as a proinflammatory adipocytokine. It has been shown to induce chemotaxis and the production of IL-1β, TNF-α, IL-6 and costimulatory molecules by CD 14+ monocytes, and to increase their ability to induce all proliferative responses in lymphocytes [6]. Fantuzzi [19] stated that, visfatin clearly represents an additional link between adipose tissue and inflammation.

The present study was designed to compare the serum levels of adiponectin, resistin and visfatin in RA patients with those in healthy controls, to investigate the correlation between their serum levels and RA disease activity and to assess SF levels of the three adipocytokines in RA patients.

**Material and Methods**

The present study was conducted on seventy adult female patients with more than 5 year history of RA. The study was carried out in the departments of Rheumatology and Rehabilitation, Internal Medicine and Clinical Pathology, Assiut University Hospitals, Assiut, Egypt from 2008-2009. RA was diagnosed based on the 1987 revised American College of Rheumatology (ACR) criteria [20]. Thirty age and BMI-matched female participants who underwent health examination at Assiut University Hospitals were enrolled in the present study. Patients treated with antihypertensive, antidiabetic, or antihyperlipidemic regimen or patients diagnosed as having hypertension, diabetes, hyperlipidemia or obesity were excluded from this study. The study was approved by the Ethical Committee of Faculty of Medicine, Assiut University and written informed consent was obtained from each participant.

**Assessment of disease activity:**

The disease activity of RA patients was assessed according to Disease Activity Score including a 28 joint count/ESR (DAS28 ESR), so the patients were classified into two sub-groups; patients with disease activity (group A) and patients in remission (group B).

All patients and controls were subjected to the following: Thorough history taking, full clinical examination, anthropometric measurements including (weight, height, BMI), fasting blood samples were collected for estimation of the following: Complete blood picture, serum glucose, kidney and liver function tests, ESR and Hs-CRP.

Synovial fluid was obtained by arthrocentesis, aseptically aspirated from affected knee joints from 39 patients with active disease and simultaneous blood samples were collected. Blood samples from 31 patients with disease remission and healthy subjects were also collected. Collected blood and synovial fluid samples were centrifuged, aliquotted, frozen and stored at –20°C until use.

Adiponectin concentrations were measured with commercially available an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer’s protocol (Ray Biotech, Inc cat, ELH adiponectin-001). Visfatin C-terminal concentration was measured by commercially available enzyme immunoassay kit according to the manufacturer’s protocol (Phenix Pharmaceuticals, Inc) with catalog number Ek-003-80. Resistin was done using ELISA kit supplied by Quantikine R, D system Inc with cat number DRSN00. Hs-CRP detection method was done on the immulite automated analyzer which is a two-site chemiluminescent enzyme immuno-metric assay with lot number LKCRPkitlot-302.

**Statistical analysis:**

Data were collected and analyzed by computer program SPSS “version 17” (The Statistical Package for the Social Science Program), Chicago, USA. Data were expressed as mean, SD, number and percentage. t-test was used to determine significance for numeric variables. Chi. square was used to determine significance for non-parametric variables and Pearson's correlation for numeric variables in the same group. Significance was considered according to the level of (p-value) as follows: p>0.05 = Non significant, p≤0.05 = Significant, P≤0.01 = Highly significant and p≤0.001 = Very highly significant.

**Results**

The mean serum levels of adiponectin, visfatin, ESR-1 st hour, Hs-CRP were significantly higher in RA patients than in controls. While, there was no significant difference between serum resistin in RA patients and control group (Table 1).

According to DAS28 we classified RA patients into patients in remission and patients with active disease. Patients with active disease had significantly higher disease duration, serum Hs-CRP, ESR 1 st hour disease activity score measured ac-
cording to DAS28-ESR, serum levels of adiponec- 
tin and visfatin and lower BMI than those in re- 
mission. While, there was no significant difference 
in serum resistin between two groups (Table 2).

Serum adiponectin, visfatin and resistin levels 
were significantly higher than their synovial fluid 
concentrations in patients with active RA (38.64 ± 
1.92 Vs. 11.81 ±0.46pg/ml, p<0.001, 27.09 ±1.79 
Vs. 7.97±0.94ng/ml, p<0.001 and 8.56±1.71 Vs. 
1.35±0.22ng/ml, p<0.001) respectively Figs. (1,2).

Correlation of adiponecin, visfatin and resistin 
with clinical and laboratory characteristics in total 
RA patients, active RA and patients in remission 
(Tables 3, 4 and 5):

Serum adiponecin correlates positively with 
serum visfatin in all groups. Both serum adiponec-
tin and visfatin are correlated positively with du-
ration of the disease, ESR1 hr, Hs-CRP, DAS28-
ESR in total RA and remission groups and with 
DAS28-ESR in active RA group. In addition, Serum 
adiponecin correlated positively with Hs-CRP in 
active RA group. Synovial visfatin showed as well 
a positive correlation with disease duration and 
DAS28-ESR in total RA and active RA groups. 
 Serum and synovial resistin showed no relation 
with adiponecin, visfatin or any other clinical and 
laboratory characteristics of RA patients.

Figs. (3,4) show the serum levels of adiponecin 
and visfatin is significantly higher in different 
patients study groups compared with the controls.

Where as the serum level of resistin shows no 
difference in all patients study groups compared 
with controls.

Table (1): Clinical and biochemical characteristics of 
the study subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RA (n=70)</th>
<th>Control (n=30)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.12±4.97</td>
<td>46.44±6.38</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>24.93±1.78</td>
<td>25.47±0.39</td>
<td>NS</td>
</tr>
<tr>
<td>Hs-CRP (mg/L)</td>
<td>3.2±0.4</td>
<td>0.35±0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESR (mm/1st hr)</td>
<td>43.02±19.34</td>
<td>7.82±9.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adiponecin (pg/ml)</td>
<td>34.51±4.96</td>
<td>9.28±0.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Visfatin (ng/ml)</td>
<td>23.97±3.91</td>
<td>2.80±0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>8.70±1.65</td>
<td>8.76±1.88</td>
<td>NS</td>
</tr>
</tbody>
</table>

RA: Rheumatoid arthritis, NS: Non significant, 
p<0.001 highly significant.

Table (2): Clinical, demographic and biochemical 
characteristics of RA patients’ groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RA with active disease (A) (n=39)</th>
<th>RA in remission (B) (n=31)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.28±5.31</td>
<td>47.93±4.58</td>
<td>NS</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>8.35±3.53</td>
<td>5.35±3.47</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>BMI(Kg/m²)</td>
<td>20.65±1.25</td>
<td>23.54±0.74</td>
<td>p&lt;0.000</td>
</tr>
<tr>
<td>DAS28-ESR</td>
<td>6.83±0.93</td>
<td>2.06±0.71</td>
<td>p&lt;0.000</td>
</tr>
<tr>
<td>Hs-CRP(mg/L)</td>
<td>4.29±1.32</td>
<td>3.52±0.27</td>
<td>p&lt;0.000</td>
</tr>
<tr>
<td>ESR (mm/1st hr)</td>
<td>57.51±11.35</td>
<td>24.80±9.01</td>
<td>p&lt;0.000</td>
</tr>
<tr>
<td>Adiponecin (pg/ml)</td>
<td>38.64±1.92</td>
<td>29.32±1.40</td>
<td>p&lt;0.000</td>
</tr>
<tr>
<td>Visfatin (ng/ml)</td>
<td>27.09±1.79</td>
<td>20.03±1.58</td>
<td>p&lt;0.000</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>8.56±1.71</td>
<td>8.86±1.57</td>
<td>NS</td>
</tr>
</tbody>
</table>

A: Group A, B: Group B, NS: Non significant.

Table (3): Correlation of the three adipocytokines with clinical and laboratory characteristics 
in all RA patients.

<table>
<thead>
<tr>
<th>Serum Adiponecin</th>
<th>Synovial Adiponecin</th>
<th>Serum Visfatin</th>
<th>Synovial Visfatin</th>
<th>Serum Resistin</th>
<th>Synovial Resistin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.121</td>
<td>0.199</td>
<td>0.142</td>
<td>0.300</td>
<td>-0.294*</td>
</tr>
<tr>
<td>Dur. of dis.</td>
<td>0.481*</td>
<td>-0.124</td>
<td>0.517**</td>
<td>0.404*</td>
<td>0.112</td>
</tr>
<tr>
<td>BMI</td>
<td>0.049</td>
<td>0.050</td>
<td>-0.043</td>
<td>-0.057</td>
<td>0.032</td>
</tr>
<tr>
<td>HS CRP</td>
<td>0.896**</td>
<td>0.046</td>
<td>0.866**</td>
<td>0.052</td>
<td>-0.076</td>
</tr>
<tr>
<td>DAS28</td>
<td>0.984**</td>
<td>-0.233</td>
<td>0.957**</td>
<td>0.385*</td>
<td>-0.098</td>
</tr>
</tbody>
</table>

Dur. of dis.: Duration of disease. BMI: Body mass index. HS CRP: High sensitive C reactive protein.

Table (4): Correlation of the three adipocytokines with clinical and laboratory characteristics in RA patients with active disease.

<table>
<thead>
<tr>
<th>Serum Adiponecin</th>
<th>Synovial Adiponecin</th>
<th>Serum Visfatin</th>
<th>Synovial Visfatin</th>
<th>Serum Resistin</th>
<th>Synovial Resistin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.276</td>
<td>0.198</td>
<td>0.304</td>
<td>0.300</td>
<td>-0.337*</td>
</tr>
<tr>
<td>Dur. of dis.</td>
<td>0.196</td>
<td>-0.156</td>
<td>0.255</td>
<td>0.404*</td>
<td>0.194</td>
</tr>
<tr>
<td>BMI</td>
<td>0.033</td>
<td>0.051</td>
<td>-0.232</td>
<td>-0.057</td>
<td>0.047</td>
</tr>
<tr>
<td>HS CRP</td>
<td>0.141</td>
<td>0.053</td>
<td>0.110</td>
<td>-0.055</td>
<td>-0.008</td>
</tr>
<tr>
<td>DAS28</td>
<td>0.407*</td>
<td>0.047</td>
<td>0.275</td>
<td>0.052</td>
<td>-0.040</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.721**</td>
<td>0.385*</td>
<td>-0.026</td>
</tr>
</tbody>
</table>

* Significant. ** Highly significant.
Table (5): Correlation of the three adipocytokines with clinical and laboratory characteristics in RA patients with remission.

<table>
<thead>
<tr>
<th></th>
<th>Serum Adiponectin</th>
<th>Serum Visfatin</th>
<th>Serum Resistin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.225</td>
<td>0.179</td>
<td>-0.222</td>
</tr>
<tr>
<td>Dur. of dis.</td>
<td>0.622**</td>
<td>0.618**</td>
<td>0.117</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.740</td>
<td>-0.792</td>
<td>0.038</td>
</tr>
<tr>
<td>ESR 1st hr</td>
<td>0.799**</td>
<td>0.789**</td>
<td>-0.058</td>
</tr>
<tr>
<td>HS CRP</td>
<td>0.671**</td>
<td>0.658**</td>
<td>-0.102</td>
</tr>
<tr>
<td>DAS28</td>
<td>0.841**</td>
<td>0.788**</td>
<td>-0.055</td>
</tr>
</tbody>
</table>

** Highly significant.

Discussion

The current working hypothesis is that adipocytokines, cytokines and other factors produced and released by white adipose tissue (WAT) are responsible for a chronic subclinic pro-inflammatory state. Changes in levels of systemic or local adipocytokines have been reported in a variety of inflammatory autoimmune conditions [13] and are now considered important players in the etiopathogenesis of numerous metabolic and inflammatory disorders including RA [21].

Our study demonstrated that significantly higher serum levels of adiponectin and visfatin in the all studied RA patients than in the controls and in active RA group than those in remission. Nevertheless, there was no significant difference in serum resistin between all RA patients and controls or between active and remission groups of RA. These results are in agreement with results of Brentano, et al. [1], Otero, et al. [13], Schaffler, et al. [7] and Š enolt, et al. [16]. Regarding disease activity, patients with active disease had significantly higher disease duration, serum Hs-CRP, ESR 1st hour, disease activity score measured according to DAS28-ESR and lower BMI than those in remission. Lower BMI in active group might be due to RA-associated wasting syndrome which characterised by a considerable loss of body cell mass. Otero, et al. [13] explained this as chronic inflammation in RA triggers body energy adjustments that include high-energy expenditure, fat mobilisation, enhanced gluconeogenesis, protein catabolism and negative nitrogen balance. These homeostatic perturbations are believed to accelerate morbidity and mortality in patients with RA and are associated with increased production of inflammatory factors, as well as disturbances of the endocrine system.
Adiponectin has various biological properties but its role in RA is controversial. Otero, et al. [13] postulated that the increased levels of adiponectin in patients with RA suggest a compensatory mechanism under catabolic or anabolic imbalance. So, an increase in adiponectin level represents an attempt to antagonise the anorexigenic and pro-inflammatory effect of leptin, suggesting that these two adipocytokines may act in parallel as opposing metabolic counterparts. In addition, Fantuzzi [19] reviewed that adiponectin reduces the production and activity of TNF-α and inhibition of IL-6 production by activated macrophage accompanied by induction of the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist (IL-1Ra). Furthermore, adiponectin reduces induction of the endothelial adhesion molecules ICAM-1 and vascular cell adhesion molecule 1 by either TNF-α or resistin.

On the other hand, it was suggested that adiponectin in particular actively participates in the process of immune response, inflammation and matrix degradation in destructive arthritides as its level was found to be increased at local sites of inflammation in RA patients [22]. Ehling, et al. [18] and Tang, et al. [23] stated that adiponectin is a potent driving force of arthritis and an enhancer of the inflammatory response in RA by inducing the inflammatory cytokines IL-6 from RA synovial fibroblasts in vitro. Another report demonstrated that adiponectin concentrations correlated negatively with the number of leukocytes in the synovial fluid of RA patients indicating that adiponectin is a counterpart of the local inflammatory process [16].

Visfatin simultaneously facilitates adipogenesis and has insulin-mimetic properties [18]. Its role in the inflammatory process of RA is unclear. Lago, et al. [17] suggested that it might involve modulation of inflammatory or immune response by visfatin, or might be part of a compensatory mechanism, or the increased levels of visfatin in RA patients could simply be an epiphenomenon. In contrast, Otero, et al. [13] could not rule out this suggestion. Brentano, et al. [1] indicated that visfatin acts as a pro-inflammatory mediator by triggering the release of cytokines, chemokines and destructive enzymes that are characteristically observed in the inflamed joints of patients with RA. Also, visfatin is a marker of inflammation, and that visfatin itself promotes inflammatory and destructive processes in the joints of patients with RA.

Resistin, apart from its putative role in insulin resistance, has been recently proposed as a new pro-inflammatory adipokine [13]. Forsblad d'Elia, et al. [24] and S enolt, et al. [25] suggested that resistin is a significant mediator in the inflammatory process in RA and the link between serum resistin, inflammation and disease activity suggest a role for resistin in the pathogenesis of rheumatoid arthritis. In our study, resistin levels in patients with rheumatoid arthritis showed no significant changes in comparison to controls. This result is identical to that reported by other authors [13,26]. So, resistin is probably not one of the main signals in the pathogenesis of RA.

Moreover, serum adiponectin, visfatin and resistin concentrations were significantly higher than their SF concentrations in patients with active RA; this is in agreement with Presle, et al. [27]. S enolt and his group [25] found that adiponectin concentration was lower in SF than that in systemic circulation whereas resistin levels were significantly higher in SF than in systemic circulation in RA patients. Nowell, et al. [28] stated that the level of synovial visfatin is consistent with its serum concentration. Presle, et al. [27] demonstrated that adipocytokines serum levels are not predictive values for SF determination. Our findings could be explained as it can be expected that higher systemic adipokines levels compared to that in synovial fluid are due to higher amounts of peripheral fat stores, which secrete adipokines into blood stream [16].

Serum adiponectin correlated positively with serum visfatin in all groups. Both serum adiponectin and visfatin are correlated positively with duration of the disease, markers of inflammation; ESR1 st hour, Hs-CRP, DAS28-ESR; in total RA and remission groups and with only DAS28-ESR in active RA group. In addition, serum adiponectin correlated positively with Hs-CRP in active RA group. Synovial visfatin showed as well a positive correlation with disease duration and DAS28-ESR in all RA and active RA groups. Strong correlation of adiponectin and visfatin with markers of inflammation provides support for an important role of these cytokines in inflammatory reactions. These findings suggest that adiponectin and visfatin are markers of inflammation in patients with RA and this is consistent with results of Brentano, et al. [1], Kitahara, et al. [29] and Moschen, et al. [6].

In contrast to our results, S enolt and his group [16] and Schäffler, et al. [7] did not find any association between adiponectin and systemic inflammatory markers. Serum and synovial resistin showed no relation with adiponectin, visfatin or any other clinical and laboratory characteristics of
RA patients. In accordance to our data, Scherer et al. [25] demonstrated that the increased serum level of resistin in RA patients did not correlate with selected adipocytokines in their study but correlated with both CRP and DAS28 and ESR [7,25].

Conclusion: Patients with RA have a marked increase in plasma levels of adiponectin and visfatin, whereas resistin levels are unmodified. Our data support the hypothesis that adiponectin and visfatin produced by WAT might play a proinflammatory and metabolic role in RA and suggest important therapeutic implications that need further investigations.

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