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Endocan: A Novel Marker of Endothelial Dysfunction in C1-Inhibitor-Deficient Hereditary Angioedema

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Keywords
C1-inhibitor deficiency · Endocan · Hereditary angioedema · Vascular cell adhesion molecules

Abstract
Background: Hereditary angioedema (HAE) related to C1-inhibitor deficiency is a rare autosomal dominant disorder. Vascular cell adhesion molecules (VCAM) are known as endothelial activation markers. Endocan (also called ESM-1) is proposed as an endothelial dysfunction indicator. We aimed to investigate endothelial activation in attack-free periods in HAE patients by measuring their levels of endocan and VCAM-1. ***Methods:*** Twenty-six HAE patients (22 female, mean age 40 ± 13 years) and 38 healthy control patients (13 female, mean age 36.9 ± 12 years) were included in the study. Peripheral blood samples were collected from HAE patients during symptom-free periods and control subjects. Endocan and VCAM-1 levels were measured using the enzyme-linked immunosorbent assay method. ***Results:*** The median serum levels of endocan (647 ± 101 ng/mL) and VCAM-1 (500 ± 79 ng/mL) in the HAE patients were significantly higher than in the control patients (391 ± 41 and 325 ± 4; $p < 0.001$ for both). ***Conclusion:*** The increased endocan and VCAM-1 levels may reflect an endothelial activation even in attack-free periods in HAE patients.

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Introduction

Hereditary angioedema (HAE) is a disease characterized by recurrent episodes of edematous swelling of the subcutaneous tissues of the extremities, trunk, and face, as well as submucosal edema of the gastrointestinal and respiratory tracts. HAE can be life threatening when it blocks airway integrity [1]. The underlying mechanism of tissue swelling in HAE is increased vascular permeability, which is stimulated by excessive bradykinin formation, the end product of an activated kinin-kallikrein cascade [2]. Complement system activation and plasma clotting cascade activation are other pathways that have been demonstrated to play a role in the overproduction of bradykinin in HAE attack [3, 4]. Furthermore, the role of the endothelial cell in HAE attacks has received significant attention in recent years [5]. Czúcz et al. [6] studied the

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endothelial cell functions of HAE patients during attack-free periods and compared those with that of healthy control subjects by monitoring 4 specific and sensitive plasma biomarkers of endothelial cells, i.e., soluble E-selectin, endothelin-1, von Willebrand factor (vWF) antigen, and vWF collagen-binding activity. They found that the majority of endothelial functions were normal during attack-free periods, although soluble E-selectin levels were elevated. However, during HAE attacks soluble E-selectin, endothelin-1, and vWF were found to increase [7]. Recently, we found that serum endothelial nitric oxide (eNOS) levels were significantly higher in HAE patients, which may reflect a sustained hyperpermeability state even in attack-free periods [8].

Endocan is a proteoglycan expressed by vascular endothelial structures. It circulates in the plasma of healthy subjects and increases during acute and severe inflammation [9]. Endocan is regulated by several cytokines and growth factors, such as the vascular endothelial growth factor (VEGF) [10]. Recently, it has been proposed as a new diagnostic marker for the demonstration of angiogenesis, endothelial function, and atherosclerosis [11]. Previous studies revealed that endocan plays an important role in inflammation, the upregulation of cell adhesion molecules, lymphocyte function, and endothelial cytoskeleton rearrangement [11–14]. Furthermore, endocan has been shown to have a prognostic value in various pathologic entities, such as cancer, sepsis, inflammatory disorders, hypertension, transplant rejection, and chronic kidney disease [13].

Cell surface adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), ICAM-1, and the endothelial leukocyte adhesion molecule (ELAM, also known as E-selectin) are accepted as markers of endothelial cell activation. Endothelial cell activation is typically induced by proinflammatory cytokines, such as TNF-α and IL-6, during many acute and chronic inflammatory conditions [15]. VCAM-1 and ICAM-1 have not been previously studied in HAE patients. We aimed to study endothelial activation in attack-free periods of HAE patients by measuring their levels of endocan and VCAM-1.

Methods

Patient Selection

This cross-sectional study was performed at Istanbul University, Istanbul Medical Faculty, and Yedikule Education and Research Hospital in Istanbul, Turkey. Approval for the study was obtained from the Medical Research Ethics Committee of the Yedikule Education and Research Hospital. The study group included 36 patients with a diagnosis of HAE who were under treatment at Istanbul Medical Faculty and Yedikule Education and Research Hospital. The control group included 50 individuals matched by age and sex, who had similar baseline characteristics. Written informed consent was obtained from all study participants (both patients and controls). Inclusion criteria necessitated being at least 18 years of age and having a diagnosis of HAE, supported on the basis of a medical history and laboratory measurements. Exclusion criteria were chronic heart and renal diseases, hypertension, diabetes, autoimmune diseases, smoking, chronic alcohol consumption, inflammatory diseases, malignancies, and other serious medical conditions.

Severity Classification of HAE Patients

In order to investigate the effect of disease severity on endocan and VCAM-1 levels, the severity classification of Agostoni et al. [1] was modified by dividing the patients into 2 groups (those with severity scores <25 and those with severity scores ≥25). We also classified HAE patients into 2 different groups based on usage of the prophylactic danazol in order to evaluate the effect of danazol on these 2 markers.

Serum Endocan and VCAM-1 Measurements

For the measurement of endocan levels, 10 mL of peripheral venous blood was drawn, put into plain blood collection tubes without any additives, immediately immersed in melting ice, and allowed to clot for 1 h before centrifugation (1,700 *g* at 4°C for 10 min). Serum was stored at –80°C until it was analyzed, and samples were melted only once. Serum endocan and VCAM-1 levels were measured using the enzyme-linked immunosorbent assay method (ESM 1-Endocan ELISA Kit and Human Soluble VCAM-1 ELISA Kit; Aviscera Bioscience Inc., Santa Clara, CA, USA).

Statistical Analysis

Statistical analysis was performed using SPSS for Microsoft Windows 15.0 (SPSS, Chicago, IL, USA). All statistical analyses were 2-tailed, and significance was set at $p < 0.05$. The Student *t* test was conducted to analyze parametric variables, whereas the Kruskal-Wallis test was used for the nonparametric variables. The Mann-Whitney U test was performed to compare 2 independent groups (C1-inhibitor-deficient HAE patients vs. healthy control participants). Correlations were calculated with the Spearman rho test.

Results

The mean age of the study group (22 female, 14 male) was 40 ± 13 years and of the control group was 36.9 ± 12 years (29 female, 21 male). Among the patient group, no patient had experienced an HAE attack in the last 72 h. Both endocan and VCAM-1 serum levels were significantly higher in the study group than in the control group (both $p < 0.001$; Table 1). The disease severity score was ≥25 in approximately half of the patients. When the patients were compared according to severity scores (<25 and ≥25), no significant differences existed in serum en-

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Table 1. Serum endocan and VCAM-1 levels (median ± SEM) of the patients and control subjects

	Patients (n = 36)	Controls (n = 50)	p value
Endocan, ng/mL	647±101	391±41	<0.001
VCAM-1, ng/mL	500±79	325±4	<0.001

Table 2. Serum endocan and VCAM-1 levels (median ± SEM) according to disease severity scores

	Severity		p value
	low (<25) (n = 19)	high (≥25) (n = 17)	
Endocan, ng/mL	581±127	680±161	0.28
VCAM-1, ng/mL	510±97	489±133	0.79

docan and VCAM-1 levels between the 2 groups (Table 2). The mean duration of the disease was 26 ± 14 years, and the duration of danazol prophylaxis was 41 ± 35 months. No correlation was found between the disease and prophylaxis duration with endocan and VCAM-1 levels (r = 0.08, p = 0.63, and r = –0.12, p = 0.46, respectively). The prophylactic treatment did not affect the endocan and VCAM-1 levels. A positive correlation was found between endocan and VCAM-1 levels (r = 0.33, p = 0.04).

Discussion

This study demonstrates that endocan and VCAM-1 levels are significantly higher in HAE patients during attack-free periods. We propose that these findings can be indicators of sustained endothelial activation in HAE.

Attack frequency and severity differ across and within patients, raising the possibility that some other factors might influence vascular permeability in this disease. In a recent study, Loffredo et al. [16] showed that plasma concentrations of VEGF-A, VEGF-C, and angiopoietins (Ang1, and Ang2) were higher in patients with C1-inhibitor-deficient HAE in remission than in healthy control subjects. Accordingly, they hypothesized that VEGFs and Angs induce a state of "vascular preconditioning" that may predispose some patients to angioedema attacks. In concordance with this finding, we previously demonstrated that eNOS, which is important in hyperpermeability processes, was higher in symptom-free HAE patients while NO metabolites were high only during attack periods [8]. In that study, although the total (nonfractionated) VEGF levels of HAE patients were higher than those of healthy subjects, this difference was not significant. Our findings, which are similar to those of Loffredo et al. [16], suggested that a vascular hyperpermeability state exists in HAE patients, even during symptom-free periods.

Endothelial dysfunction associated with an impaired protective regulatory balance and altered endothelial barrier permeability is a hallmark of many inflammatory responses. A newly proposed endothelial marker, endocan, was found to be correlated with vascular inflammatory responses in various conditions, such as sepsis, cancer, and even atherosclerosis [17–20]. In a recent, comprehensive study, Lee et al. [14] demonstrated that endocan elicits proinflammatory responses, such as hyperpermeability, cytoskeleton rearrangement, upregulation of the cellular adhesion molecule expression, leukocyte adhesion and migration via interaction with mitogen-activated protein kinase, signaling pathways, and NF-kB. These researchers also showed that endocan-induced VEGF-A could facilitate the binding of VEGF-A to VEGFR-2. Additionally, in their study, endocan was shown to induce a prominent decrease in the expressions of the cytoskeleton-associated proteins occludin and ZO-1, which are important elements of tight junctions in vascular endothelium. In light of these findings, our results showing high endocan levels in HAE patients during attack-free periods can be interpreted as sustained endothelial activation, which may predispose patients to angioedema attacks. Although endocan is usually proposed as a marker of endothelial inflammation, previous studies could not find evidence for endothelial inflammation as a cause of an increase in vascular permeability in HAE [8–13, 15, 16]. Accordingly, it can be hypothesized that other factors exist which affect endothelial integrity, even in attack-free HAE patients.

Similar to endocan, the VCAM-1 levels of HAE patients in attack-free periods were found to be increased in our study, which is an expected result considering that Lee et al. [14] demonstrated that endocan is related to the upregulation of cellular adhesion molecules. This result can be interpreted as indicating that VCAM-1 can be an additional marker to show that endothelial activation exists even in attack-free periods in HAE.

In a previous study, we demonstrated coronary flow reserve reduction in HAE patients and proposed that there is a microvascular endothelial dysfunction in this disease [21]. If endocan reflects an impaired vascular

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state, coronary flow reserve reduction can be the consequence of this condition.

In conclusion, endocan and VCAM-1 seem to be sensitive markers in HAE patients for sustained endothelial activation and/or dysfunction in attack-free periods. The relation of C1-inhibitor deficiency and some other factors with this activation and/or dysfunction needs to be further studied.

Ethics Statement

Clinical Trial registration is not applicable.

Disclosure Statement

The authors declare that they have no conflicts of interest.

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Author Contributions

Each author contributed to the conception and design of the study, generation, analysis, and interpretation of the data, and preparation or critical revision of the manuscript.

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