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Evaluation of carotid intima-media thickness with vascular endothelial growth factor and malondialdehyde levels in patients with sarcoidosis

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KEYWORDS
Carotid intima-media thickness;
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Abstract
Purpose: To assess the impact of sarcoidosis on endothelial function by measuring carotid intima-media thickness (CIMT) and serum levels of malondialdehyde and vascular endothelial growth factor (VEGF).
Materials and methods: We prospectively analyzed 41 patients with sarcoidosis (9 men, 32 women) with a mean age of 44.9 ± 10.2 (SD) years and 34 healthy subjects (9 men, 24 women) with a mean age of 37.26 ± 8.9 (SD) years who served as a control group. Sarcoidosis patients receiving steroids were included in Group 1 while those not under steroid treatment were included in Group 2. CIMT measurements were performed using B-mode ultrasound. Malondialdehyde and VEGF serum levels were obtained in all sarcoidosis patients and control subjects.
Results: Both right and left CIMT was significantly higher in Group 1 and Group 2 than in control subjects. Serum levels of malondialdehyde and VEGF in Group 1 and Group 2 were significantly higher than in healthy subjects. No differences in CIMT, malondialdehyde and VEGF were found between Group 1 and Group 2.

Abbreviations: VEGF, Vascular Endothelial Growth Factor; CIMT, Carotid intima-media thickness; TBARS, Thiobarbituric acid reactive substances; FMD, flow-mediated dilatation.

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Conclusion: Sarcoidosis results in increased CIMT, VEGF and malondialdehyde serum levels. However, there was no difference in terms of CIMT, VEGF and malondialdehyde levels between sarcoidosis patients with or without steroid treatment, suggesting that new treatment strategies for sarcoidosis vascular involvement should consider this result.

Sarcoidosis is an inflammatory disorder with unknown etiology, which commonly involves the lungs and lymph nodes [1]. Except for the lungs and lymph nodes, sarcoidosis may involve many organs including heart, eyes, nervous system and liver [1]. Noncaseating granulomas and increased inflammatory mediators are the main characteristic features of sarcoidosis [1,2]. Since sarcoidosis is an inflammatory disorder, involvement of the vascular system is not unexpected in patients with this condition. However, until recently, vascular involvement in patients with sarcoidosis has received little attention.

Atherosclerosis is an inflammatory disease that is characterized by changes in the arterial wall and formation of plaques in large and medium elastic and muscular arteries, due to endothelial dysfunction [3,4]. These changes may be unnoticed for a lifetime, but in some patients they may present as acute vascular diseases [3—5]. In the early stages of arterial remodeling, no luminal changes are present, thus radiological modalities that mainly give information about luminal changes of the vessels such as angiography and Doppler ultrasound do not provide useful information [6,7]. In contrast, carotid intima-media thickness (CIMT) obtained by B-mode ultrasound is a validated and accepted surrogate marker for early atherosclerosis. By measuring CIMT, radiologist could detect every stage of atherosclerosis by visualizing the arterial wall itself [8—13]. Correlation of CIMT and increased risk of cardiovascular events has been studied in various disease and conditions [10—20]. However to our knowledge, CIMT in patients with sarcoidosis patients has never been studied.

Malondialdehyde is a highly toxic product that results from lipid peroxidation of polyunsaturated fatty acids [21]. Malondialdehyde is a well-known marker that shows oxidative stress [21]. Vascular endothelial growth factor (VEGF) is a well-known marker of angiogenesis, which was found to be associated with some granulomatous diseases such as Crohn disease, tuberculosis and Wegener granulomatosis [22]. Increased level of VEGF was also detected in pulmonary lesions of sarcoidosis patient [22].

The goal of this study was to assess vascular involvement of sarcoidosis by evaluating CIMT with serum levels of malondialdehyde, and VEGF.

Material and methods

Patients

In this study, we prospectively analyzed 41 patients with sarcoidosis (9 men, 32 women) with a mean age of 44. 9 ± 10.2 (SD) years [range: 28—66 years] and 34 healthy subjects without sarcoidosis (9 men, 25 women) with a mean age 37.3 ± 8.9 (SD) who served as a control group.

The study was performed from December 2014 to October 2015 in Haseki training and research hospital. In all patients, the diagnosis of sarcoidosis was proven by presence of noncaseating granulomas in various tissue specimens such as lung, lymph nodes or skin. Patients with systemic disease, renal insufficiency, diabetes mellitus, known or suspected neoplasm, hematological malignancies, immunosuppression, surgery during the previous 6 months, pregnancy, lactation and history of myocardial infarction or stroke were excluded from the study. Subjects were asymptomatic patients without medical treatment, no smoking habit and had a normal physical examination, a normal resting electrocardiogram, normal blood pressure, normal blood count, and routine serological tests, and no history of systemic diseases.

Patients with sarcoidosis were divided into two groups according to treatment status. Sarcoidosis patients under cortisone treatment were included in Group 1 while those who did not receive steroids were included in Group 2. In Group 1, patients were receiving 5 to 60 mg of prednisone (Deltacortril®, Pfizer) for at least 9.4 ± 7.0 (SD) months.

The study was conducted according to the recommendations set forth by the Declaration of Helsinki on biomedical research involving human subjects. Approval for this prospective study was obtained from the local ethics committee and every participant gave written informed consent following explanation by the principal investigator.

Carotid intima-media thickness measurement

The same radiologist (C.S.) with an experience of 5 years in ultrasound performed all CIMT measurements blinded to clinical data of the patients. Patients and healthy controls were examined using a LOGIQ9 (General Electric Healthcare system, Milwaukee, WI, USA) equipped with a linear transducer (8L) with a frequency ranging from 8—13 MHz.

All CIMT examinations were performed according to the American Society of Echocardiography Carotid Intima-Media Thickness Task Force recommendations [23]. The patients were placed in a comfortable supine position with slightly hyperextended and contra-laterally rotated neck for the US examination. Longitudinal images were taken of the carotid system. Ultrasound images were captured at whatever time of the cardiac cycle and CIMT measurements were performed from the posterior wall of the both of the common carotid artery, 10 mm proximal to the initiation of the carotid bulb. The zoom levels were adjusted according to comfort of the operator during examinations. All data were recorded in our local picture archiving and communication

system (Extremepacs, Ankara, Turkey). Fig. 1 shows technique for caliper placement for CIMT measurements.

Biochemical findings

Measurements of the malondialdehyde in plasma samples were performed with the thiobarbituric acid reactive substances (TBARS) assay kit. (San Diego, USA, Cell Biolabs. Inc.) It's a tool for the direct quantitative measurement of malondialdehyde in biological sample. The unknown malondialdehyde containing samples or malondialdehyde standards were first reacted with thiobarbituric acid reactive at 95 °C. After a brief incubation, the samples and standards read spectrophotometrically. The malondialdehyde content in unknown samples were determined by comparison with predetermined malondialdehyde standard curve.

A commercial human VEGF immunoassay kit (Biosource, CA, USA) was used for the analysis of VEGF levels. The ELISA kit is a solid-phase sandwich immunoassay that is designed for the detection of VEGF isoform 165.

Statistical analysis

Statistical analyses were performed using the SPSS software (version 16.0. SPSS. Chicago. Ill). Categorical variables were expressed as proportions and percentages Continuous variables were expressed as mean, standard deviation (SD) and range. Independent comparisons between two groups were performed with Student *t* test or Mann-Whitney U test. Ratios between groups of categorical variables were tested by χ^2 test. The relationship between numeric variables was examined with Spearman correlation analysis because parametric test condition was not provided. Statistical significance was asset at $P < 0.05$.

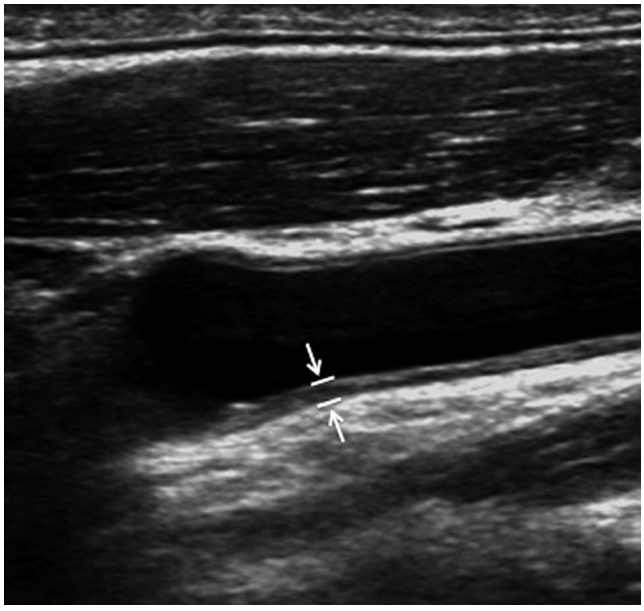

Figure 1. Grey-scale ultrasound image of carotid artery wall in a 40-year-old woman with sarcoidosis. The distance between the arrows from the lumen—intima interface to the media—adventitia interface indicates an intima-media thickness of 0.9 mm.

Results

Demographic characteristics, CIMT values, VEGF and malondialdehyde levels of patients and control subjects are listed in the Table 1.

Patients in Group 1 (47.56 ± 11.37 years) were older than control subjects (37.26 ± 8.93 years) ($P = 0.001$). No difference in terms of age were found between Group 1 (47.56 ± 11.37 years) and Group 2 (42.39 ± 8.80 years) ($P = 0.204$) and Group 2 (42.39 ± 8.80 years) and control subjects (37.26 ± 8.93 years) ($P = 0.121$). No difference in gender distribution was observed between the different groups of patients.

After matching for age and gender, the mean malondialdehyde serum levels of patients in Group 1 (47.62 ± 36.14 μmol/L) and Group 2 (66.98 ± 40.31 μmol/L) were greater than those in control subjects (44.56 ± 41.88 μmol/L) ($P = 0.030$ for both comparison). However, there was no difference between Group 1 (47.62 ± 36.14 μmol/L) and Group 2 (66.98 ± 40.31 μmol/L) in terms of mean malondialdehyde serum level ($P = 0.115$). Mean VEGF serum levels in Group 1 (26.12 ± 14.23 pg/mL) and Group 2 (27.75 ± 12.34 pg/mL) were greater than in control subject (19.38 ± 12.59 pg/mL) ($P < 0.009$ for both comparison). No difference was found between Group 1 (26.12 ± 14.23 pg/mL) and Group 2 (27.75 ± 12.34 pg/mL) in terms of mean VEGF serum level ($P = 0.674$).

Mean right and left CIMT were significantly greater in Group 1 and Group 2 than in control subjects ($P < 0.001$) (Table 1). However no differences were found between Group 1 and Group 2 in terms of left CIMT ($P = 0.821$) and right CIMT ($P = 0.932$).

Discussion

In this present study, we have demonstrated that CIMT is increased in sarcoidosis patient compared to healthy controls. Furthermore, sarcoidosis patients have increased plasma levels of malondialdehyde and VEGF compared to healthy controls. However, there was no difference between sarcoidosis patients with or without cortisone treatment in terms of all these variables.

Many markers have been expected to reflect the activity or the prognosis of sarcoidosis [24—27]. Corticosteroids are generally administered to sarcoidosis patients with progressive parenchymal lung involvement or when extrathoracic organ involvement is present. Sekiya et al. found higher serum levels of VEGF in sarcoidosis patients under cortisone treatment compared to sarcoidosis patients without steroid treatment and suggested that serum VEGF might be a prognostic indicator in sarcoidosis patients [28]. In our study, we demonstrated that patients with sarcoidosis have higher serum levels of VEGF compared to control subjects. However, we found no difference in VEGF levels of sarcoidosis patient with or without cortisone treatment. We believed that polymorphism of *VEGF* genes might be the main factor that are causing discrepancies between these two studies [29].

Recent studies revealed increased levels of malondialdehyde in diabetes mellitus, dyslipidemia, and metabolic syndrome [21,30,31,32]. These studies also suggested

Table 1 Demographic characteristics, carotid intima-media thickness and serum levels of vascular endothelial growth factor and malondialdehyde in sarcoidosis patients and control subjects.

	Group 1 (patients under cortisone treatment)	Group 2 (patients without cortisone treatment)	Controls
Age	47.56 ± 11.37 [28–66]	42.39 ± 8.80 [29–61]	37.26 ± 8.93 [19–53]
Gender (M/F)	4/14	5/18	9/25
Left CIMT (mm)	0.78 ± 0.17 [0.5–1.1]	0.76 ± 0.12 [0.57–0.96]	0.57 ± 0.11 [0.4–0.8]
Right CIMT (mm)	0.74 ± 0.14 [0.51–1]	0.73 ± 0.12 [0.47–0.9]	0.57 ± 0.10 [0.4–0.69]
VEGF (pg/mL)	26.12 ± 14.23 [6.788–49.845]	27.75 ± 12.34 [12.961–53]	19.38 ± 12.59 [1.2–49.4]
Malondialdehyde (μmol/L)	47.62 ± 36.14 [6.263–131]	66.98 ± 40.31 [4.171–131.25]	44.56 ± 41.88 [0.62–127.8]

All values are expressed as mean ± SD. Numbers in brackets are ranges. CIMT: intima-media thickness; VEGF: vascular endothelial growth factor.

that malondialdehyde might be important biomarker for assessing cardiovascular risk [33,34]. In our study, we found increased serum levels of malondialdehyde in our sarcoidosis patients compared to control subjects, which clearly demonstrated harmful effect of sarcoidosis on the cardiovascular system. However, we found no difference in serum level of malondialdehyde in sarcoidosis patients with or without steroid treatment. Ohira et al. showed beneficial effects of metformin over serum malondialdehyde levels in diabetic patients [35]. Animal studies showed effectiveness of anti-hyperlipidemic drugs in decreasing serum malondialdehyde levels [36]. We suggested that new treatment strategies other than steroids should be developed to reduce the cardiovascular risk by lowering serum malondialdehyde levels in sarcoidosis patients.

Beside our study, there are only two studies that documented the effects of sarcoidosis on cardiovascular system by directly evaluating endothelial function and arterial wall properties [37,38]. We demonstrated impairment of endothelial function and enhanced atherosclerosis in patients with sarcoidosis in accordance with the literature. However, in our study there was no difference between CIMT values in sarcoidosis patients with and without treatment. So according to our results, although steroid is a valuable treatment option for sarcoidosis patient with parenchymal, neurological, cardiac and sight-threatening ocular involvement, is not seen as a reliable treatment strategy for cardiovascular involvement of sarcoidosis.

Our study has some limitations. First we have small number of sarcoidosis patients, so future studies with larger series are required. Also, we did not evaluate consistency of CIMT and flow-mediated dilatation, which is a well-known marker of increased cardiovascular risk and has been shown as a feasible method to detect sarcoidosis vascular involvement, so future studies that evaluate compatibility of these diagnostic modalities should be needed [37,38]. Also, assessment of new preventive and treatment modalities for sarcoidosis patients, and efficiency of these new strategies could also be evaluated by both of these diagnostic modalities.

In conclusion, we demonstrated the presence of vascular involvement in sarcoidosis by showing increased CIMT, VEGF and malondialdehyde values in sarcoidosis patients compared to healthy controls. More importantly, we demonstrated that there are no differences in CIMT and plasma malondialdehyde levels between sarcoidosis patients with or without steroid treatment, so we emphasize that new treatment strategies for sarcoidosis vascular involvement should consider this result.

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The authors have no relevant financial interest in this article.

Disclosure of interest

The authors declare that they have no competing interest.

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