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Evaluation of serum endothelial cell-specific molecule-1 levels and carotid intima-media thickness in patients with ankylosing spondylitis

Ankilozan spodilitli hastalarda serum endothelial cell-specific molecule-1 ve karotis intima-media kalınlığının değerlendirilmesi

Abstract: Objective: Ankylosing spondylitis (AS) is a chronic inflammatory disease and the increased mortality in these patients is largely caused by cardiovascular diseases. Endothelial cell-specific molecule-1 (ESM-1) is a novel marker to assess endothelial dysfunction and expressed by the vascular endothelium. In this study, the serum ESM-1 levels in patients with AS and the possible association between serum ESM-1 and carotid intima-media thickness (CIMT) as a marker of atherosclerosis was evaluated.

Methods: A total of thirty-seven patients with AS and thirty healthy control subjects were included in this study. ESM-1, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and CIMT were measured in all subjects. ESM-1 levels were measured by ELISA method. The disease activity of patients with AS were assessed using questionnaires Bath Ankylosing Spondylitis Functional Index (BASFI) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI).

Results: Serum ESM-1 levels were lower in AS patients than in healthy controls. However, there was no statistically significant difference between ESM-1 levels (304.3±185.2 vs. 373.9±206.9 ng/L, respectively; p=0.064). Patients with AS had significantly higher CIMT levels compared with controls (0.77±0.16 vs. 0.53±0.09 mm, respectively; p<0.001). While a statistically significant positive correlation was detected in all subjects between CIMT levels and ESR, CRP (r=0.378, p=0.002, r=0.547, p<0.001, respectively), no significant correlation was detected between serum ESM-1 levels and ESR, CRP, BASDAI, BASFI and CIMT.

Conclusion: The results showed that CIMT values in AS patients were increased when compared to control group. There was no correlation among ESM-1 levels, disease activity and CIMT. In order to reveal the pathological role of the ESM-1 levels in patients with AS need more studies.

Keywords: Ankylosing spondylitis, endothelial cell-specific molecule-1, carotid intima-media thickness, CRP

Özet: Amaç: Ankilozan spondilit (AS) kronik inflamatur
bir hastalıktır ve bu hastalarda artış mortalite büyük oranda kardiyovasküler hastalıklardan kaynaklanmaktadır. Endothelial cell-specific molecule-1 endotel tarafından exprase edilen ve endotel disfonksiyonu değerlendirmek için yeni bir belirteçtir. Çalışmamızda, AS hastalarında serum ESM-1 düzeylerini ve aterosklerozun bir göstergesi olarak Karotis İntima-Media Kalınlığı (KIMK) arasındaki olası ilişkisini değerlendirmeyi amaçladık.

Metod: Çalışmaya 37 AS’li hasta ve 30 sağlıklı kontrol grubu dahil edildi. Her iki grupta ESM-1, eritrosit sedimentasyon hızı (ESH), C-reactive protein (CRP) ve KIMK ölçümleri yapıldı. ESM-1 düzeyleri eliza yöntemiyle ölçüldü. Hastalık aktivitesini değerlendirmek için BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) ve BASFI (Bath Ankylosing Spondylitis Functional Index) indeksleri kullanıldı.

Bulgular: Serum ESM-1 düzeyleri, AS hastalarında sağlıklı kontrollere göre düşüktü. Bununla birlikte, ESM-1 düzeyleri arasında istatistiksel olarak anlamlı fark yoktu (304.3±185.2 ve 373.9±206.9 ng/L, p=0.064). AS’li hastalar kontrolle karşılaştırıldığında anlamlı olarak yüksek KIMK düzeylerine sahipti (0.77±0.16 ve 0.53±0.09 mm, p<0.001). KIMK düzeyleri ile ESH ve CRP arasında istatistiksel olarak pozitif korelasyon tespit edilirken (r=0.378, p=0.002, r=0.547, p<0.001), ESM-1 düzeyleri ile ESH, CRP, BASDAI, BASFI ve KIMK arasında anlamlı korelasyon tespit edildi.

Sonuç: Sonuçlar kontrol grubuna kıyasla AS hastalarında KIMK değerleri arttuğunu gösterdi. ESM-1 düzeyleri, hastalık aktivitesi ve CIMT arasında korelasyon saptanmadı. AS hastalarında ESM-1 düzeylerinin patolojik rolünü ortaya çıkarmak için daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler: Ankilozan Spondilit, endothelial cell-specific molecule-1, Karotis İntima-Media Kalınlığı, CRP

Introduction

Ankylosing spondilitis (AS) is a chronic inflammatory disease generally affecting the spine and causing severe pain and stiffness in patients. Affecting both genders, though the majority of patients are male, it is known to be a spondyloarthropathy related to HLA-B27. Though AS typically affects the axial skeleton, it can be related to involvement of the peripheral joints and entheses (tendon, capsule and ligament). Apart from this, signs of extra articular involvement of the eye, intestine, lung, heart, skin, bone and kidneys may be observed. During the course of the disease, the incidence and severity of extra articular involvement may change [1–4]. Additionally it has been reported that compared to the general population chronic inflammatory diseases such as AS can increase accelerated atherosclerosis along with cardiovascular morbidity and mortality [5,6].

The increased mortality in patients with AS is largely caused by cardiovascular diseases. It has been suggested that patients with AS have higher risk factors for ischemic heart disease, peripheral vein disease, atherosclerosis, congestive heart failure and cardiovascular diseases compared to healthy controls. Also the relationship between subclinical atherosclerosis and severity of inflammatory response has been clearly observed in arthritic patients [3,7].

Endothelial dysfunction forms an important step at the beginning of atherosclerosis and occurs in the early period of the disease. As a result the link between systemic inflammatory diseases and atherosclerosis may be understood at the endothelial level [5]. Measurement of the carotid intima-medial thickness (CIMT) with ultrasound is a non-invasive, sensitive and repeatable technique to determine the risk of cardiovascular disease (CVD) and atherosclerotic burden. Carotid intima-media thickness is widely used as a prominent marker for both systemic inflammation and cardiovascular diseases [8].

Endocan, known as Endothelial cell-specific molecule-1 (ESM-1), is a soluble dermatan sulfate proteoglycan containing 165 amino acids and weighing 50 kDa. Recent studies have emphasized that ESM-1, expressed by vascular endothelium, may have a potential structural and functional role in cancer and inflammatory diseases [9,10].

The increased cardiovascular disease risk in AS patients and the important role of endothelial dysfunction in the development of atherosclerosis have increased the importance of identifying subclinical atherosclerosis in the early period. In our study we aimed to determine the serum ESM-1 levels in patients with AS and the possible association between serum ESM-1 and CIMT as a marker of atherosclerosis is evaluate.

Method

Population of the study

The study comprised 37 patients applying to the Physical Medicine and Rehabilitation clinic with AS diagnosis according to the modified New York Criteria, and a control
group of 30 healthy volunteers with no cardiovascular and metabolic diseases and similar age and gender [11]. Before the study permission was obtained from the local ethics committee (Ethical No: 2014-10/28.05.2014). Patients and healthy controls were informed about the study and a consent form was provided. To evaluate the disease activity of the patient group, the laboratory and clinical parameters of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Bath Ankylosing Spondylitis Functional Index (BASFI) were used. Individuals with acute and chronic infection that could affect ESM-1 levels, malignancy, diabetes, hypertension, acute and chronic kidney failure, chronic liver disease, pregnancy, any other inflammatory rheumatism disease and those with history of allergic diseases were excluded from the study.

Biochemical analysis

Blood samples were collected from all subjects after fasting by venous puncture technique into Vacutainer (BD Diagnostics, Plymouth, UK) tubes with ethylenediaminetetraacetic acid (EDTA), sodium citrate and no anticoagulants for biochemical determinations. Serum samples were obtained after the centrifugation of blood samples at 4000 rpm for 10 minutes. Serum CRP levels were performed in the same day with immunoturbidimetric method on the Cobas c501 autoanalyzer (Roche Diagnostics, Indianapolis, IN). ESR was determined by the Westergren method.

Serum samples collected for ESM-1 were stored at -20°C until analyses. Serum ESM-1 levels were measured using an ESM-1 enzyme-linked immunosorbent assay (ELISA) kit (Cat. No: CK-E90514, Hangzhou Eastbiopharm Co. Ltd., Hangzhou, China) according to the manufacturer’s instructions. The intra-assay and inter-assay coefficients of variations were <10% and <12% for ESM-1 (ng/L) respectively.

Carotid intima-media thickness measurements

Carotid intima-media thickness was examined after 15 minutes rest in a supine position with head turned 20–30 degrees in the opposite direction. Images were obtained by a radiology expert using a Toshiba Apio XG model Doppler ultrasonograph and 12 MHz linear transducer. Carotid intima-media thickness was measured 1 cm proximal to the bulbous common carotid artery and on the posterior wall. The carotid artery lumen-intima and media-adventitia edges were identified as a double line.

Statistical analysis

SPSS version 15.0 program was used for statistical analysis. The power analysis was performed to determine the minimum sample size in this study. The minimum sample size to achieve a significance level of 0.05 and 90% power was calculated to be 24 subjects (per group). Continuous variables are shown as mean±SD or median (min–max), variable conformity with normal distribution was investigated using the Kolmogorov-Smirnov/Shapiro-Wilk tests. The independent t test was used to compare normally distributed variables between groups, and the Mann-Whitney U test for non-normally distributed variables. For the comparison of categorical data, chi-square test was used. Correlation between constant variables in the case group was examined using Spearman’s correlation test. p<0.05 was accepted as statistically significant.

Results

The study comprised 37 AS patients and 30 controls, a total of 67 individuals. The clinical and laboratory data of patient and control groups are given in Table 1. Of participants in the study 26.9% (n=18) were women and
73.1% (n=49) were male. The average age of participants was 37.8±9.0 years, with the average age of AS patients 39.4±10.0 years and the average age of the control group 35.9±7.3 years. Of AS patients 29.7% (n=11) were female and 70.3% (n=26) were male, while in the control group 23.3% (n=7) were female and 76.7% (n=23) were male. There was no statistically significant difference in terms of age or gender (p=0.110 and p=0.557, respectively).

The average duration of disease in AS patients was 11.2±9.4 years (median=8, min-max=1–36). While 30 patients (81.1%) were receiving non-steroidal anti-inflammatory medication (NSAID), 7 patients (18.9%) were receiving anti-TNF treatment.

When the ESM-1 levels of the patient and control groups were compared, there was no statistically significant difference found (304.3±185.2, 236.5 (151.7–1014.8) vs. 373.9±206.9, 280.1 (176.8–965.2) respectively; p=0.064). Data have been indicated as Average of the data standard deviation, median (min-max). AS: Ankylosing spondylitis, ESM-1: Endothelial cell-specific molecule-1.

When the ESM-1 levels of the patient and control groups were compared, there was no statistically significant difference found (304.3±185.2, 236.5, min-max=151.7–1014.8 ng/L vs. 373.9±206.9, median=280.1, min-max=176.8–965.2 ng/L, respectively; p=0.064) (Fig 1). When the relationship between ESM-1 levels and the clinical parameters of ESR, CRP, BASDAI, BASFI and CIMT was investigated, there was no statistically significant correlation found (Table 2). There was no statistically significant difference in ESM-1 levels between patients receiving conventional NSAID and anti-TNF treatment (307.4±195.1, median=235.0, min-max=151.7–1014.8 ng/L vs. 290.9±146.9, median=246.6, min-max=153.5–608.6 ng/L, respectively; p=0.805).

The mean CIMT in the ankylosing spondylitis patient group was higher at a statistically significant level compared to the control group (0.77±0.16 vs. 0.53±0.09, respectively; p<0.001) (Fig 2). When the relationship between carotid intima-media thickness and the clinical parameters were investigated, while there was a statistically significant positive correlation of CIMT with ESR (r=0.378, p=0.002) and with CRP (r=0.547, p<0.001) there was no correlation found with age, BASDAI, BASFI and ESM-1 parameters. There was no statistically significant difference in CIMT levels between patients receiving conventional NSAID and anti-TNF treatment (0.78±0.17, median=0.80, min-max=0.40–1.20 mm vs. 0.71±0.12, median=0.70, min-max=0.60–0.90 mm, respectively; p=0.243).

Table 2: Correlation of study parameters with carotid intima media thickness and ESM-1 levels in all subjects.

|        | ESM-1   |        | CIMT   |        |
|--------|---------|--------|--------|--------|
| r      | p       | r      | p      |
| ESM-1  | -0.094  | NS     | -0.094 | NS     |
| CIMT   | -0.109  | NS     | -0.547 | <0.001 |
| CRP    | -0.054  | NS     | 0.378  | 0.002  |
| ESR    | -0.190  | NS     | -0.191 | NS     |
| BASDAI | 0.110   | NS     | -0.080 | NS     |

ESM-1: Endothelial cell-specific molecule-1; CIMT: Carotid intima media thickness; NS: Not significant; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index.
Discussion

In our study no statistically significant difference was found when the ESM-1 levels of AS patients and the control group were compared. Additionally there was no significant relationship found between ESM-1 levels and clinical parameters. Another finding of our study is that the CIMT measured in AS patients was higher compared to the control group and there was a statistically significant positive correlation of CIMT with ESR and CRP. To our knowledge, this is the first study evaluating the relationship between ESM-1 level and atherosclerosis in AS patients.

AS is a chronic disabling inflammatory disease characterized by sacroiliitis, spinal inflammation and enthesitis. Also the disease may affect peripheral joints, eye, gut and aorta [12]. Recently, it has been suggested that AS is closely related to impaired endothelial function, which is an onset of the underlying mechanisms in atherosclerosis [13,14]. Serological and sonographic reports have demonstrated the endothelial dysfunction of AS patients [15–17].

Several biomarkers are used to for the assessment of endothelial function. Endocan, known as Endothelial cell-specific molecule-1 (ESM-1), is a soluble dermal sulfate proteoglycan secreted by vascular endothelium. ESM-1 is a novel marker of endothelial activation, tumor angiogenesis, invasion, and tumor progression, cancer, inflammation and sepsis [18]. ESM-1 may reflect endothelial dysfunction in many conditions such as hypertension, inflammation and chronic kidney disease [19–21]. It has been demonstrated that expression of ESM-1 in arthritic synovial tissues from patients with rheumatoid arthritis or osteoarthritis was increased [22]. In contrast, we found decreased levels of ESM-1 in the AS group when compared to the healthy group (p>0.05). Also we determined no statistically significant correlation between the serum ESM-1 level of patients and ESR, CRP, BASDAI and BASFI. ESM-1 levels can be affected by NSAID and anti-TNF treatment which altered the production of inflammatory cytokines. Because the overexpression of ESM-1 in human endothelial cells induced by inflammatory cytokines including TNF-alpha, and pro-angiogenic growth factors such as VEGF, FGF-2 and HGF/SF [10]. Gonzalez et al. determined that IL-1 and TNF-alpha levels reduced in a study investigating the effects of long-term NSAID use on cytokines and inflammatory mediators in osteoarthritis patients [23]. In another study, it has been reported that the VEGF levels increased in AS patients not treated, however these levels were lower in patients treated with corticosteroids [24]. Ozmen et al have investigated the effects of conventional therapy on the patients of AS. Their results indicated that the novel biomarkers related with vascular injury in patient with AS have not been affected by conventional therapy of 2 months’ duration, but BASDAI and acute phase reactants have been shown significant decrease during this period [6].

It is known that measurement of CIMT by ultrasonography is a noninvasive method used to assess endothelial function which is the initial step of atherosclerosis [25]. In a systematic review and meta-analysis of 68 comparisons, a significant increase in CIMT has been observed with rheumatic diseases populations (ie, rheumatoid arthritis, systemic lupus erythematosus, and systemic sclerosis) compared with age- and sex-matched healthy controls [26]. It has been demonstrated that CIMT was slightly increased in the in patients with Behcet’s disease without vascular involvement compared to the control, but the difference was not significant statistically [27]. Furthermore, Karaoglan et al. were evaluated endothelial dysfunction of rheumatoid arthritis (RA) patients and detected CIMT and Asymmetric dimethylarginine (ADMA) values were higher in patients with rheumatoid arthritis than the control group [28]. It has been shown that CIMT was elevated in AS patients than healthy controls and it was correlated with the disease index BASMI (Bath Ankylosing Spondylitis Metrology Index). In addition, no correlation was observed between CIMT and CRP levels in patients of AS [29]. Similarly, in the present study CIMT was found to be possibly associated with AS. We revealed the CIMT values of AS patients were higher than the healthy control group. Also while we found a significant positive correlation between CIMT and ESR and CRP, there was no correlation with ESM-1 levels. In an observational study, association between CRP, CIMT and P-wave dispersion (PWD) in obese premenopausal women was evaluated. The investigators reported that increased PWD values in obese patients were correlated positively with CRP, CIMT and abdominal obesity [30]. Resoruł et al indicated that CIMT and epicardial adipose tissue thickness as a novel indicator of atherosclerosis were higher in the AS patients compared to the control group [31]. According to a recent report, subclinical atherosclerosis in patients with AS was assessed and epicardial fat thickness, CIMT and aortic stiffness index were significantly elevated in AS patients than in healthy controls [32].

Systemic inflammation plays an important role to development of cardiovascular complications in chronic inflammatory rheumatic diseases [26]. Increased levels of inflammatory mediators such as CRP and interleukin (IL)-6 have been shown to correlate with adverse events in cardiovascular disease. Besides, these mediators were associated with endothelial dysfunction. Serum CRP
levels elevated in RA patients and correlated with activity of the disease. In AS patients, circulating levels of inflammatory mediators including CRP, IL-6, TNF-α, tissue factor and fibrinogen were higher than control group [33]. In the present study, serum CRP levels were elevated when compared to control group (p<0.001). Also, we found a statistically significant positive correlation of CIMT with CRP (r=0.547, p<0.001). Indeed, CIMT and CRP appears to be beneficial markers to determine inflammation linked to subclinical atherosclerosis in AS patients.

Conclusions

In conclusion, the aim of this study was to determine ESM-1 and CIMT values in AS patients and reveal whether there was a relationship between the two or not. In our study there was no difference in ESM-1 levels while CIMT values were increased and it was shown that there was no relationship between them. However, we found a positive correlation of CIMT with CRP. When the results of the study are evaluated with previous studies, CIMT and CRP may be precursors of cardiovascular diseases in AS patients without cardiovascular risk factors. However to state that ESM-1 levels in AS patients may be related to inflammation and atherosclerosis more clinical studies including more AS patients who are newly-diagnosed, untreated and with high disease activity are required.

Conflict of Interest: The authors have no conflict of interest.

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