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Assessment of serum trace element levels in rheumatic heart disease: A case-control study

Romatizmal kalp hastalığında serum eser element seviyelerinin değerlendirilmesi: Bir vaka kontrol araştırması

Mehmet Hakan Öncel [1], Cemal Tuncer [2]

Abstract

Aim: Some trace elements play important roles in various heart diseases. In this study, we aimed to determine the changes in some trace element concentrations in the serum of patients with rheumatic heart disease (RHD).
Methods: Sixty-one patients with RHD and 60 healthy subjects were included in the study. Six subgroups were defined in the study group according to the Wilkins score, the degree of aortic involvement, and the presence or absence of pulmonary hypertension. Selenium (Se), Zinc (Zn), and Cupper (Cu) levels were measured, and transthoracic echocardiography was performed in all participants.
Results: Serum Se and Zn concentrations were significantly lower in the patients compared to the controls (mean±SD and p 43.08±1.83 µg/dL vs. 60.75±2.44 µg/dL; t=-5.305, p=0.001 and 64.65±2.77 µg/dL vs. 87.34±3.33 µg/dL; t=-5.458, p=0.001, respectively). However, the serum Cu concentration was significantly higher in the patient group than in the control group (84.50±3.50 µg/dL vs. 74.23±3.08 µg/dL; t=2.309, p=0.023). Similarly, the Cu/Zn ratio in the patient group was found to be significantly higher than the control group (1.4±0.09 µg/dL vs. 0.9±0.04 µg/dL; t=5.267, p=0.001). In the patient group, there was no significant relationship between the Wilkins score, aortic involvement, pulmonary hypertension, the serum trace element concentrations, and Cu/Zn ratio (p>0.05 for all).
Conclusions: We conclude that the changes in the concentrations of the measured trace elements can predispose to RHD and play a role in the formation of certain factors that leads to the development of the disease. Besides, serum Cu levels and Cu/Zn ratio can be used as inflammatory process markers.

Keywords: rheumatic heart disease; trace elements; selenium; zinc; copper

Öz

Amaç: Değişik kalp hastalıklarında bazı eser elementlerin önemli olduğu bilinmektedir. Bu çalışmada Romatizmal Kalp Hastalığı (RKH) olan hastaların serumunda bazı eser element konsantrasyonlarındaki değişiklikleri belirlemek ve bunun kronik RKH ve kapak tutulumunun şiddetiyle olan ilişkisinin ortaya konması amaçlanmıştır.
Metod: RKH olan 61 hasta ve 60 sağlıklı birey çalışma kapsamına alındı. Bu çalışmaya alınan hasta grup, Wilkins skoruna, aort tutulumunun derecesine ve ayrıca pulmoner hipertansiyonun varlığı ve yokluğuna göre olmak üzere toplam altı alt gruba daha ayrıldı. Hastalara ayrıntılı fizik muayene ve laboratuvar incelemesinin yanında kan Se, Zn ve Cu analizleri yapıldı. Hasta ve kontrol guruplarına transtorasik ekokardiografi ile ölçümler standart görünümleri üzerinden yapıldı.
Bulgular: Hasta grubunda serum Se ve Zn konsantrasyonu kontrol grubuna göre anlamlı ölçüde düşük bulundu (sırasıyla 43,08±1,83 µg/dL; 60,75±2,44 µg/dL; p<0,001) ve 64,65±2,77 µg/dL; 87,34±3,33 µg/dL; p<0.001). Serum Cu konsantrasyonu hasta grubunda kontrol grubuna göre anlamlı ölçüde yüksek bulunmuştur (84,5±3,5 µg/dL'ye karşılık 74,23± 3,08 µg/dL, p<0.05). Benzer şekilde Cu/Zn oranı hasta grubunda kontrol grubuna göre anlamlı ölçüde yüksek bulunmuştur (1,4±0,09 µg/dL'ye karşılık 0,9±0,04 µg/dL, p<0.001). Hasta grubunda serum eser element konsantrasyonları ve Cu/Zn oranı ile hastalığın tutulum şiddetini gösteren Wilkins skoru, aort tutulumu ve pulmoner hipertansiyon arasında anlamlı bir ilişki bulunmamıştır (p>0,05).
Sonuç: Eser element konsantrasyonundaki değişiklikler RKH'nı predispoze edebileceği gibi hastalığın gelişimine yol açan bazı faktörlerin ortaya çıkmasına da katkı sağlayabilir. Ek olarak artmış serum Cu seviyesi ve Cu/Zn oranı devam eden inflamatuvar proçes markırları olarak kullanılabilir.

Anahtar kelimeler: Romatizmal kalp hastalığı; eser element; selenyum; çinko; bakır.

[1] Şanlıurfa Mehmet Akif İnan Education Hospital, Department of Cardiology, Şanlıurfa, Turkey.
[2] Kahramanmaraş Sütçü İmam University, Medical Faculty, Department of Cardiology, Kahramanmaraş, Turkey.

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Sorumlu yazar / Corresponding author:

Mehmet Hakan Öncel
Şanlıurfa Mehmet Akif İnan Education Hospital, Department of Cardiology
Şanlıurfa, Turkey
e-posta: mhakanoncel@gmail.com
Tel/Phone: +905316703352

Introduction

Acute rheumatic fever (ARF) is classified as a connective or collagen tissue disease. In this ailment, there is damage in the collagen fibrils and connective tissue. The disease presents with inflammatory reactions in many organs, including the heart, joints, and the central nervous system [1]. The most significant complication of ARF is fibrosis in the heart valves, which may lead to hemodynamic disturbance and chronic heart disease, causing acquired heart disease in children and young adults [2].

The effects of antioxidant trace elements on the response of cardiac tissue to oxidative stress is long known [3]. It has been suggested that abnormal immunoglobulin inflammatory responses may be related to changes in the trace element levels [4].

The role of oxidative stress in the etiology of cardiovascular disease has been regarded as promising concerning antioxidant therapies [5]. Despite the huge accumulation of knowledge, there are still conflicting reports on the significance of trace elements. Antioxidant supplementations were reported to be associated with no effect or even adverse disease outcomes [6].

We hypothesized that the serum Selenium (Se), Zinc (Zn), and Cupper (Cu) levels are lower in patients with rheumatic heart disease. Thus, the aim of this study was to determine some trace element concentrations in the serum of patients with RHD and to demonstrate the association of chronic RHD and the severity of valvular involvement.

Material and methods

Study design

A retrospective case-control study was conducted. Study reporting was done according to the STROBE criteria [7]. Ethical permit for conducting the study was obtained from the local ethics committee at the Kahramanmaraş Sütçü İmam University Medical Faculty (IRB number: 2006/4-3, date: 06/04/2006). The Helsinki Declaration was followed during all study processes.

Setting

The study was executed at the Kahramanmaraş Sütçü İmam University, Department of Cardiology between June 2005 and June 2006. During the study period, a total of 12,375 outpatients were served by the department.

Participants

Sixty-one patients (Group I) who applied to the cardiology polyclinics within one year meeting the revised Jones criteria [8] and 60 healthy controls (Group II) were included in the study. Healthy controls were selected from the visitors or caregivers of the study group. All control cases were interviewed and examined concerning the absence of the outcome. Six subgroups were defined among the patients, consisting of two according to Wilkins score [9] (Wilkins score 1-8 and ≥9), two according to the degree of aortic involvement (no or mild involvement and moderate or severe involvement) [10], and two according to the state of pulmonary hypertension (present or absent).

Patients with known coronary artery disease, degenerative valve disease, congestive heart failure, cardiomyopathy, primary pulmonary hypertension, uncontrolled hyperthyroidism or hypothyroidism, chronic obstructive pulmonary disease, active ARF, active myocarditis, pericardial diseases, congenital heart diseases, renal or hepatic insufficiency, acute or chronic inflammation, infectious diseases, known malignancy, cerebrovascular disease, presence of major systemic disease, and age younger than 18 were excluded from the study. The study procedures were explained and written consent was obtained from all participants. The patients underwent a detailed physical examination and transthoracic echocardiography.

Variables

The main study outcomes were serum Selenium (Se), Zinc (Zn), and Cupper (Cu) levels. Samples for zinc (Zn) and copper (Cu) measurements were diluted with glycerol. Standards were prepared at concentrations of 50, 100, 200, and 400 μg/dL. Samples were measured and evaluated in a flame photometer against standard concentrations. Other variables studied were age, sex, systolic blood pressure (mmHg), diastolic blood pressure (mmHg), presence of hypertension or diabetes mellitus, smoking status, blood Ca (mg/dL), Mg (mg/dL), P (mg/dL), alanine aminotransferase (U/L), aspartate transaminase (U/L), glucose (mg/dL), creatinine (mg/dL), total cholesterol (mg/dL), triglycerides (mg/dL), high-density lipoprotein (mg/dL), low-density lipoprotein (mg/dL), hemoglobin (g/dL), and hematocrit (%) measurements.

Sample collection and storage was done while the participants were in the sitting position, drawing 10 ml of venous blood from the forearm cubital vein using vacutainer tubes. After being kept in the room temperature for 30 minutes, the blood samples were centrifuged at 4000 rpm for 15 minutes. Serum samples were transferred to another tube and stored in the deep freeze at -20◦C for analysis. For complete blood count (CBC), sufficient blood was obtained to an ethylenediaminetetraacetic acid (EDTA) tube. CBC, glucose, creatinine, aspartate transaminase (AST), alanine aminotransferase (ALT), total cholesterol, triglyceride, HDL (High-density lipoprotein), low-density lipoprotein (LDL), Ca, Mg, P, and Fe levels were analyzed in the same day with the Behring RXL autoanalyser.

Samples for selenium (Se) measurement were diluted with 5% Triton X 100. A mixture of palladium (Pd) and Mg (NO3)2 (magnesium nitrate) was used as matrix modifier. Standards were prepared at concentrations of 10, 20, 30, and 40 μg / L. Samples were run on an atomic absorption spectrophotometer using a graphite bath and the concentrations were evaluated according to the standard curve.

Transthoracic echocardiography was performed with the Acuson-Aspen® (Acuson Computer Sonography, Mountain View, California) device using a 3.5 MHz probe. Echocardiographic examinations were performed using standard parasternal long axis, parasternal short axis, apical two spaces, apical four space, and apical five space views. Measurements of left ventricular (LV) end-diastole diameter, end-systolic diameter, posterior wall thickness, interventricular septum thickness, ejection fraction, fractional shortening, left atrium (LA) and aortic root were done by the M-mode echocardiography as defined by the American Echocardiography Society [11]. The systolic pulmonary artery pressure was calculated from the tricuspid insufficiency jet flow using the Bernoulli equation by adding the right atrial pressure. In addition, grading of the mitral valve characteristics by echocardiographic examination was performed using the Massachusetts General Hospital Score for Mitral valve morphology (Wilkins scoring) [9]. Two-dimensional, M-mode and double echocardiography methods were used in the assessment of aortic involvement [12–15]. Transesophageal echocardiography (TEE) was performed in patients where transthoracic echocardiography was inadequate due to a poor acoustic window [16].

Study size

Sample size calculation revealed that a total sample of 120 cases (60 study+60 controls) would be enough to detect a

difference of 1.5 µg/dl difference in the serum Zn levels between the study and control groups using the Independent Samples t-test with a two-tailed hypothesis given a mean 1 of 60 µg/dl and mean 2 58.5 µg/dl, standard deviation of 2 (effect size 0.6), alpha error of 5% with a power of 90% [17].

Statistical methods

Clinical, laboratory, and echocardiographic data were compared between Group I and Group II. Data were presented as mean ± standard deviation (SD) or median (min-max). The numerical variables were checked for normal distribution. Variables with normal distribution were analyzed with the Independent Samples t-test whereas the Mann-Whitney U test was used for skewed data. The Chi-Square test was used for the comparisons between categorical variables. All statistical analyzes were done with the statistical package program SPSS (version 13.0). A p-value less than 0.05 was accepted as statistically significant.

Results

Participants

A total of 121 participants (Group I: n=61; Group II: n=60 controls) were included in the study. Of the 61 patients in Group I, 15 (24.6%) were males, and 46 (75.4%) were females, while from the 60 controls in Group II 18 (30%) were males and 42 (70%) were females.

Descriptive data

The mean age of the participants was 37.02 ± 1.25 years in Group I and 38.95 ± 1.44 years in Group II. There was no significant difference between the groups concerning the demographic and clinical characteristics (p> 0.05 for all) (Table 1).

Table 1: Comparison of demographic features and blood chemistry between Group I and Group II.

Variable	Group I (n=61)	Group II (n=60)	p
Age (years)¥	38.95±1.44 (18-64)	37.02±1.25 (18-61)	0.313
Male/female	15/46	18/42	0.508
Systolic blood pressure (mmHg)¥	119.18±1.83 (100-160)	120.08±1.80 (90-160)	0.855
Diastolic blood pressure (mmHg)¥	73.28±1.32 (50-100)	75.68±1.21 (60-100)	0.116
Hypertension β	5/61 (8.2)	4/60 (6.7)	0.751
Diabetes mellitus β	3/61 (4.9)	2/60 (3.3)	0.665
Smokers β	6/61 (9.8)	7/60 (11.7)	0.551
Ca (mg/dL)¥	8.96±0.06 (7.9-10)	9.03±0.06 (7.8-10)	0.381
Mg (mg/dL)¥	1.83±0.03 (1.20-2.30)	1.87±0.07 (1.40-5.70)	0.729
P (mg/dL)¥	3.47±0.08 (2.30-5.90)	3.29±0.06 (2.30-4.60)	0.090
ALT (U/L)¥	38.38±2.10 (15-105)	40.05±1.80 (16-93)	0.096
AST (U/L)¥	25.92±1.55 (14-72)	24.26±1.31 (12-66)	0.249
Glucose (mg/dL)¥	94.20±1.79 (69-137)	92.87±1.67 (75-134)	0.173
Creatinine (mg/dL)¥	0.79±0.03 (0.10-1.40)	0.74±0.02 (0.10-1.40)	0.093
Total cholesterol (mg/dL)¥	162.35±4.54 (95-240)	172.06±3.96 (117-228)	0.193
Triglycerides (mg/dL)¥	118.34±8.45 (26-339)	135.93±9.15 (33-306)	0.160
HDL (mg/dL)¥	43.62±1.45 (22-71)	42.00±1.53 (26-96)	0.444
LDL (mg/dL)¥	98.58±3.4 (45.8-189.2)	108.33±3.84 (44.8-175.2)	0.206
Hemoglobin (g/dL)	12.92±0.17 (8.7-17.1)	13.40±0.20 (9.03-16.50)	0.876
Hematocrit (%)¥	38.46±0.75 (24.37-47.30)	39.75±0.56 (27.70-46.90)	0.468

¥: Mean±standard deviation (range), β: n(%), RHD: Rheumatic Heart Disease; AST: Aspartate transaminase; ALT: Alanine aminotransferase; HDL: High-density lipoprotein, LDL: Low-density lipoprotein

Outcome data

The two groups were compared regarding cardiac functions. There was no significant difference in the LV end-diastole diameters, aortic root diameters, posterior wall and septum thicknesses between Group I and Group II with echocardiographic measurements (p> 0.05 for all). However, significantly higher values for LV end-systolic diameter, lower ejection fraction levels, lower fractional shortening, and higher left atrium diameters were observed in the Group I compared to the Group II (Table 2).

Table 2: Comparison of transthoracic echocardiography measurements between the groups.

Variable	Group I (n=61)	Group II (n=60)	p
LV end-diastole diameter (cm)¥	4.69±0.08 (2.24-6)	4.57±0.06 (3.66-5.36)	0.591
LV end-systole diameter (cm)¥	2.95±0.06 (2.00-4.40)	3.67±0.06 (1.90-3.51)	0.976
Ejection fraction¥	65.75±1.08 (45.0-79.0)	69.83±0.93 (58.0-83.0)	0.542
Fractional shortening¥	37.26±0.88 (25.0-48.0)	40.84±1.03 (31.0-51.0)	0.388
Interventricular septum thickness (cm)¥	1.03±0.02 (0.70-1.50)	0.98±0.02 (0.66-1.30)	0.758
LV posterior wall thickness (cm)¥	0.91±0.02 (0.64-1.40)	0.87±0.02 (0.58-1.10)	0.930
Left atrium diameter (cm)¥	3.84±0.14 (2.30-7.30)	2.90±0.07 (2.18-4.10)	0.546
Aorta diameter (cm)¥	2.62±0.05 (1.83-3.30)	2.68±0.05 (2.05-3.47)	0.907

¥: Mean±standard deviation (range), LV: the left ventricle

Serum Se and Zn concentrations in the Group I were significantly lower than in Group II. Se values for the patient and control groups were 43.08±1.83 and 60.75±2.44, respectively (t=-5.305, p<0.001), while the Zn values were 64.65±2.77 and 87.34±3.33, respectively (t=-5.458, p<0.001). Serum Cu concentrations were significantly higher in the Group I compared to the Group II (84.5±3.5 vs. 74.23±3.08, respectively) (t=2.309, p=0.023) (Figure 1). Similarly, the Cu/Zn ratio was significantly higher in the Group I than the Group II (1.4±0.09 vs. 0.9±0.04), (Z=4.588, p=0.001) (Table 3).

Figure 1: Comparison of the mean serum trace element values between the groups.

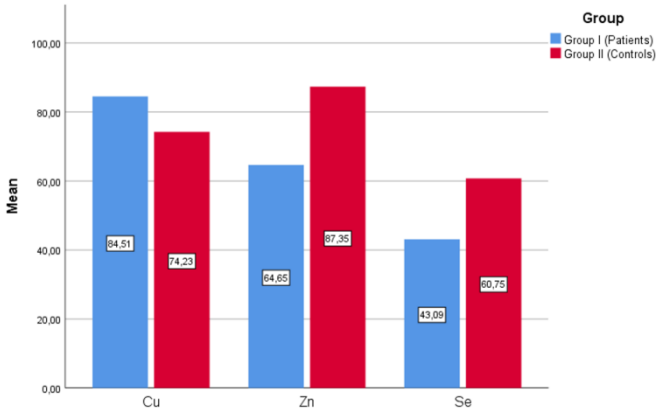

Table 3: Comparison of serum trace element levels between the groups.

Variable	Group I (n=61)	Group II (n=60)	p
Se (µg/dL)¥	43.08 ± 1.83 (9.77-79.65)	60.75±2.44 (10.77-107.88)	0.001
Zn (µg/dL)¥	64.65±2.77 (30.15-109.4)	87.34±3.33 (57.16-166.84)	0.001
Cu (µg/dL)¥	84.50±3.5 (37.32-167.92)	74.23±3.08 (38.20-133.10)	0.023
Cu/Zn (ratio)¥	1.40±0.09 (0.58-2.84)	0.90±0.04 (0.04-1.82)	0.001

¥: Mean±standard deviation (range)

Mean serum Se and Zn values were slightly higher (45.74 µg/dl vs. 42.29 µg/dl and 73.49 µg/dl vs. 61.98 µg/dl, respectively), while the serum Cu concentrations were slightly lower (85.53 µg/dl vs. 81.59 µg/dl) among the patients with a Wilkins score of >8. However, there was no significant relationship between the serum trace element concentrations and Cu/Zn ratio depending on the Wilkins score groups in the patients (p>0.05) (Table 4).

Table 4: Comparison of serum trace element levels between Wilkins score groups.

	Wilkins Score 1-8 (n=47)	Wilkins Score >8 (n=14)	p
Se (µg/dL)¥	42.29±2.06 (9.77-72.15)	45.74±4.06 (23.97-79.65)	0.444
Zn (µg/dL)¥	61.98±2.96 (30.15-109.40)	73.49±6.43 (43.68-108.44)	0.060
Cu (µg/dL)¥	85.53±4.48 (37.32-167.92)	81.59±4.41 (38.98-97.30)	0.263
Cu/Zn ratio¥	1.47±0.11 (0.58-2.84)	1.20±0.14 (0.72±2.22)	0.392

¥: Mean±standard deviation (range)

There was no significant relationship between the serum trace element concentrations and Cu/Zn ratio with aortic involvement in the Group I (p>0.05) (Table 5).

Table 5: Comparison of serum trace element levels in the Group I based on aortic involvement.

	No or mild aortic involvement (n=30)	Medium or severe aortic involvement (n=31)	p
Se (µg/dL) ¥	43.09±2.89 (9.77-72.15)	43.08±2.29 (17.12-79.65)	0.315
Zn (µg/dL) ¥	65.27±3.65 (30.15-108.64)	64.04±4.22 (32.63-109.40)	0.061
Cu (µg/dL) ¥	84.72±4.95 (40.70-157.14)	84.31±5.03 (37.32-167.92)	0.147
Cu/Zn ratio ¥	1.37±0.14 (0.65-2.84)	1.42±0.12 (0.58±2.57)	0.159

¥: Mean±standard deviation (range)

There was no significant relationship between serum trace element concentration and Cu/Zn ratio and pulmonary hypertension in the patient group (p>0.05) (Table 6).

Table 6: Comparison of serum trace element levels in the Group I based on pulmonary hypertension status.

Variable	Absence of pulmonary hypertension (n=29)	Presence of pulmonary hypertension (n=32)	p
Se (µg/dl) ¥	43.08±2.78 (15.78-79.65)	43.10±2.45 (9.77-72.15)	0.932
Zn (µg/dl) ¥	58.69±3.06 (34.00-103.04)	63.22±3.79 (30.15-99.48)	0.659
Cu (µg/dl) ¥	87.25±4.93 (40.70-157.14)	81.98±4.99 (37.32-167.92)	0.225
Cu/Zn ratio ¥	1.51±0.13 (0.65-2.84)	1.28±0.12 (0.58±2.66)	0.214

¥: Mean±standard deviation (range)

Discussion

This study demonstrated that rheumatic heart disease (RHD) is accompanied by decreased Se and Zn concentrations and increased Cu concentrations.

Deteriorated trace element levels in cardiac diseases such as atherosclerosis, idiopathic dilated cardiomyopathy, and chronic heart failure have been reported in the literature [18–21]. Likewise, possible changes in the trace element levels in rheumatic heart disease (RHD) have been reported [22,23]. However, serum trace element levels have not been studied in detail. Nutritional deficiencies and infectious diseases are common and show complex interactions leading to poor clinical effects. Such combinations are evident in developing countries, especially in rural areas. Many nutritional elements (such as Se, Cu, and Zn) modulate the immune function and affect the sensitivity of the host to the infection [24,25]. It is also essential for optimal functioning of organs and tissues. For this reason, trace elements can play a critical role in cardiovascular diseases.

Selenium is an essential trace element for protecting the immune system and oxidative functions. It acts as both an antioxidant and an anti-inflammatory agent. Because hydrogen reduces peroxide and phospholipid hydroperoxidase, it also reduces the levels of free radicals and reactive oxygen species [26].

Zn and Cu, like Se, have similar effects on immunological systems [24]. Increased Cu levels and Cu / Zn ratio are indicative of the severity of an inflammatory process, high levels demonstrating a more serious inflammatory process. It has been suggested that high levels of serum Cu and low Zn (therefore, high Cu/Zn ratio) are low-rate acute phase reactants in patients with sclerotic aortic valves. Studies with similar patient groups have also been supported by moderately elevated C-reactive protein levels [27]. Using serum trace element levels of healthy individuals, sclerotic aortic valves of operated patients, and aortic valves from autopsies, Nyström-Rosader et al. [28] supported that serum Cu and Zn concentrations are indicative of the severity of infection and inflammatory process.

Various immunologic and inflammatory changes have long been reported to play a key role in the pathogenesis and prognosis of RHD. According to many theories, the pathogenesis of RHD is explained by an abnormal immunological response [1, 29]. It was also stated that these responses are related to changes in trace element levels. Previous studies have focused on the trace element levels (especially Zn) in the tissue samples of RHD [22, 23]. These studies have shown that the Zn levels in the samples taken from heart valves of postmortem cases of RCC are significantly lower than those of the control group. Govindarajo et al. [22] have shown that low levels of Zn in RHD may affect cell-mediated immunity and increase rheumatic activity and patients' susceptibility to infections. Koşar F. and colleagues [4] observed that serum Zn and Se concentrations were lower and Cu and Cu / Zn ratios were significantly higher in RHD, and they observed that these were important parameters in the development of the rheumatic process, but they did not find a meaningful relationship with the severity of the functional class, which is indicative of the severity of the disease. Chlamydia pneumonia contributes to the development of atherosclerosis and RHD. Nyström-Rosader and colleagues observed that serum and sclerotic occult elemental levels play an important role in this contribution of Chlamydia pneumonia [30], facilitating the development of active infection by Chlamydia pneumonia and adversely affecting the immune system of the brain.

Low Se levels have been demonstrated in the inflamed heart in acute viral myocarditis [31]. Especially feeding with nutrients deficient from Se is known by its negative effect on the immune system. As a result, more virulent virus variants are selected and they contribute to a heavier disease transmission [32]. In addition, Se deficiency has been associated with many different cardiovascular diseases such as Keshan and Chagas [33].

In our study, it was determined that the serum concentrations of Se and Zn were significantly lower in patients with RHD than in the healthy control group, and that these patients had a significantly higher serum Cu level and a higher Cu/Zn ratio than the control group. However, there was no difference between the groups in which trace element concentrations and Cu/Zn ratios were classified according to the presence of Wilkins score, aortic valve involvement, and presence of pulmonary hypertension, which are echocardiographic findings showing the severity of patients' illnesses. We found that the echocardiographic parameters LV end-systolic diameter and left atrium diameters were significantly larger in the patient group than the control group, and the ejection fraction and fractional shortening were significantly lower.

However, we could not explain an underlying mechanism by which serum Zn and Se element levels could be reduced, and we could not detect a situation that would increase oxidative stress or inflammation and affect serum Zn or Se levels. Because RHD is an inflammatory condition, it is not surprising that low levels of Se and Zn are seen in our patients. In other words, it can be said that the changes in trace element levels in these patients may be the result of the current inflammatory process, as well as the inadequate intake of dietary trace elements. A second finding in this study is that the increase in serum Cu concentration and Cu/Zn ratio reflects an increased or persistent inflammatory process in this disease, because the Cu concentrations and Cu/Zn ratios are significantly higher in these patients compared to the control group. There was no significant relationship between the third trace element profile and the groups we classified according to Wilkins score, aortic valve involvement, and the presence of pulmonary hypertension, which were echocardiographic findings showing the severity of the disease. It is known and expected that among the echocardiographic parameters the left atrium diameter is significantly larger in the patient group than in the control group. Although the ejection fraction and fractional shortening measurements were significantly lower than the control group,

we observed that left ventricular function was maintained in the patient group.

As a result, we report that RHD is accompanied by decreased Se and Zn concentrations and increased Cu concentrations. We can say that changes in trace element concentration may predispose to RHD, but may also give rise to the development of some factors, which may lead to the disease. As they may contribute to the development of many cardiovascular diseases, the low serum levels of Se and Zn may have also significantly contributed to the development of RHD. In childhood, malnutrition and poor hygienic conditions contribute to the decrease of Se and Zn levels [32]. Of course, it is the most economical way to prevent the development of RHD, which is still a significant health problem in Turkey. Therefore, it can be expected that rich and balanced nutrition in terms of trace elements can contribute to the reduction of the incidence of RHD by providing support for a healthy immune system.

When we translate our results into clinical practice, some limitations of this study must be kept in mind. First, our sample is from a restricted area and a limited number of carefully selected participants. Although the results of a study from the same area [4] supports our findings, there is a need for a wider range of data to verify the accuracy and precision of these results. Secondly, the serum levels of the measured elements depend on the nutritional status and oral intake such as mineral supplements and medications. For this reason, it is difficult to make final conclusions by a one-time analysis. Also the time gap between data collection and study reporting, which was due to personal problems of the main author, can be mentioned as a limitation.

In conclusion, assessment of serum trace element levels in patients with well-settled RHD may not be clinically useful, but evaluation of serum trace element levels and providing nutritional trace element support in the acute phase may reduce the development of RHD. Besides, elevated serum Cu levels and Cu / Zn ratio can be used as ongoing inflammatory process markers. For this reason, evaluation of serum trace element levels seems feasible in patients with RHD.

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