Research Article / Araştırma Makalesi

Comparison of Serum Nesfatin-1 and Visfatin Levels Between Patients with Rheumatoid Arthritis and Healthy Controls

Romatoid Artritli Hastalar ile Sağlıklı Kontroller Arasında Serum Nesfatin-1 ve Visfatin Düzeylerinin Karşılaştırılması

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Abstract: Even though elevated nesfatin-1 levels were reported in patients with rheumatoid arthritis (RA) previously, the possible role of body mass index (BMI) was not excluded. The aim of the present study was to compare serum nesfatin-1 and visfatin levels between patients with RA and healthy controls who have normal BMI values (18.5<BMI<30.0). Forty-four patients with RA (mean age: 49.2 ± 10.2 years) and 42 age-sex matched healthy controls (mean age: 45.9 ± 7.8 years) were included in this retrospective study. Nesfatin-1 and visfatin levels were determined by using enzyme-linked immunosorbent assay kits according to manufacturer's instructions. Serum C-Reactive protein levels and erythrocyte sedimentation rate were 1.1 ± 1.8 mg/dL, and 29.3 ± 20.0 mm/hour, respectively, in patients with RA. Although there was a trend toward higher visfatin levels in RA patients (3.0 ± 2.7 vs 2.4 ± 1.3 ng/mL) it did not reach statistical significance (p= 0.972). Nesfatin-1 levels were found significantly different between groups (p<0.001). No significant relationships were detected between nesfatin-1 and visfatin levels in patients with RA (p>0.05). It seems that nesfatin-1 levels are significantly higher in RA patients compared to healthy peers. However, nesfatin-1 levels were not found related to disease activity.
Keywords: Adipokines, arthritis, body mass index

Özet: Romatoid artritli (RA) hastalarda nesfatin-1 düzeylerinin yükseldiği daha önce bildirilmiş olmasına rağmen vücut kütle indeksinin (BMI) olası rolü dışlanmamıştır. Bu çalışmanın amacı BMI değerleri normal (18,5<BMI<30,0) RA'lı hastalar ile olan sağlıklı kontroller arasında serum nesfatin-1 ve visfatin düzeylerini karşılaştırmaktı. Retrospektif tarzdaki bu çalışmaya 44 RA'lı hasta (ortalama yaş: 49,2 ± 10,2 yıl) ve yaş-cinsiyet uyumlu 42 sağlıklı kontrol (ortalama yaş: 45,9 ± 7,8 yıl) dahil edildi. Nesfatin-1 ve visfatin düzeyleri, enzim bağlantılı immünosorbent test kitleri kullanılarak üretici firmanın talimatlarına göre belirlendi. RA hastalarında serum C-Reaktif protein düzeyi ve eritrosit sedimantasyon hızı sırasıyla 1,1 ± 1,8 mg/dL ve 29,3 ± 20,0 mm/saat olarak belirlendi. RA hastalarında visfatin düzeyleri açısından daha yüksek bir eğilim olmasına rağmen (3,0 ± 2,7 vs 2,4 ± 1,3 ng/mL) bu durum istatistiksel anlamlılığa erişmedi (p= 0,972). Nesfatin-1 düzeyleri gruplar arasında anlamlı farklılık gösterdi (p<0,001). RA hastalarında nesfatin-1 ile visfatin düzeyleri arasında anlamlı ilişki saptanmadı (p>0,05). RA hastalarında nesfatin-1 düzeylerinin sağlıklı yaşıtlarına göre anlamlı derecede yüksek olduğu görülmektedir. Ancak nesfatin-1 düzeylerinin hastalık aktivitesiyle ilişkisi saptanamadı.
Anahtar Kelimeler: Adipokinler, artrit, vücut kütle indeksi

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1. Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease particularly affecting small peripheral joints symmetrically and may lead to joint damage (1). Chronic inflammation and joint damage in RA were associated with cytokines, myokines, and recently with adipokines (2,3). In terms of adipokines, leptin, adiponectin, visfatin, and resistin have been studied extensively in patients with RA (3). According to a recent meta-analysis, increased serum visfatin levels were reported in patients with RA, also higher serum visfatin levels were associated with higher RA disease activity (3).

Visfatin is adipocyte hormone binding to the insulin receptor. It causes hypoglycaemia by reducing glucose release from liver and stimulating glucose utilization in cells. While the upregulation of the visfatin was meditated by hypoxia, inflammation, and hyperglycaemia, it is downregulated by insulin, somatostatin and statins (4). Visfatin can be induced by inflammatory factors such as TNF-α, IL-1, IL-6 (5).

NEFA/nucleobindin2 (NUCB2) is a 396 amino acid peptide, which was originally described as a secreted protein of unknown function (6,7). NUCB2 was suggested as an appetite regulating molecule in immortalized cell lines and in the hypothalamus of rodents (8). The N-terminal fragment, named nesfatin-1, was subsequently investigated in many studies to reveal its role not only in the regulation of food intake but also in other physiological functions. Serum nesfatin-1 levels were found to be higher in subjects with osteoarthritis (OA) compared to healthy subjects, and it was suggested that serum nesfatin-1 levels may be associated with radiographic damage in knee OA (9,10). In addition, in-vitro studies with chondrocytes from humans and rats have shown that nesfatin-1 triggers the secretion of pro-inflammatory cytokines such as IL-6, IL-8, COX-2 and contributes to the inflammatory process (11). Some recent studies have reported elevated nesfatin-1 levels in patients with RA compared to healthy controls. As it is an adipokine, body mass index (BMI) might have a potential role on nesfatin-1 levels. However, the possible role of BMI was not investigated in previous studies in RA patients.

The aim of this study was to compare nesfatin-1 levels which might have a potential role in the inflammatory processes, and visfatin levels which were showed as an indicator of disease activity between patients with RA and healthy controls with normal BMI values.

2. Materials and Methods

The data was retrospectively collected in the present study. The patient files were screened retrospectively, and the blood samples which were preserved in study center were analyzed prospectively.

2.1. Patients

Patients with RA according to 2010 ACR/EULAR Rheumatoid Arthritis Classification criteria (12) who were between 18 and 65 years of age were included. Age, sex, and BMI matched subjects without any known co-morbidity participated as healthy controls. Patients and controls were excluded if they had; uncontrolled hypertension, renal failure (glomerular filtration rate <60 ml/min), diabetes mellitus, history of severe hyperlipidemia (total cholesterol 0.300 mg/dL, triglyceride 0.400 mg/dL or lipid-lowering drug use), obesity (body mass index>30), coronary artery disease or heart failure, history of cerebrovascular disease history, liver dysfunction, and pregnancy. Disease duration, pain, patient global status, and Disease Activity Score-28 (DAS-28) were recorded for patients with RA.

2.2. Laboratory Analyzes

Venous blood samples were collected from the forearm between 08.00 and 10.00 hours and were stored at -80 °C until analysis time. Serum nesfatin-1 and visfatin levels were studied by using commercially available enzyme-linked immunosorbent assay (ELISA)

kits following the manufacturer's recommendations.

2.3. Statistical Analysis

Statistical analysis was performed by using SPSS 16.0. Descriptive statistics were presented as median (interquartile range: 25/75). Mann-Whitney U test was employed to compare groups. The relationships between serum nesfatin-1 and other parameters were investigated by using Spearman's correlation. A *p* value of <0.05 was accepted as meaningful.

3. Results

A total of 44 patients (35 female [80%]) with RA, and 42 healthy controls (35 female [83%]) were included in the study. Median disease duration was 48 months (min-max: 3-360 months) for patients with RA. Twenty-four (55%) patients with RA were using methotrexate, 23 of them (52%) were using corticosteroids, 19 of them (43%) were using non-steroidal anti-inflammatory drugs, 10 of them (23%) were using leflunomide, 9 of them (21%) were using hydroxychloroquine, and 8 of them (18%) were using sulfasalazine. Nesfatin-1 levels were found significantly different between groups (p<0.05, Table 1). Although, there was a trend toward higher visfatin levels in RA patients, it did not reach statistical significance (p>0.05, Table 1). Nesfatin-1 levels were not correlated to visfatin or disease related characteristics (p>0.05, Table 2).

Table 1. Demographics, disease related characteristics and serum adipokine levels in study groups

	RA (n: 44) Median (IQR 25/75)	**Control (n: 42) Median (IQR 25/75)**	**p***
Age (years)	49.5 (41.0/57.5)	48.0 (38.0/50.0)	0.109
BMI (kg/m^2)	25.9 (23.3/27.5)	25.1 (22.9/27.3)	0.688
Pain (0-100)	20.0 (10.0/50.0)	NA	NA
Patient Global (0-100)	30 (10.0/52.5)	NA	NA
DAS-28 (score)	3.3 (2.3/4.5)	NA	NA
CRP (mg/dl)	0.4 (0.2/0.9)	NA	NA
ESR (mm/hour)	23.5 (15.0/35.0)	NA	NA
Nesfatin-1 (ng/ml)	34.7 (15.7/83.7)	14.3 (11.6/28.9)	**p<0.001**
Visfatin (ng/ml)	2.3 (0.6/4.1)	2.1 (1.5/3.5)	0.972

**: Mann-Whitney U test, BMI: Body-Mass Index, DAS-28: Disease Activity Score 28, CRP: C-Reactive Protein, ESR: Erythrocyte Sedimentation Rate, NA: Not Applicable, p<0.05.*

Table 2. The relationships between serum nesfatin-1 levels and other parameters in patients with RA

n: 44	**Nesfatin-1 (ng/ml)**	
	rho	**p**
Age (years)	0.013	0.934
BMI (kg/m^2)	-0.091	0.559
Disease Duration (months)	-0.266	0.081
Pain (0-100)	-0.064	0.678
Patient Global (0-100)	-0.010	0.678
DAS28 (score)	0.105	0.500
CRP (mg/dl)	0.110	0.477
ESR (mm/hour)	0.042	0.788
Visfatin (ng/ml)	0.280	0.060

rho: Spearman's rank correlation coefficient, BMI: Body-Mass Index, DAS-28: Disease Activity Score 28, CRP: C-Reactive Protein, ESR: Erythrocyte Sedimentation Rate, p<0.05.

4. Discussion

Nesfatin-1 is an adipokine involved in satiety induction and energy homeostasis, however, it may also have a pro-inflammatory role in RA (5). In the present study, significantly higher nesfatin-1 levels were detected in RA patients compared to healthy subjects. However, no relationships were determined between nesfatin-1 levels and visfatin levels, DAS-28 scores, CRP, ESR, patient global score, pain or physical characteristics (age, BMI).

This might be explained by the lack of significant difference in visfatin levels between groups in the present study. Visfatin levels were defined as a well-documented indicator of the inflammation in patients with RA previously, however, no significant difference in visfatin levels were detected in the present study, indicating a low inflammation activity (3). Furthermore, low level of inflammatory markers (CRP< 0.5 mg/dl, ESR ≈ 20 mm/hour) supported our idea. Contrary, DAS-28 scores suggested moderate disease activity for the patients with RA in our study. This might be resulted by the other components of DAS-28 such as patient global score, and number of sensitive/swollen joints.

Even though, it is not correlated to disease activity, nesfatin-1 can still be considered as a disease biomarker, as it is found significantly higher in patients with RA compared to healthy controls. Similarly, adiponectin, another adipokine, was also found high in the patients with RA, despite of having no relationship with disease activity in a meta-analysis (3).

Previous studies have suggested some relationship between nesfatin-1 and the immune response. Some studies reported that nesfatin-1 might be a pro-inflammatory adipokine, others suggested that it may have anti-inflammatory properties. In subarachnoid hemorrhage model, nesfatin-1 showed anti-apoptotic and anti-inflammatory features in rats. However, it was also reported that serum nesfatin-1 levels were higher in patients with knee OA than healthy controls (9,10). Moreover, nesfatin-1 serum levels were also found to be related with radiographic severity (9). It has also been suggested that nesfatin-1 may be associated with systemic inflammation in patients with emphysema-type COPD (13). Therefore, one can speculate that serum nesfatin-1 levels might be increased due to preventing inflammation or as a result of inflammation. However, the causal link needs to be explained in future studies.

The debate related to the role of nesfatin-1 in the inflammation associated with RA continues as well. Robinson et al. reported that nesfatin-1 levels are associated with reduced carotid intima-media thickness which is an early indicator of atherosclerosis in patients with RA (14). These authors also reported a moderate correlation between nesfatin-1 and visfatin levels (Spearman's rho: 0.516), which we cannot determine between these two adipokines in the present study. On the other hand, Kvilizdze et al. reported that nesfatin-1 levels related to severity of clinical manifestations in patients with RA (15). Considering the role of nesfatin-1 in the energy homeostasis, increased BMI and adipose tissue might lead the change in nesfatin-1 levels. Therefore, obese patients were excluded in the present study. Most of the previous studies did not evaluate the role of BMI in serum nesfatin-1 levels in RA and the discrepancies between our results and other studies might be related to this difference. However, in a recent study by Naghashian et al, no relationship was established between BMI and nesfatin-1 levels in patients with RA (16). Similarly, no significant relationship was found between nesfatin-1 and BMI patients in the present study. However, nesfatin-1 levels were found elevated in patients with RA.

This study has some limitations, sample size is relatively small compared to other studies in literature. Also, measuring the fat mass percentage by using valid and reliable methods such as bioelectric impedance or dual-energy x-ray absorptiometry might provide additional information, which was not available in our study.

5. Conclusion

The present study has provided more evidence about the nesfatin-1 levels difference between patients with RA and healthy controls. In addition, no significant relationship was found between disease activity parameters and nesfatin-1 levels. Elimination of obese individuals has possibly strengthened the reliability of our results. However, other studies should be conducted to reveal the exact role of the nesfatin-1 in patients with RA.

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Ethics

Ethics Committee Approval: The study was approved by the İzmir Katip Çelebi University Ethical committee with the number 106 - 22.06.2017

Informed Consent: This study did not require informed consent.

Authorship Contributions: SG, MÖ, DS, and SA designed the study, ÖG, and ATK collected the data, LDK analyzed the data, , SG composed the manuscript. All authors contributed to writing the manuscript.

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