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ORIGINAL ARTICLE

Could Endocan, a Marker of Inflammation and Endothelial Dysfunction, be a New Diagnostic Marker for Fibromyalgia?

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SUMMARY

Background: **Although the pathophysiology of fibromyalgia has not yet been fully clarified, inflammation and endothelial dysfunction have been suggested to occur in patients with fibromyalgia. In recent years, endocan has been reported as an important biomarker for inflammation and endothelial dysfunction. In this study, we investigated this mechanism by measuring the serum endocan levels in fibromyalgia patients.**
Methods: **The serum samples collected from 37 female patients diagnosed with fibromyalgia for the first time and 43 healthy women were analysed in terms of the endocan levels and laboratory parameters, and the values from the two groups were compared.**
Results: **In the patient group, the serum endocan level was found to be higher ($p < 0.0001$). Endocan had a significantly high sensitivity and specificity for the diagnosis of fibromyalgia (area under curve: 0.897, cutoff value: 9.81 ng/mL, sensitivity: 88.5%, specificity: 89.7%).**
Conclusions: **Subclinical inflammation and endothelial dysfunction are important in the pathophysiology of fibromyalgia. Increased endocan not only elucidates this mechanism but also presents as an important potential marker for fibromyalgia.**
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KEY WORDS

Endocan [endothelial cell-specific molecule-1 (ESM-1)], fibromyalgia, inflammation, endothelial dysfunction

INTRODUCTION

Fibromyalgia is characterised by widespread pain, stiffness, fatigue, sleep disturbance, and cognitive dysfunction [1]. Although the pathophysiology of fibromyalgia has not yet been fully understood, predisposing factors such as genetic and familial predisposition, autonomic nervous system dysfunction, infection, inflammation, physical trauma, psychological factors, and stress have been implicated [2]. Several parameters have been reported to increase in fibromyalgia patients, including proinflammatory cytokines, tumor necrosis factor-α (TNF-α) [3], interleukin-6 (IL-6) [4,5], and inflammato-

ry chemokines (a special type of pro-inflammatory cytokines); e.g., thymus and activation-regulated chemokine (TARC), and monokine induced by gamma-interferon (MIG) [6]. Furthermore, it has been suggested that inflammatory activation mediates oxidative stress, which plays an important role in the pathophysiology of fibromyalgia [7,8]. In a similar vein, fibromyalgia has been associated with endothelial function and arterial stiffness [9,10]. Endothelial cell-specific molecule-1 (ESM-1) also known as endocan is a soluble proteoglycan produced by the vascular endothelium and controlled by several cytokines [11,12]; thus, it provides information about the inflammatory process and endothelial function [13]. It is considered that endocan is particularly associated with cancer, sepsis, and cardiovascular diseases [14-16].

In this study, we investigated the relationship between fibromyalgia and endocan, which is a new and significant marker for inflammation and endothelial function, two important factors for the etiopathogenesis of fibromyalgia.

MATERIALS AND METHODS

The sample of the study consisted of 37 female patients diagnosed for the first time with fibromyalgia by the physical therapy and rehabilitation outpatient clinic of our hospital and 43 healthy women that were not taking regular medication. All the participants were over 18 years of age. The study was conducted following the principles for medical research provided by the guidelines of the Declaration of Helsinki and the International Conference on Harmonization Guideline for Good Clinical Practice. Written informed consent was obtained from all the participants.

Subjects

Patients with fibromyalgia

The criteria for including the patients were: age between 19 and 65, female, and a diagnosis of fibromyalgia based on the classification criteria provided by the American College of Rheumatology (ACR) 1990 [17]. The patients with chronic diseases such as hypertension, diabetes, cancer, cardiovascular diseases, osteoarthritis, or other severe somatic/psychiatric disorders, those who smoked or consumed alcohol, and those who were not able to refrain from analgesics, NSAID or hypnotics for 15 days prior to the examination were excluded from the study.

Blood sampling

Fasting peripheral blood samples were obtained from the patient and control groups between 08:30 and 09:30. Immediately after collection, 1,500 g of the samples were centrifuged for 20 minutes. Then, the sera were placed into Eppendorf tubes, aliquoted, and stored at -80°C.

Determination of laboratory markers

The complete blood counts were obtained using a Sysmex XN-1000 automatic hematology analyser. The serum biochemical parameter concentrations were analysed with a Beckman Coulter Olympus AU2700 analyser. The nephelometric assay of the serum C-reactive protein (CRP) level was performed using a Siemens BN II system. The endocan levels of the samples were determined using the ELISA method [Cusabio Human Endothelial Cell-Specific Molecule 1 (ESM-1) ELISA Kit]. For human ESM-1, the minimum dose that can be detected is typically less than 0.039 ng/mL. The precision values were obtained as follows: intra-assay coefficient of variation CV% < 8%, inter-assay CV% < 10%. The results were recorded in ng/mL.

Statistics

The Statistical Package for Social Sciences Software for Windows version 18.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis of the demographic and clinical data of the sample. A chi-square test was undertaken to evaluate the difference between the groups concerning the categorical variables. Data that showed normally distributed differences between the fibromyalgia and control groups were evaluated using Student's unpaired *t*-test. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

The demographic characteristics of the samples are given in Table 1. The patient and control groups did not differ significantly in terms of age, height, weight, and body mass index (BMI). There were also no significant differences between the two groups with regard to their alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, CRP, glucose, hemoglobin, lymphocyte, mean platelet volume (MPV), neutrophil, platelet distribution width (PDW), platelet, urea, uric acid, and white blood cell counts (Table 2). However, the serum endocan levels were higher in the patient group compared to the control group ($p < 0.0001$, Figure 1). The receiver operating characteristic (ROC) curve showed a significant correlation between the levels of endocan [area under curve (AUC): 0.897]. For endocan, the cutoff values based on 9.81 ng/mL were calculated as 88.5% sensitivity and 89.7% specificity (Figure 2).

DISCUSSION

To the best of our knowledge, this is the first study in the literature to demonstrate the elevated serum endocan levels in patients with fibromyalgia. This finding indicates the important role of subclinical inflammation and endothelial dysfunction in the pathophysiology of fibromyalgia.

Table 1. Demografic data of the groups.

Parameter	Group						
	Fibromyalgia			Control			p-value
	Mean	95% CI	SD	Mean	95% CI	SD	
BMI (kg/m^2)	25.5	24.5 - 26.5	3.2	23.9	22.4 - 25.3	4.6	0.065
Lenght (m^2)	1.62	1.60 - 1.64	0.05	1.58	1.50 - 1.66	0.25	0.296
Age (years)	39.1	34.3 - 43.9	13.0	35.7	33.8 - 37.5	6.2	0.184
Weight (kg)	67.4	65.0 - 69.8	7.4	62.9	58.9 - 66.9	12.7	0.061

Abbreviations: BMI - body mass index, CI - confidence interval, SD - standard deviation.

Table 2. Biochemical and haematological data of the groups.

Parameter	Group						
	Fibromyalgia			Control			p-value
	Mean	95% CI	SD	Mean	95% CI	SD	
ALT (U/L)	16.1	13.9 -18.3	6.7	20.7	15.2 - 26.3	19.2	0.1232
AST (U/L)	18.6	17.0 - 20.1	4.7	21.6	18.7 - 24.4	9.8	0.0642
Cr (mg/dL)	0.80	0.75 - 0.84	0.13	0.78	0.75 - 0.82	0.12	0.2627
CRP (mg/L)	4.0	1.8 - 6.2	6.6	3.7	2.2 - 5.1	5.0	0.7888
Endocan (ng/mL)	11.7	11.1 - 12.3	1.8	5.4	3.9 - 6.8	4.1	< 0.0001 [a]
Glucose (mg/dL)	91	88 - 94	9	87	84 - 91	11	0.0974
Hgb (g/dL)	13.1	12.7 - 13.5	1.1	13.4	13.0 - 13.8	1.3	0.2313
Lym (10^3/µL)	2.2	2.0 - 2.4	0.5	2.2	2.0 - 2.3	0.5	0.8877
MPV (fL)	10.6	10.3 - 10.9	0.9	10.7	10.4 - 11.0	0.9	0.4764
Neu (10^3/µL)	3.9	3.6 - 4.3	1.1	4.1	3.8 - 4.5	1.2	0.4527
PDW (%)	12.5	11.8 - 13.1	1.9	12.9	12.2 - 13.5	2.1	0.3529
Plt (10^3/mm^3)	294	272 - 317	68	283	264 - 301	63	0.4117
Urea (mg/dL)	28.7	26.1 - 31.2	7.8	26.4	25.0 - 27.8	4.7	0.1176
Uric acid (mg/dL)	4.3	4.0 - 4.6	0.95	4.1	3.8 - 4.3	0.99	0.2669
WBC (10^3/mm^3)	6.8	6.4 - 7.3	1.4	7.3	6.8 - 7.8	1.4	0.13371

A - p < 0.0001, Abbreviations: ALT - alanine aminotransferase, AST - aspartate aminotransferase, CI - confidence interval, Cr - creatinine, CRP - C-reactive protein, Hgb - hemoglobin, Lym - lymphocyte, MPV - mean platelet volume, Neu - neutrophil, PDW - platelet distribution width, Plt - platelet, SD - standard deviation, WBC - white blood cell.

Topal et al. found that asymmetric dimethylarginine (ADMA) levels associated with endothelial dysfunction were increased in fibromyalgia patients. In addition, the authors reported that ADMA had a positive correlation with elevated 8-iso-prostaglandin F2α (8-iso-PGF2α), a significant indicator of oxidative stress and lipid peroxidation, and TNF-α, a proinflammatory cytokine [3]. Other studies have also shown that increased levels of IL-6 and IL-8 in fibromyalgia cases are correlated with the severity of the symptoms [18], and elevated high-sensitivity C-reactive (hsCRP) levels are correlated with BMI, IL-6, IL-8, and ESR [19]. Similarly, the increased presence of inflammatory chemokines such as TARC, MIG, macrophage-derived chemokine, interferon-inducible T-cell alpha chemoattractant and eotaxin suggests that inflammation is an important factor in the pathogenesis of fibromyalgia [6]. Sánchez et al. reported a correlation between the TNF-α levels measured in skin biopsies and serum of fibromyalgia patients and suggested that increased oxidative stress led to inflam-

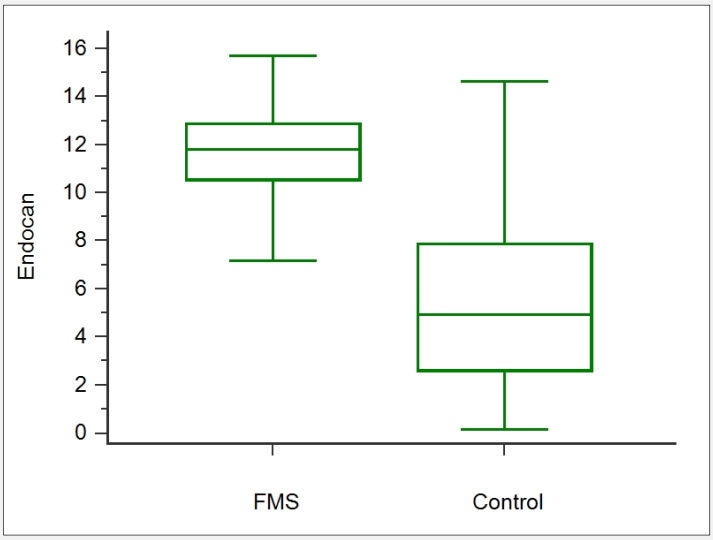

Figure 1. Mean differences of serum endocan levels between fibromyalgia and control groups on the y-axis (ng/mL).

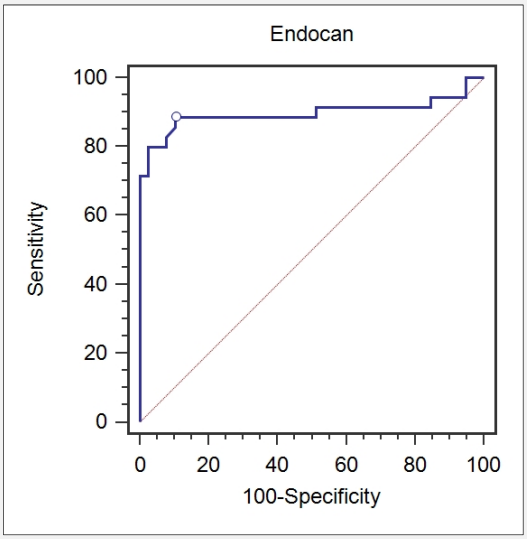

Figure 2. Receiver operating characteristic analysis of endocan for the diagnosis of fibromyalgia (area under curve: 0.897, cutoff value: 9.81 ng/mL, sensitivity: 88.5%, specificity: 89.7%).

mation [7].

In a recent review, it was emphasized that in fibromyalgia cases, high levels of cytokines, particularly those of IL-6, IL-8, and IL-1RA, decreased the pain threshold of patients by increasing the expression of substance P. Therefore, the authors suggested that the relationship between this chemokine-cytokine network and fibromyalgia should be further investigated to identify effective

treatment methods [20].
In fibromyalgia patients, brachial artery flow-mediated dilatation (FMD) was reported to be reduced and this decrease was an indication of endothelial dysfunction [9]. In another study, the FMD of fibromyalgia patients was measured and their brachial-ankle pulse wave velocity (baPWV) was calculated to determine arterial stiffness based on the status of large and small arteries in the lower extremity. As a result, the authors reported increased arterial stiffness and endothelial dysfunction [10].
In addition to cancer, sepsis, and cardiovascular diseases, serum endocan levels have also been associated with several other diseases such as diabetes, systemic sclerosis, psoriasis, and Behçet's syndrome [13-16,21, 22]. All these results clearly demonstrate the importance of inflammation and endothelial dysfunction in the pathophysiology of fibromyalgia. The current study further explored this etiopathogenesis using the new marker of the endocan level.
Furthermore, the current study also showed that the serum endocan level could be an exclusive marker for the diagnosis of fibromyalgia presenting with meaningful and significant percentages of sensitivity and specificity (Figure 2). It should also be noted, however, that the endocan assay method utilized in this study (ELISA method with Cusabio® Endocan ELISA kit) was only for experimental purposes and is not appropriate for clinical use.

CONCLUSION

In conclusion, the serum endocan levels were found to be increased in patients with fibromyalgia. These results demonstrate the presence of increased subclinical inflammation and endothelial dysfunction in fibromyalgia and offer insight into an important mechanism in the pathophysiology of fibromyalgia. However, this study was conducted on a relatively small number of patients. Therefore, for future work, it is suggested that the serum endocan levels are standardised in multicenter studies with larger series of fibromyalgia patients.

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Ethical Approval:
The required permissions were obtained from the clinical research ethics committee of Erzincan University (dated 27/07/2016; number: 33216249-604.01.02-E.29 220).

Contributorship:
CM planed the study. All authors were involved in gaining ethical approval, patient recruitment, and data analysis. CM wrote the manuscript after all authors reviewed and edited the manuscript and approved the final version of the manuscript.

Declaration of Interest:
None declared.

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