Original Article

Serum Endocan Levels in Children with Acute Rheumatic Fever

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Received: 08-Nov-2023;
Revision: 24-Apr-2024;
Accepted: 21-Aug-2024;
Published: 30-Sep-2024

ABSTRACT

Background: Acute rheumatic fever is an immunologically delayed autoimmune sequel of throat infection caused by group A streptococcus. The aim of this study was to evaluate endocan levels in patients with acute rheumatic fever and compare with the control group. **Aim:** The aim of this study was to evaluate endocan levels in patients with acute rheumatic fever and compare with the control group. **Methods:** Twenty-three children with acute rheumatic fever (11 men, 12 females; mean age 13 ± 2.7 years; range 5 to 15 years) and a healthy control group of 31 children (16 men, 15 females; mean age 13.8 ± 2.4 years; range 5 to 15 years) were recruited. The sedimentation rate, C-reactive protein, antistreptolysin-O titres, and endocan levels were examined in each group. **Results:** Before anti-inflammatory therapy, endocan levels in the acute rheumatic fever group were not statistically significant to those in the control group, respectively (200.64 ng/L, 120.71 ng/L, $P = 0.208$). After anti-inflammatory therapy, endocan levels were significantly higher in the acute rheumatic fever group than in the control group, respectively (260.87 ng/L vs. 120.71 ng/L, $P < 0.01$). A significant difference was found in endocan levels before and after anti-inflammatory therapy in the group of acute rheumatic fever, respectively (200.64 ng/L vs. 260.87 ng/L, $P = 0.033$). Endocan levels after anti-inflammatory therapy were statistically higher in the severe carditis group compared to those of the mild carditis group, respectively (344.56 ng/L vs. 191.01 ng/L, $P < 0.01$). **Conclusion:** Our study showed that serum endocan levels increased during the subacute phase of acute rheumatic fever. We suggest that serum endocan level can be used as a new biomarker to identify the degree of cardiac involvement in acute rheumatic fever.

Keywords: *Acute rheumatic fever, carditis, children, endocan*

Introduction

Acute rheumatic fever is an autoimmune disease caused by the group A streptococcus that follows a nontreated throat infection in vulnerable children. In developing countries, rheumatic heart disease remains a major cause of cardiovascular morbidity and mortality.[1,2] Patients with acute rheumatic fever may develop varying degrees of pancarditis with associated valve disease, heart failure, and pericarditis.

Heart valves are composed mainly of extracellular matrix, fibroblasts, smooth muscle, and endothelial cells. Endocan is a specific soluble glycoprotein secreted by human endothelial cells. Endocan plays a key role in vascular inflammation.[3,4] Serum endocan levels have also been shown to increase inflammatory diseases such as inflammatory bowel disease, Behçet's disease, familial Mediterranean fever in adulthood and juvenile idiopathic arthritis in childhood.[5-8] To our knowledge, no data on serum endocan levels were revealed in children with acute rheumatic fever. Therefore, the objective of this study was to evaluate endocan levels in patients with acute rheumatic fever and compare with the control group.

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_783_23

Methods

Study population

This prospective case-control study was conducted between March 20 and November 30, 2020 after

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How to cite this article: Doğan MT, Can U, Alp H, Ayguneş U. Serum endocan levels in children with acute rheumatic fever. Niger J Clin Pract 2024;27:1051-6.

obtaining ethics committee approval (issue number: 2020/2379). Participants were informed about the study according to the Declaration of Helsinki and their written consent was obtained.

A total of 54 children (ages 5 to 15 years) including 23 in the case group and 31 in the control group, were included in the study. Patients with any chronic disease, associated infections and congenital valve disease that may have an effect on endocan levels were excluded from the study. The control group consisted of healthy individuals who presented for routine cardiological examination.

All patients underwent detailed medical history, physical examination, laboratory evaluation, electrocardiogram and echocardiography. The diagnosis of acute rheumatic fever was based on Jones criteria, update 2015.[9] The acute rheumatic fever group was split into two groups: patients with carditis and those with isolated arthritis. Non-steroid anti-inflammatory was administered to patients with isolated arthritis or mild carditis. Oral prednisolone was used in patients with severe carditis and heart failure.[10] In the acute rheumatic fever group, the same pediatric cardiologist performed an echocardiographic examination twice. The first analysis was performed at the time of diagnosis, and the second analysis was performed at the normalization of acute inflammatory reactants (subacute phase) on anti-inflammatory therapy.

Echocardiographic examination

Echocardiographic studies were performed with guided two-dimensional M-mode echocardiography according to the methods established by the American Society of Echocardiography with 5-MHz transducer frequencies using a Vivid S60 ultrasonic imager.[11] Pathological valvular insufficiency was evaluated according to the criteria of the World Heart Federation, as mentioned in the literature.[9] Doppler methods, including the assessment of the characteristics of the regurgitant jet, were used in the evaluation of the severity of valvular regurgitation.[12,13]

Blood sample collection and biochemical assay

Sedimentation rate, C-reactive protein, antistreptolysin-O titres and endocan levels were examined just before and immediately after the anti-inflammatory treatment given for ARF in each group. Blood samples were collected from a cubital vein into blood tubes. Serum was separated from cells by centrifugation at 4000 rpm for 10 min and then divided into aliquots for storage at -80°C until analysis, and serum endocan levels were measured using commercially available kits based on enzyme-linked immunosorbent assay methods. The results were expressed as ng/L.

Statistical analysis

Analysis of the data and relationships between the variables were examined using the "SPSS for Windows 22.0" package program. Descriptive statistics were presented as mean ± standard deviation or median (minimum-maximum) for continuous variables and number of cases and (%) for categorical variables. Differences between the frequencies of categorical variables were investigated using the chi-square test. Whether the distribution of continuous variables was close to normal was investigated using the Kolmogorov-Smirnov and Shapiro-Wilk test. Differences between groups were evaluated using the Student's *t* test for normally distributed continuous variables and the Mann-Whitney U test for nonnormally distributed continuous variables. If the comparison of two dependent numerical variables showed a normal distribution, the paired two-sample T-test was used, and if it did not show a normal distribution, the Wilcoxon test was used. The results were considered statistically significant for $P < 0.05$.

Results

Twenty-three patients with acute rheumatic fever (12 female, mean age =13 ± 2.7 years), 31 control patients (15 female, mean age =13.8 ± 2.4 years) were included in the study. The mean weight was 45,11 ± 10 kg and the mean height was 149,13 ± 10 cm in the acute rheumatic fever group. The mean weight was 44,7 ± 20 kg and the mean height was 147,13 ± 20 cm in the control group. Age, sex and anthropometric values were similar between acute rheumatic fever and control groups.

The rate of sedimentation and C-reactive protein levels were higher in the acute rheumatic fever group before anti-inflammatory therapy compared to the acute rheumatic fever group after anti-inflammatory therapy and in the control group ($P < 0.01$). Antistreptolysin-O titres were higher in the acute rheumatic fever group than in the control group ($P < 0.01$).

When the echocardiological measurements of the groups were compared, the left ventricular internal diameter at end diastole and the left ventricular internal diameter at end systole was higher in the group of acute rheumatic fever before anti-inflammatory therapy than in the other groups. Interventricular septal thickness at end diastole, interventricular septal thickness at end systole, ejection fraction and fractional shortening were higher in the acute rheumatic fever group after anti-inflammatory therapy compared to the control group and in the acute rheumatic fever group before anti-inflammatory therapy. The laboratory findings and echocardiographic

Table 1: Comparison of laboratory findings and echocardiographic measurements between subgroups

	Before Anti-Inflammatory Therapy	After Anti-Inflammatory Therapy	Control Group	P1	P2	P3
LVIDd (mm)	41 (34-47)	39 (31-47)	39 (32-50)	0,004	0,049	0,429
IVSd (mm)	7,13±1,18	7,57±1,59	7,39±1,43	0,026	0,678	0,535
IVSs (mm)	8,96±1,3	9,3±1,22	8,9±1,25	0,046	0,914	0,125
LVIDs (mm)	25,26±2,51	24,26±3,09	23,19±3,15	0,021	0,009	0,087
LVPWs (mm)	10 (7-12)	10 (8-13)	9 (6-12)	0,053	0,319	0,047
LVPWd (mm)	8 (5-10)	8 (6-11)	7 (5-10)	0,396	0,156	0,045
EF (%)	65 (60-75)	70 (63-78)	69 (62-75)	0,005	0,029	0,679
FS (%)	36,52±3,72	38,96±2,85	36,81±2,29	0,007	0,528	0,007
C-reactive protein	99,0 (54,0– 214,0)	2,6 (1,0-13,3)	3,0 (1,0–9,0)	<0.001	<0.001	0,720
Erythrocyte sedimentation rate	57,34±14,33	11,17±5,26	5,22±2,65	<0.001	<0.001	<0.001
Anti-streptolysin-O	627,0 (340,0-2100,0)	602,0 (370,0-2980,0)	198,0 (58,0-330,0)	<0.201	<0.001	<0.001

P1 (Before Anti-Inflammatory Therapy - After Anti-Inflammatory Therapy); P2 (Before Anti-Inflammatory Therapy-Control); P3 (After Anti-Inflammatory Therapy-Control); EF=Ejection fraction; FS=shortening fraction; IVSd=Interventricular septal thickness at end-diastole; IVSs=Interventricular septal thickness at end-systole; LVIDd=Left ventricular internal diameter at end-diastole; LVIDs=Left ventricular internal diameter at end-systole; LVPWd=Left ventricular posterior wall thickness at end-diastole; LVPWs=Left ventricular posterior wall thickness at end-systole

Table 2: Comparison of serum endocan levels before and after anti-inflammatory therapy between moderate and severe carditis

	Median (min-mak)		*P*
	Mild carditis	Severe carditis	
Before Anti-Inflammatory Therapy	121,26 (96,73-310,74)	127,57 (70,09-198)	0,245
After Anti-Inflammatory Therapy	141,24 (106,54-352)	358,31 (261,36-412,85)	0,001

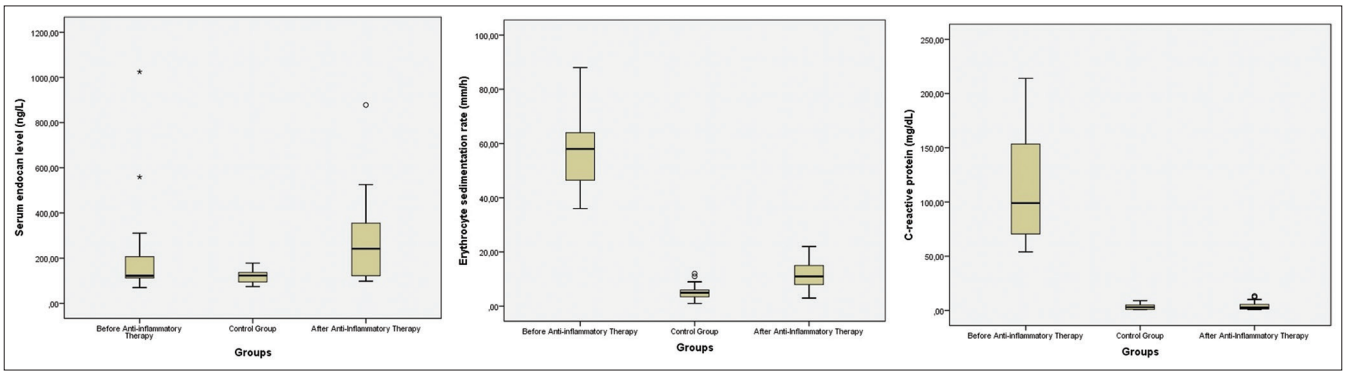

Figure 1: Serum endocan level, C-reactive protein, erythrocyte sedimentation rate comparison of the groups

measurements of the patients included in the study are shown in Table 1.

Before anti-inflammatory therapy, endocan levels in the acute rheumatic fever group were not statistically significant compared to those of the control group (*p*:0.208). After anti-inflammatory therapy, endocan levels were higher in the group with acute rheumatic fever group than in the control group ($P < 0.01$). There was no correlation between the duration of therapy and endocan levels. A significant difference in endocan levels was found before and after anti-inflammatory therapy in the group with acute rheumatic fever (*p*:0.033). Endocan levels after anti-inflammatory therapy were significantly higher in the severe carditis group when compared with the after anti-inflammatory therapy in the mild carditis group ($P < 0.01$) [Table 2].

Eight children had isolated arthritis. Serum endocan levels were 191.35 ± 157.63 ng/L and 157.49 ± 149.69 ng/L before and after anti-inflammatory therapy, respectively. No statistical differences were detected between before and after anti-inflammatory therapy in this group ($P = 0.666$). Furthermore, no statistical differences were detected between the carditis and isolated arthritis groups for serum endocan levels before anti-inflammatory therapy. However, there is a significant statistical difference between the carditis and

isolated arthritis groups for serum endocan levels after anti-inflammatory therapy ($P < 0.001$).

The serum endocan level, C- reactive protein and sedimentation rate distribution of the patients included in the study are shown in Figure 1.

Discussion

To our knowledge, no study had previously evaluated serum endocan levels in patients with acute rheumatic fever and compared the results with healthy controls. In this study, we showed that serum endocan levels were significantly higher in patients with acute rheumatic fever after anti-inflammatory therapy compared to the control group. Furthermore, there was a statistically significant difference between endocan levels before and after anti-inflammatory therapy in the group with acute rheumatic fever group. We suggest that serum endocan levels increase during the subacute phase of acute rheumatic fever. Endocan levels after anti-inflammatory therapy were statistically significantly higher in the severe carditis group compared to endocan levels after anti-inflammatory therapy in the mild carditis group.

Endocan, originally known as endothelial cell-specific molecule-1[13,14] is a proteoglycan expressed by endothelial cells. During inflammation, endocan plays an inhibitory role in leukocyte adhesion, migration, and eventually extravasation by binding lymphocyte function-associated antigen 1 and blocking its integration with ligands on the intravascular endothelium.[4] There is strong evidence to support its role in several chronic diseases and this suggests that it is an important biomarker of endothelial function.[15-17] Endocan is associated with endothelial damage, and since endothelial dysfunction is a key component of acute rheumatic fever pathogenesis, endocan is expected to be a significant biomarker for patients with acute rheumatic fever.[7] We found that after anti-inflammatory therapy in patients with severe carditis, endocan levels were higher than those of mild carditis, which may be speculated that endocan may be an option to detect the severity of heart involvement of acute rheumatic fever.

In a study of patients with juvenile idiopathic arthritis, higher serum endocan levels were detected in the patient group compared to the control group.[8] Furthermore, serum endocan levels were lower in those in active stages than in those in clinical remission. Voiosu *et al*.[5] found that in patients with inflammatory bowel disease, there was no significant correlation between disease activity and serum endocan levels. We also found that endocan levels before anti-inflammatory therapy were lower in the acute rheumatic fever group than endocan levels after anti-inflammatory therapy. Omma *et al*.[7] found that in patients with familial mediterranean fever serum endocan levels were similar during an attack period in patients with familial Mediterranean fever, although the sedimentation rate and the C- reactive protein levels were significantly different. In our study in the acute rheumatic fever group, serum endocan levels were higher in the subacute phases. Endocan may be more useful in detecting a long-lasting, chronic inflammatory state rather than rapid and brief hyper-inflammatory reactions, as endocan from inflammatory cells is not a quick process.[7] In previous studies, authors suggested that rheumatic heart disease exhibits various signs of granulomatous myocarditis in addition to infiltration of the myocardium and endocardium with monocytes, neutrophils and lymphocytes.[18,19] By producing oxygen-free radicals, these phagocytic cells can contribute to the disease's progression.[20,21] When TNF-α, IL-8 and IL-6 levels increased significantly during the acute phase of acute rheumatic fever, Yegin *et al*.[22] found that these cytokines might have a pathogenic function in rheumatic fever. In a previous study, IL-8 was considerably elevated in patients with acute rheumatic fever during the clinically active phase, according to Kutukculer and Narin's findings.[23] In the acute phase of acute rheumatic fever, Narin *et al*.[24] demonstrated that IL-1 and IL-2 levels were elevated. Recently, an increase in oxidative stress has been reported in patients with acute rheumatic fever compared to healthy control.[25] Chiu-Braga *et al*.[26] observed elevated levels of advanced oxidation protein products and high sensitive C-reactive protein in patients with chronic rheumatic heart disease, and have also shown the involvement of oxidative stress and systemic inflammation in the pathogenesis of chronic rheumatic heart disease. Endocan binds to lymphocyte function-associated antigen 1 and prevents it from integrating with ligands on the intravascular endothelium during inflammation, inhibiting leukocyte adhesion, migration and ultimately extravasation. We did not measure cytokine levels in this study; but we showed that endocan levels increased during the subacute phase of acute rheumatic fever.

Limitations

There were some limitations of our study. First, our study had a small sample size due to the significant decrease in the number of patients with acute rheumatic fever during the pandemic. Second, it was not a longitudinal study.

Conclusions

Our findings showed that after anti-inflammatory therapy endocan levels were higher in the acute rheumatic fever

group when compared to the control group. We also found that severe carditis endocan levels were higher after anti-inflammatory therapy than mild carditis endocan levels, raising the possibility that endocan could be used to identify cardiac involvement in acute rheumatic fever. These results suggest that serum endocan may be a useful biomarker for identifying the degree of cardiac involvement in acute rheumatic fever. Longitudinal studies with a large number of patients are needed to assess the role of serum endocan levels in acute rheumatic fever.

Authors' contributions

M.T.D. planned the study, interpreted the data, and wrote the manuscript. H.A., Ü.C and U.A. provided medical care to the patient and critically reviewed the manuscript. All authors read and approved the final manuscript.

Ethical standards

This prospective case-control study was conducted between March 20 and November 30, 2020 after obtaining the approval of the ethics committee (issue number: 2020 / 2379). Participants were informed about the study according to the Declaration of Helsinki and their written consents were obtained.

Financial support and sponsorship

This research received a grant from the Scientific Research Projects Committee of Health Science University Konya Health Application and Research Centre.

Conflicts of interest

There are no conflicts of interest.

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