


# Serum Endocan Levels and Subclinical Atherosclerosis in Behçet's Syndrome

Eman Mostafa Nassef<sup>1</sup>, Hemmat Ahmed Elabd<sup>2</sup>, Basma Mohamed Mohamed Ali El nagger<sup>2</sup>, Hala Mohamed Elzomor<sup>2</sup>, Hend Gamal Kotb<sup>1</sup>, Seham Sabry<sup>1</sup>, Boshra Ahmed Zaghloul<sup>3</sup>, Asmaa S Hassan<sup>4</sup> , Eman El Sayed Mohamed<sup>4</sup>

<sup>1</sup>Internal Medicine Department, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt; <sup>2</sup>Rheumatology and Rehabilitation Department, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt; <sup>3</sup>Radiodiagnosis Department, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt; <sup>4</sup>Clinical Pathology Department, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt

Correspondence: Eman Mostafa Nassef, Internal Medicine Department, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt, Tel +20 1098002232, Email nassef.eman@yahoo.com

**Background and Aim:** Behçet disease (BD) is a rare chronic relapsing-remitting inflammatory systemic vasculitis. BD patients were reported to have marked acceleration of subclinical atherosclerosis (SCA). Endocan is a soluble proteoglycan mainly secreted by the activated endothelium. The present study aimed to assess the relation between serum endocan levels and SCA in BD patients.

**Subjects and Methods:** The study included 40 adult BD patients in addition to twenty age- and sex-matched healthy controls. BD was diagnosed according to International Study Group criteria. Upon recruitment, all participants were subjected to careful history taking and thorough clinical examination. BD activity was assessed using Behçet Syndrome Activity Score. Measurement of serum endocan was performed using quantitative double-antibody sandwich ELISA kit. CIMT measurement was done using B-mode ultrasound.

**Results:** Comparison between patients and controls regarding serum endocan levels revealed significantly higher endocan levels in BD patients [median (IQR): 155.0 (69.3–610.0) versus 73.8 (51.9–94.6)]. Using ultrasound assessment, SCA was found in 14 BD patients (35.0%). Comparison between patients with SCA and patients without regarding the clinical and laboratory data revealed that the former group had significantly higher CRP [median (IQR): 36.5 (26.8–43.5) versus 21.0 (11.8–26.8) mg/dL,  $p < 0.001$ ] and endocan [median (IQR): 622.0 (107.4–974.8) versus 104.5 (64.0–342.0) mg/dL,  $p = 0.004$ ] levels. Logistic regression analysis recognized endocan [OR (95% CI): 1.0 (1.0–1.012),  $p0.035$ ] levels as significant predictor of SCA in multivariate analysis.

**Conclusion:** The present study identified the clinical value of serum endocan levels as a possible early marker of vascular involvement in BD patients.

**Keywords:** Behçet's disease, subclinical atherosclerosis, endocan

## Introduction

Behçet disease (BD) is a rare chronic relapsing-remitting inflammatory systemic vasculitis with roughly equal sex distribution. The disease is claimed to be more common in countries located along the so-called “Silk Road” passing from East Asia to the Mediterranean through the Middle East.<sup>1</sup> The exact pathological mechanisms involved in BD remain largely unknown. However, some evidence links BD to certain environmental, genetic and immunological alterations.<sup>2</sup>

Clinically, BD is characterized by the hallmarks of oral and genital ulcers. BD associated pathology may extend to include gastrointestinal tract, cardiovascular system, bony skeleton and central nervous system with some observed gender-related differences.<sup>3,4</sup> However, growing evidence suggests that BD presentations can be classified into distinctive phenotypes. Vascular phenotype affects ~40.0% of patients with clear male predominance. Vascular affection entails small and large vessels of the venous and arterial systems.<sup>5,6</sup> In fact, vascular complications may be one of the earliest manifestations leading to the diagnosis of BD.<sup>7</sup>

Besides these pronounced vascular complications, BD patients were reported to have a marked acceleration of subclinical atherosclerosis (SCA) when compared with their age-matched healthy counterparts. SCA was reported in BD patients even in the absence of major atherosclerosis risk factor<sup>8</sup> and one study identified SCA in 32.0% of BD patients in comparison to only 7.0% in healthy individuals.<sup>9</sup>

Unfortunately, mechanisms involved in the development of SCA in BD patients are not fully understood and the identification of SCA in those patients may help predict future cardiovascular events.

Endocan is a soluble proteoglycan mainly secreted by the activated endothelium. Up-regulation of endocan is controlled by proangiogenic factors and proinflammatory cytokines and it is suggested that endocan itself may serve as a proinflammatory mediator contributing to endothelial dysfunction.<sup>10,11</sup> In BD, endocan levels were found to be related to disease activity.<sup>12,13</sup> Interestingly, previous reports documented an association between elevated endocan levels and SCA in many conditions, including systemic lupus erythematosus,<sup>14</sup> type 2 diabetes mellitus<sup>15</sup> and psoriatic disease.<sup>16</sup>

The present study aimed to assess the relation between serum endocan levels and SCA in BD patients.

## Subjects and Methods

The present study was conducted at Al-Azhar University Hospitals, Cairo, Egypt in the time from January, through December, 2021. The study protocol was approved by the ethics committee of Al-Azhar Faculty of Medicine and informed consent was obtained from all participants before enrollment in line with the Declaration of Helsinki on clinical research involving human subjects.

The study included 40 adult BD patients in addition to twenty age- and sex-matched healthy controls. BD was diagnosed according to International Study Group criteria.<sup>17</sup> Patients were excluded from the study if they had any cardiovascular or cerebrovascular diseases or possible related risk factors (eg diabetes, dyslipidemia, smoking). Other exclusions criteria were chronic conditions (eg chronic kidney disease, malignancy, autoimmune diseases). Pregnant women were also excluded.

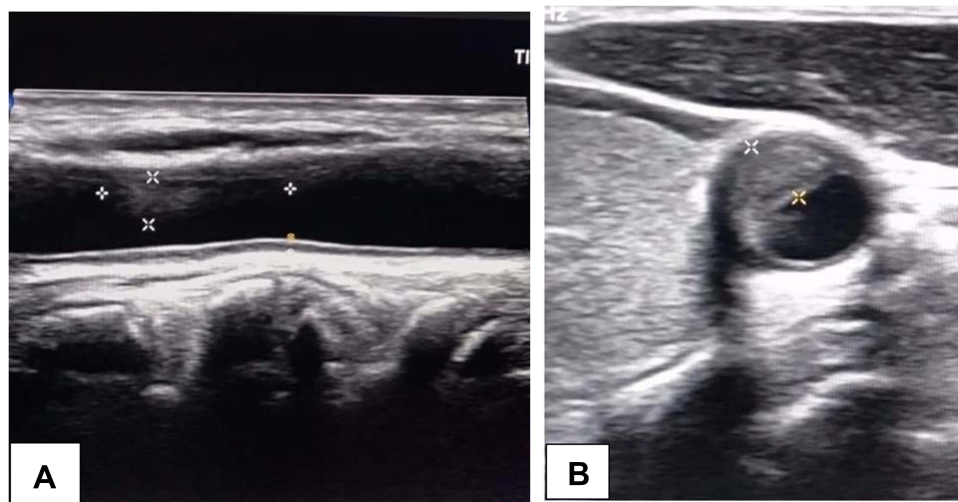
Upon recruitment, all participants were subjected to careful history taking and thorough clinical examination. BD activity was assessed using the 10-item patient-reported Behçet Syndrome Activity Score.<sup>18</sup> For laboratory assessment, 4 mL of peripheral venous blood was withdrawn from each individual and divided into two aliquots; 2 mL were collected in an EDTA tube for CBC. The remaining part was collected in a serum separator tube, centrifuged at 3500 rpm for 10 min and divided into two parts; the first part was used for biochemical tests and the remaining part of the serum was frozen at  $-20^{\circ}\text{C}$  for analysis of endocan. Measurement of serum endocan was performed using quantitative double-antibody sandwich ELISA kit (Bioassay Technology Laboratory, China, Cat. No. E3160Hu). CIMT measurement was done using B-mode ultrasound with a high-definition L12-5 linear wideband probe (Philips HDI 5000, Bothell, Washington, USA). Patients were categorized as having SCA if their CIMT measurement was  $\geq 0.9$  mm or in the presence of carotid plaques (Figure 1).<sup>19</sup>

Data were computerized and analyzed using IBM SPSS software package version 20.0. (IBM Corp, USA). Quantitative data were described using mean and standard deviation (SD) and were compared using *t* test. Qualitative data were presented in numbers and percentages and were compared using Fisher's exact test or chi-square test as appropriate. Correlation analysis was achieved using Pearson's correlation coefficient. P value less than 0.05 was considered statistically significant. Receiver operator characteristic (ROC) curve analysis was used to identify the diagnostic value of the investigated marker. Binary logistic regression was used to identify predictors of SCA. P value less than 0.05 was considered statistically significant.

## Results

The present study included 40 adult BD patients and 20 age- and sex-matched healthy controls. Clinical and laboratory findings in the studied patients are shown in Table 1. Comparison between patients and controls regarding serum endocan levels revealed significantly higher endocan levels in BD patients [median (IQR): 155.0 (69.3–610.0) versus 73.8 (51.9–94.6),  $p < 0.001$ ].

Using ultrasound assessment, SCA was found in 14 BD patients (35.0%). Comparison between patients with SCA and patients without regarding the clinical and laboratory data revealed that the former group had significantly higher CRP



**Figure 1 (A)** Longitudinal B-mode scan of the right common carotid artery at the level of atheromatous plaque showing about 50% stenosis. **(B)** Transverse B mode scan of the same lesion.

[median (IQR): 36.5 (26.8–43.5) versus 21.0 (11.8–26.8) mg/dL,  $p < 0.001$ ] and endocan [median (IQR): 622.0 (107.4–974.8) versus 104.5 (64.0–342.0) mg/dL,  $p = 0.004$ ] levels (Table 1).

Correlation analysis identified a significant correlation between serum endocan levels and CIMT measurements ( $r = 0.47$ ,  $p = 0.002$ ) (Table 2). Logistic regression analysis recognized serum CRP [OR (95% CI): 1.27 (1.04–1.56),  $p = 0.019$ ] and endocan [OR (95% CI): 1.0 (1.0–1.012),  $p = 0.035$ ] levels as significant predictors of SCA in multivariate analysis (Table 3).

ROC curve analysis showed good performance of serum endocan levels as a possible diagnostic marker of SCA in BD patients at a cut-off of 109.5 mg/dl: [AUC (95% CI): 0.78 (0.61–0.94), sensitivity: 78.6%, specificity: 53.8%] (Figure 2).

## Discussion

Using ultrasound assessment, the present study diagnosed SCA in 35.0% of the studied BD patients. This figure is close to that reported by the Turkish study of Uslu Yurteri et al.<sup>9</sup> In their work, they could diagnose SCA in 32.0% of their 100 BD-patient study.

However, the present study identified no significant relation between SCA and the clinical data in the studied patients, in contrast to Uslu Yurteri et al.'s<sup>9</sup> study, which noted a significant marginal association between SCA and vascular phenotype and major organ involvement. On the other hand, our study revealed significantly higher CRP levels in BD patients with SCA as compared to their counterparts without SCA. Uslu Yurteri et al.<sup>9</sup> study failed to document such relation. In support of our conclusions, elevated CRP levels were frequently linked to SCA in many clinical conditions including rheumatoid arthritis,<sup>20</sup> newly diagnosed diabetes<sup>21</sup> and psoriasis.<sup>22</sup>

In our work, endocan levels were significantly elevated in BD patients as compared to healthy controls. In accordance with these findings, Balta et al.<sup>12</sup> noted significantly higher endocan levels in BD patients in comparison to controls. In addition, they found a significant relation between endocan levels and disease activity.

The present study recognized a possible clinical value of serum endocan levels in the identification of SCA in BD patients as shown by regression and ROC curve analysis. As previously mentioned, other studies noted similar findings in other clinical conditions.

In systemic lupus erythematosus, endocan levels were significantly correlated with CIMT measurements and showed significant association with SCA.<sup>14</sup> Likewise, in diabetic patients, endocan levels expressed significant correlation with

**Table 1** Clinical and Laboratory Data in Patients with and without SCA

	All Patients N=40	SCA +ve n=14	SCA -ve n=26	p value
Age (years) mean $\pm$ SD	36.9 $\pm$ 5.5	38.6 $\pm$ 5.1	35.9 $\pm$ 5.6	0.13
Male/female n	34/6	12/2	22/4	0.93
Disease duration (years) median (IQR)	2.5 (1.0–4.0)	2.5 (1.0–4.0)	2.5 (1.8–4.3)	0.69
<b>Clinical data n (%)</b>				
Oral ulcer	36 (90.0)	13 (92.9)	23 (88.5)	0.66
Genital ulcer	28 (70.0)	9 (64.3)	19 (73.1)	0.56
Ocular affection	30 (75.0)	9 (64.3)	21 (80.8)	0.25
Skin affection	18 (45.0)	7 (50.0)	11 (42.3)	0.64
CNS affection	24 (60.0)	7 (50.0)	17 (65.4)	0.34
GIT affection	6 (15.0)	3 (21.4)	3 (11.5)	0.4
Joint affection	8 (20.0)	2 (14.3)	6 (23.1)	0.51
Major vessels affection	8 (20.0)	1 (7.1)	7 (26.9)	0.14
Headache	20 (50.0)	8 (57.1)	12 (46.2)	0.51
Active disease	25 (62.5)	10 (71.4)	15 (57.7)	0.39
<b>Laboratory findings median (IQR)</b>				
WBCs ( $\times 10^3$ /ml)	8.1 (7.0–9.0)	7.4 (6.9–9.0)	8.2 (7.2–8.9)	0.69
Hb (gm/dl)	12.7 (11.6–14.9)	12.7 (12.0–13.5)	12.7 (11.5–14.0)	0.99
Platelets ( $\times 10^3$ /ml)	270.0 (224.5–309.5)	267.0 (217.5–305.0)	275.0 (233.5–312.0)	0.39
Bilirubin (mg/dl)	0.9 (0.8–1.0)	0.9 (0.8–0.9)	0.9 (0.8–1.0)	0.81
Albumin (gm/dl)	4.7 (4.3–4.7)	4.7 (4.1–4.7)	4.7 (4.4–4.7)	0.92
Creatinine (mg/dl)	0.5 (0.4–0.8)	0.6 (0.5–0.8)	0.5 (0.4–0.7)	0.22
Urea (mg/dl)	26.5 (23.0–28.0)	26.5 (22.8–28.3)	26.5 (23.0–28.0)	0.88
ESR (mm/hr)	7.5 (5.3–9.8)	7.0 (5.8–9.3)	8.0 (5.0–10.0)	0.66
CRP (mg/dl)	25.0 (18.0–35.8)	36.5 (26.8–43.5)	21.0 (11.8–26.8)	<0.001
Endocan (mg/dl)	155.5 (69.3–610.0)	622.0 (107.4–974.8)	104.5 (64.0–342.0)	0.004
<b>Therapeutic interventions n (%)</b>				
Colchicine	16 (40.0)	7 (50.0)	9 (34.6)	0.34
Remicade	14 (35.0)	7 (50.0)	7 (26.9)	0.14
Steroids	14 (35.0)	4 (28.6)	10 (38.5)	0.53
Azathioprine	6 (15.0)	2 (7.1)	4 (15.4)	0.93
Cyclophosphamide	2 (5.0)	2 (7.1)	1 (3.9)	0.65
Methotrexate	2 (5.0)	2 (7.1)	1 (3.9)	0.65
Rituximab + methotrexate	4 (10.0)	2 (7.1)	3 (11.5)	0.66

**Abbreviations:** CNS, central nervous system; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GIT, gastrointestinal tract; Hb, hemoglobin; SCA, subclinical atherosclerosis; WBCs, white blood cells.

**Table 2** Correlation Between Endocan Levels and Clinical and Laboratory Data

	Endocan	
	r	p
WBCs	-0.13	0.43
Hb	0.24	0.13
Platelets	-0.17	0.3
Bilirubin	-0.15	0.34
Albumin	-0.11	0.49
Creatinine	0.015	0.93
Urea	-0.04	0.82
ESR	0.0	0.99
CRP	0.3	0.063
CIMT	0.47	0.002

**Abbreviations:** CIMT, carotid intima-media thickness; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; WBCs, white blood cells.

**Table 3** Predictors of SCA in the Studied Patients

	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	p value	OR	95% CI	p value
<b>Age</b>	1.18	1.02–1.36	0.028	1.27	0.98–1.64	0.077
<b>Sex</b>	0.92	0.15–5.76	0.93	–	–	–
<b>Active disease</b>	1.8	0.45–7.41	0.4	–	–	–
<b>CRP</b>	1.17	1.06–1.28	0.002	1.27	1.04–1.56	0.019
<b>Endocan</b>	1.0	1.0–1.006	0.003	1.0	1.0–1.012	0.035

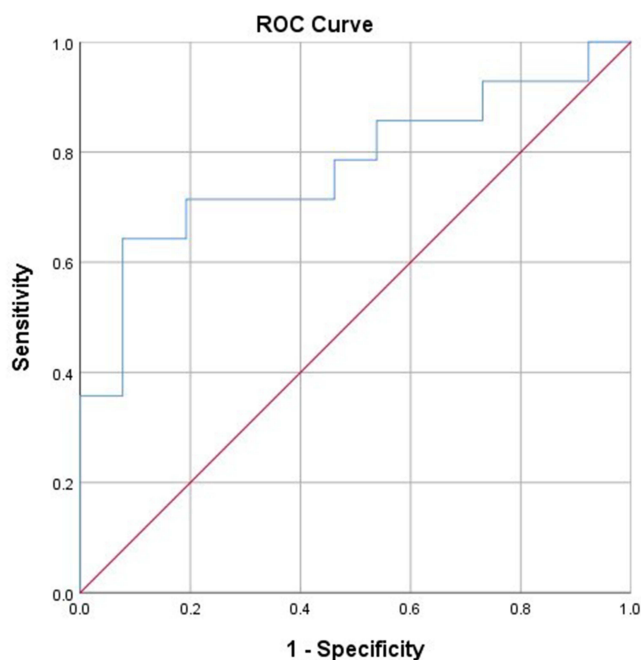
**Abbreviation:** CRP, C-reactive protein.

CIMT measurements and were a significant predictor of SCA.<sup>15</sup> Similar findings were found in patients with moderate-severe psoriasis vulgaris patients.<sup>16</sup>

The role of endocan in the development of SCA is attributed to many factors. Overexpression of endocan is related to endothelial activation, dysfunction and neovascularization.<sup>23</sup> Moreover, elevated endocan levels are associated with augmented oxidative stress.<sup>24</sup> In addition, endocan is known to promote proinflammatory mediators in the context of cardiovascular diseases.<sup>25</sup>

## Conclusion

In conclusion, the present study suggests serum endocan levels as a marker of subclinical atherosclerosis in BD patients. These conclusions, however, are limited by the relatively small sample size of the studied population. Further studies with larger sample size are recommended to confirm these conclusions.



**Figure 2** Serum endocan in diagnosis of SCA.

## Data Sharing Statement

Data from this research will be available from the corresponding author upon reasonable request.

## Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article was submitted, gave final approval for the version to be published, and agreed to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in relation to this work.

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