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Original Article

Evaluation of Serum Endocan Levels in Sensorineural Hearing Loss

Erdoğan Gültekin, Tolga Ersözlü, Oral Burak Demirel, Özlem Kaymaz, Birol Topçu, Zafer Çiftçi

Department of Otorhinolaryngology and Head and Neck Surgery, Namik Kemal University School of Medicine, Tekirdağ, Turkey (EG, TE, OBD, ZÇ)
Department of Biochemistry, Namik Kemal University School of Medicine, Tekirdağ, Turkey (ÖK)
Department of Biostatistics, Namik Kemal University School of Medicine, Tekirdağ, Turkey (BT)

ORCID IDs of the authors: E.G. 0000-0002-8017-3854; T.E. 0000-0001-8629-6022; O.B.D. 0000-0001-7066-2294; Ö.K. 0000-0001-5710-3316; B.T. 0000-0003-0771-2505; Z.Ç. 0000-0003-3307-085X.

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OBJECTIVES: The aim of this study was to reveal the possible role of endothelial dysfunction in sensorineural hearing loss (SNHL) by determining the serum endocan levels of patients with varying degrees of SNHL.

MATERIALS and METHODS: Patients with documented SNHL and healthy controls were included in the study, whereas those with a known history of chronic inflammatory condition were excluded. In addition, a recent history of use of glucocorticoids, nonsteroid anti-inflammatory drugs, or any ototoxic medications was also considered as an exclusion criterion due to its potential impact on endocan synthesis and metabolism. Following overnight fasting, blood samples were collected, and serum endocan levels were measured. For statistical analysis of the data, PASW Statistics for Windows version 18 was used.

RESULTS: The comparison of the subgroups yielded no statistically significant difference between the control and mild-to-moderate SNHL groups. Despite the increase in hearing loss, the difference between the endocan levels in these patients did not increase proportionately and was not statistically significant ($p>0.05$). The patients in the severe SNHL group had a higher level of serum endocan than those in other groups, and the difference was statistically significant ($p<0.05$).

CONCLUSION: The serum endocan levels failed to show a proportionate increase with increasing degree of SNHL, indicating that there is no precise association between SNHL and serum endocan levels. The serum endocan levels of patients with SNHL did not significantly differ from those of the healthy controls.

KEYWORDS: Sensorineural hearing loss, endocan, endothelial dysfunction, microvascular dysfunction

INTRODUCTION

Sensorineural hearing loss (SNHL) is known to be a major debilitating factor for subjects at any age. It is usually considered as age-related, noise-induced, or idiopathic. To date, several theories, such as viral diseases, immune-mediated processes, and vascular compromise, have been proposed to explain the underlyng mechanisms leading to hearing loss [1-4]. Among the proposed theories, diminished blood supply to the inner ear secondary to microvascular endothelial dysfunction has gained increasing acceptance. According to the literature, endothelial dysfunction is triggered or induced by several factors, such as redox protein p66, soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVACM-1), factor V Leiden polymorphism, prothrombin G20210A variants, and P2X4 activation [5-8].

In a study by Cox et al. [9], endocan, a novel serum endothelial cell-specific molecule 1, was found to be a more reliable marker than its predecessors in determining the severity of experimentally induced endothelial dysfunction. The implication of endocan in the diagnosis and prognosis of several other endothelial dysfunction-associated diseases has been well-documented [10, 11]. However, to the best of our knowledge, the association between serum endocan levels and presence or severity of SNHL has not been investigated.

To reveal the possible role of endothelial dysfunction in SNHL, we aimed to determine the serum endocan levels of patients with varying degrees of SNHL and compared them with those of healthy subjects. We also discussed the implications of the study findings under the scope of the existing literature.

Corresponding Author: Erdoğan Gültekin E-mail: erdogangultekin@hotmail.com

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MATERIALS AND METHODS

This prospective study was conducted in a tertiary research setting between February and May 2017. Patients with a known history of chronic inflammatory condition, diabetes mellitus, atherosclerosis, ischemic heart disease, stroke, uncontrolled hypertension, malignancy, chronic otitis media, or any metabolic disorder were excluded from the study. Despite a negative medical history, patients with a documented abnormality in blood biochemistry, including high fasting glucose, cholesterol, triglyceride, and creatinine levels, were also excluded from the study. In addition, a recent history of glucocorticoids, nonsteroid anti-inflammatory drugs, or any ototoxic medications use was also accepted as an exclusion criterion due to its potential impact on endocan synthesis and metabolism.

Table 1. Mean serum endocan levels in the control and the study groups (ng/mL)

	Group	n	Mean±sd	p
Endocan	Control	25	4.400.62	0.001
	Mild SNHL*	27	4.440.73	
	Moderate SNHL*	31	4.550.51	
	Severe SNHL*	31	6.150.65[a1,b1,c1]	

[a]Comparison of control and severe SNHL groups.
[b]Comparison of mild and severe SNHL groups.
[c]Comparison of moderate and severe SNHL groups.
[1]p<0.01
SNHL: Sensorineural hearing loss

Table 2. Tukey Multiple Comparison results

					95% Confidence Interval
(I) grup	(J) grup	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound
Control	Mild	-.04150	.17589	.995	-.5003
	Moderate	-.14898	.17034	.818	-.5934
	Severe	-1.74837*	.17034	.001	-2.1927
Mild	Control	.04150	.17589	.995	-.4173
	Moderate	-.10749	.16682	.917	-.5427
	Severe	-1.70687*	.16682	.001	-2.1420
Moderate	Control	.14898	.17034	.818	-.2954
	Mild	.10749	.16682	.917	-.3277
	Severe	-1.59938*	.16096	.001	-2.0193
Severe	Control	1.74837*	.17034	.001	1.3040
	Mild	1.70687*	.16682	.001	1.2717
	Moderate	1.59938*	.16096	.001	1.1795

*The mean difference is significant at the 0.05 level.

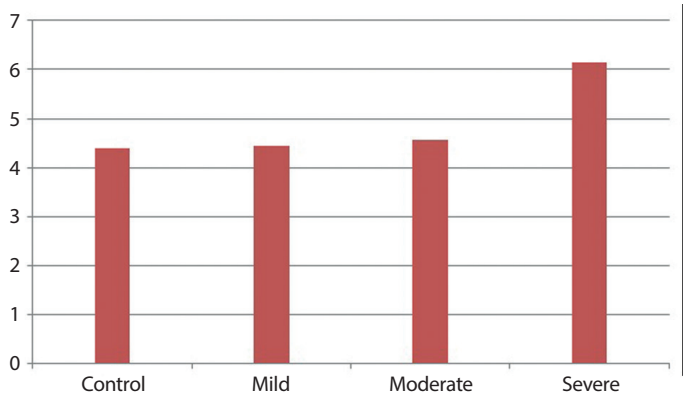

Figure 1. Mean serum endocan levels in the control and study groups.

All pure tone thresholds were measured by the same senior audiometrist in a high-standard silent audiometry cabin. For the testing, a two-channel audiometer was used (Interacoustics AC 40, Denmark). Prior to the audiometric analysis, all the subjects underwent an otoscopic examination, and those with abnormal otoscopic findings were excluded. Patients with documented SNHL (total, 89; 42 female and 47 male) and 25 healthy controls (14 female and 11 male) were included in the study. Patients with SNHL had varying degrees of hearing loss and were classified as having mild (n=27; 12 female, 15 male), moderate (n=31; 19 female, 12 male), or severe (n=31; 11 female, 20 male) SNHL according to the American Speech-Language-Hearing Association (ASHA) guideline [12]. The mean ages of the control, mild, moderate, and severe SNHL groups were 57.420, 57.221, 58.640, and 58.114 years, respectively. The control subjects were enrolled as healthy volunteers following pure tone threshold testing. Informed consent of all the subjects who were enrolled in the study was obtained. The research ethics committee of Namık Kemal University granted ethics approval to conduct this research (Date: 26/01/2017; No: 2017/16).

Following overnight fasting, blood samples were collected from the patients and controls. The antecubital vein was used to draw blood samples, which were then collected into vials containing 3.8% sodium citrate in a proportion of 9:1. Whole blood samples were centrifuged at 300 rpm, and plasma samples were obtained. The samples were stored at −86°C. The serum endocan levels were measured using Elabscience ELISA kit (Elabscience Biotechnology Co., Ltd, Wu-Han, China). The intra- and inter-assay variabilities of the ELISA kit were 6.36% and 6.09%, respectively.

Statistical Analysis

For the statistical analysis of the data, PASW Statistics for Windows version 18 was used. Categorical variables were expressed as the mean, standard deviation, frequency, and percentage. For the comparison of variables that showed a normal distribution, analysis of variance (ANOVA) was used. For the comparison of the subgroups, the Tukey test was used. The associations between variables were tested using the Pearson correlation analysis. A $p<0.05$ was considered statistically significant.

RESULTS

The mean serum endocan levels of patients in the control and study groups are summarized in Table 1. The comparison of the subgroups showed that there was a statistically significant difference between the severe SNHL and other groups ($p<0.001$). However, no statistically significant difference was observed between the control, mild SNHL, and moderate SNHL groups ($p>0.05$; Table 2). Despite the gradually increasing hearing loss observed in the mild and moderate SNHL groups compared with the control group, the difference between the groups was not statistically significant ($p>0.05$; Figure 1). However, the serum endocan levels in the severe SNHL group were higher than those in the other groups, and this difference was statistically significant ($p<0.001$). A post hoc power analysis along with

Table 3. Post-hoc power analysis

Difference	Size	Power
3	25	0.978296
3	27	0.987082
3	31	0.995655
3	31	0.995655

α=0.01 Assumed standard deviation=2 d=3 Factors: 1 Number of levels: 4

one-way ANOVA indicated that the groups had 97%-99% power, with an alpha value of 1% (Table 3).

DISCUSSION

Endocan is a proteoglycan derived from the endothelium and has been recently discovered by Lassalle et al. [13] in 1996 as a new human endothelial cell-specific molecule. The molecule ESM-1 was found to be specifically distributed in the vascular endothelial cells, and subsequent studies identified it as a novel member of the proteoglycan family and named it as endocan [14]. Ongoing studies have shown that endocan can be isolated not only from the vasculature of the lung tissue but also from the adipose tissue, skin, and heart [15-17].

Endocan can bind a number of biologically active molecules that participate in cellular migration, adhesion, and proliferation. The roles of endocan in healing, inflammation, and tumorigenesis have been previously documented [18, 19]. A literature review revealed that serum endocan level was a reliable marker for both the diagnosis of various diseases and surveillance of their post-therapeutic responses [20, 21].

In a recent study, Lv et al. [17] proposed that serum endocan levels could be used as a useful marker for the early diagnosis of subclinical atherosclerosis in patients with type 2 diabetes mellitus. Moreover, in his review, Azimi et al. [21] pointed out the role of endocan as a reliable marker for renal tubular injury and suggested it to be a potential equivalent of troponin in the field of nephrology. Furthermore, the prognostic implication of endocan in triple negative breast cancer was evaluated in a study by Sagara et al. [22], who concluded that endocan could be used as a blood-based biomarker for patients with breast cancer. In another study, serum and urinary levels of endocan were found to be higher in patients with bladder cancer [23].

To reveal the role of endocan in various clinical settings associated with endothelial dysfunction, including endotoxemia, obstructive sleep apnea, and migraine, numerous studies have been performed [24-26]. Elevated levels of endocan have been found to be associated with disease severity.

From an otorhinolaryngological point of view, the possible role of endothelial dysfunction in SNHL has been investigated in the literature. In their study, Berjis et al. [27] concluded that endothelial dysfunction is associated with SNHL. They also emphasized that this association was independent of other cardiovascular risk factors [27]. The reversal of peripheral endothelial dysfunction by rheopheresis in patients with SNHL was found to be beneficial in a study by Balletshofer et al. [28], in which patients with SNHL showed endothelial dysfunction evidenced by diminished flow-mediated vasodilatation.

Specifically, the role of soluble adhesion molecules in the pathogenesis of idiopathic sudden SNHL was investigated in two prospective studies. Ramunni et al. [29] hypothesized that the reduction of adhesion molecule levels by low-density lipoprotein-apheresis could have a role in the treatment of SNHL. In another study, Quaranta et al. [30] evaluated 37 patients with sudden SNHL and indicated the role of elevated sICAM-1 and sVACM-1 levels in these patients.

Although many studies have shown such an association, some studies have denied the role of endothelial dysfunction in SNHL. Haubner et al. [31] reported that they did not observe any increase in the serum levels of endothelial dysfunction markers, including ICAM-1, VCAM-1, E-selectin, and monocyte chemoattractant protein 1 (MCP-1) in patients with SNHL. They denied the presence of an association of vascular endothelia dysfunction with hearing loss and concluded that further studies should be conducted to elucidate the role of vascular endothelial dysfunction in this disorder [31]. sICAM1, sVCAM-1, E-selectin, IL-6, IL-8, and MCP-1 levels were not significantly elevated in the patient group. Despite the findings of other researches, Haubner's study revealed no association between sudden SNHL and typical vascular risk factors. Soluble adhesion molecule levels were not elevated in the ISSHL group. The study concluded that the association between increased levels of soluble adhesion molecules and SNHL pathogenesis remains unclear.

The possible role of endothelial dysfunction in SNHL has been previously suggested; however, to the best of our knowledge, the implication of the novel endothelial dysfunction marker endocan has not been previously investigated. Endocan is accepted as one of the most potent immune inflammatory markers for the diagnosis and surveillance of many endothelial disorders. Endocan is known to have a crucial role in cell adhesion regulation, and increased levels in the plasma are associated with endothelial dysfunction [32]. The null hypothesis of the present study was that the possible association between SNHL and endothelial dysfunction could be clearly demonstrated using the novel and reliable marker endocan in patients with varying degrees of SNHL. Serum endocan levels were found to be increased in the mild and moderate SNHL groups compared with the control group, but the difference was not statistically significant. However, the severe SNHL group showed a statistically significant difference compared with the control group.

The secretion of endocan is restricted to the endothelial cells, and preanalytical variations may be observed when samples are collected at different time intervals following endothelial cell injury. The onset of hearing loss ranged from 6 months to 10 years in our study, which may be considered a limitation. Further studies with more homogenous study groups would be more convenient in evaluating the impact of endothelial dysfunction.

Senility alone can negatively affect endothelial dysfunction, which in turn contributes to organ failure [33]. The aging process is also associated with neurodegenerative disorders, which may independently be a risk factor for SNHL [34, 35]. The relatively small size of the control and study groups and the relative shift toward increasing age of the study population may also be considered as limitations of this study.

CONCLUSION

A statistically significant difference was observed in the serum endocan levels of patients with severe SNHL compared with other groups in this study. This finding would support the substantial implication of endothelial dysfunction in SNHL. Further clinical researches with larger populations should be conducted to delineate the significance

of this association. A better understanding of the underlying processes in the pathogenesis of SNHL may be beneficial for both the prevention and treatment of the disorder.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Namik Kemal University (Date: 26/01/2017; No: 2017/16).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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