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Trace elements in patients with aortic valve sclerosis

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Abstract

Background: Aortic valve sclerosis (AVSc) is defined as the thickening and calcification of aortic valve cusps, in the absence of obstruction of ventricular outflow. AVSc is linked with a clear imbalance in some trace elements.

Aims: The objective of this study was to investigate the relationship between AVSc and serum levels of iron (Fe), zinc (Zn), selenium (Se), and copper (Cu). Additionally, this research aimed to explore the clinical significance of human serum zinc, selenium, copper, and iron concentrations as a potential new biomarker for AVSc patients and to clarify the pathophysiological role in individuals at risk of developing AVSc.

Patients and methods: The study included 40 subjects with AVSc (25% male and 75% female) who were compared with a healthy control group with the same gender ratio. AVSc was based on comprehensive echocardiographic assessments. Blood samples were taken and Zn and Cu concentrations were determined through the use of atomic absorption spectroscopy. Se was measured using an inductively coupled plasma mass spectrometry device and Fe was measured using a Beckman Coulter instrument.

Results: There was a significant difference in the prevalence of diabetes, blood pressure levels, and body mass index between the patients and the healthy subjects ($p < 0.05$). The differences between the serum Fe, Se, and Cu levels of the AVSc patients and the healthy subjects ($p > 0.05$) were recorded. The serum Zn of AVSc patients when compared was significantly lower compared with that of the control group ($p < 0.01$).

Conclusion: Patients with AVSc had an imbalance in some of the trace elements in their blood. The patient group's valves had higher serum Cu levels and lower serum Se, Zn, and Fe concentrations compared with the healthy group's valves. In the valve patients as compared, AVSc had a high prevalence of obesity, hypertension, and diabetes.

Keywords: aortic valve sclerosis, copper, iron, selenium, zinc

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Introduction

Aortic valve sclerosis (AVSc) is defined as the thickening and calcification of the aortic valve leaflets[1] in the absence of obstruction of ventricular outflow,[2] with a gradient of <20–25 mmHg.[3] AVSc is characterized by increased stiffness, thickness, and calcification of the aortic leaflets without fusion of commissures.[4,5] AVSc is the most common valve disorder in developed countries[6] and is prevalent in Western countries.[7] About 25% of people aged 65 years and above have AVSc[8] and this increases to 50% at the age of 80 years.[7,9] Mild, moderate, and severe AVSc have been classified as a protuberance to the thickness of 2–4 mm, >4 mm, and 6 mm respectively[10] with a transaortic flow rate of <2.5 m/s.[11] AVSc is the late outcome of a long-lasting inflammatory process caused by various pathophysiological mechanisms and not a result of normal ageing[12] possibly leading to life-threatening conditions.[13] Aortic valve sclerosis is linked with a clear imbalance in some trace elements of well-known significance for the immune and cardiovascular function, such as iron (Fe), zinc (Zn),

selenium (Se), and copper (Cu). These trace elements are fundamental components in the metabolic operation in both sickness and health.[14] The pathogenesis of several heart disorders has been linked to changes in the equilibrium of certain trace elements.[15] Trace elements are essential nutritional components of human enzymatic systems.[16–18] They are therefore compounds that must be present in the human diet to preserve normal physiological functions.[19] They are involved in several biochemical pathways[18] and are fundamental in the functioning, activation, and maturation of host defence mechanisms.[20] Trace elements such as Se, Zn, and Cu are catalytic, regulatory, and structural ions for enzymes, transcription factors, and proteins. Thus these elements are critical for a variety of the body's different homeostatic mechanisms.[21] In addition, Fe is an active micronutrient and arguably the body's most important biological catalyst, which is essential for the synthesis of myoglobin[22,23] and the haemoglobin molecule.[24] It is also an important co-factor for enzymatic reactions[25–27] required for oxidative metabolism, including that occurring in the myocardium.[22,23] Zn, Se, Cu, and Fe exert significant protective or enhancing effects on the development of several diseases.[19] They are also collectively related to protection against reactive nitrogen and oxygen species in cytosolic defence.[28]

Nyström-Rosander *et al.*[15] examined Cu, Zn, Se, and Fe levels in the serum and tissues of AVSc patients who have been operated on for aortic stenosis. Likewise, Nyström-Rosander *et al.*[29] examined Zn and Fe levels in the tissues and Cu and Zn levels in the serum of AVSc patients undergoing open-heart surgery for the replacement of stenotic aortic valves due to advanced aortic stenosis. Nyström-Rosander *et al.*[14] measured the levels of Cu, Zn, Fe, and Se in the aortic valve tissues of patients undergoing surgical aortic valve replacement due to aortic stenosis. Lis *et al.*[30] studied Cu, Fe, and Zn levels in the tissues of early and late lesions of calcified human aortic valves, and Ilyas and Shah[31] evaluated Fe, Cu, and Zn levels in the blood of patients with valvular heart disease.

The major goals of this study were to explore the clinical significance of serum Se, Zn, Fe, and Cu levels as potential new biomarkers for patients with AVSc, and to clarify the pathophysiological role in individuals at risk of developing AVSc.

Methods

Subjects and study design

This study involved 80 subjects divided into two groups; 40 subjects (30 female, 10 male) with AVSc aged between 18 and 65 years were placed in the AVSc patient group and 40 healthy subjects with the same gender ratio were placed in the control group. AVSc was diagnosed by the cardiologist based on echocardiography scans. Similarly, an echocardiographical evaluation was carried out for the healthy individuals who had no cardiac pathology to thus make up the control group. The control subjects had normal aortic valve leaflets and did not have any cardiovascular disorder or other diseases (e.g. cancer, diabetes, kidney disorder, hypertension, liver disorder). Pregnant subjects and subjects with severe diseases, such as rheumatoid arthritis, liver disease, cancer, renal dysfunction, blood disease, lung disease, congenital heart disease, and other heart diseases, were also excluded from our study to prevent any ulterior impact on serum biochemical parameters from affecting the results. The clinical evaluation of AVSc was performed during visits to a single cardiac centre (Gaziantep University General Hospital, Gaziantep, Turkey) between February 2018 and April 2018. The protocol of the present study was approved by the Clinical Research Ethics Committee of Gaziantep University on 26.12.2017 with no. 2017/429. In addition, the AVSc and healthy subjects provided their written, informed consent to participate in this study.

Clinical measurements

Important information on the clinical patients was collected, including their age, gender, the existence of hypertension and diabetes, height, weight, body mass index (BMI), consumption of drugs and alcohol, and smoking habits. Detailed clinical checks, including laboratory parameters and echocardiography scans, were recorded and combined with a physical examination (conducted by specialist cardiologists). The standard for diagnosing AVSc was based on haemodynamic and morphologic findings in the echocardiographic study showing a protuberance of 2–6 mm in a minimum of one abnormal leaflet per aortic valve with a transaortic flow rate of <2.5 m/s. Anthropometric measurements of body weight and height were recorded

Table 1. Descriptive analyses of the study groups.

	n	Minimum	Maximum	Mean	SD
Age (years)	80	39.0	65.0	56.6	7.2
BMI (kg/m^2)	80	21.1	54.69	31.12	6.08
Systolic blood pressure (mmHg)	80	108.0	168.00	132.13	16.83
Diastolic blood pressure (mmHg)	80	54.0	99.00	69.26	10.51
Iron (µg/dL)	80	11.00	131.00	64.63	30.61
Copper (µg/dL)	80	35.60	167.10	90.38	24.63
Selenium (µg/L)	80	41.70	92.90	61.17	11.41
Zinc (µg/dL)	80	60.10	146.00	86.87	23.16

BMI, body mass index.

with a digital instrument, ensuring the patients were barefoot and wore light clothing (Davi & Cia Weighing Equipment, Barcelona, Spain). Furthermore, the equation of BMI = weight/height^2 (kg/m^2) was used to determine the BMI.

Blood samples and biochemical analysis

Blood samples were collected in the Venesection Unit of Gaziantep University Medical Faculty Hospital. With the subject seated, the antecubital area of the forearm was sterilized by being wiped with cotton soaked in an alcoholic solution. Five millilitres of venous blood was drawn using a BD Vacutainer needle, then transferred to and gathered in tubes (VACUETTE® Tube, 8 ml, US, Z Serum Separator Clot Activator containing microscopic silica particles to stimulate coagulation). The blood samples were then allowed to coagulate for 10–15 min at room temperature. The specimens were centrifuged for 10 min at 4000 rev/min in a centrifuge. The serum was then separated and preserved in a micro Eppendorf tube and stored at –80°C. Zn and Cu levels were measured using atomic absorption spectroscopy (Shimadzu AA-6800 spectrometer), Se levels were measured using inductively coupled plasma mass spectrometry (NexION® 350 ICP-MS spectrometer), and Fe levels were measured using a Beckman Coulter® device (Au5800, Japan, 2007). These analyses were carried out at the Central Laboratory of Gaziantep University's Medical Faculty Hospital.

Statistical analysis

The normality of the distribution of the continuous variables was tested using the Shapiro–Wilk test. The Student's t test was used to compare two independent groups of variables with a normal distribution and the Mann–Whitney U test was used to compare two independent groups of variables with a non-normal distribution. A chi-squared test was used to assess the relationship between categorical variables. Statistical analysis was performed with SPSS for Windows version 22.0 and a p-value < 0.05 was accepted as statistically significant.

Results

The results of the descriptive analyses are given in Table 1. The AVSc patient group ranged from 39 to 65 years of age, whereas the control group ranged between 42 and 65 years. BMI was found to be higher in the patient group (~23–55 kg/m^2) compared with the control group (~21–49 kg/m^2). The subjects also had different heavy metal values in their serum.

The mean categorical variables of the subjects are given in Table 2. BMI was found to be significantly higher in the females in the AVSc patient group (34.37 kg/m^2) compared with the females in the control groups (29.47 kg/m^2) ($p = 0.003$), but this difference was not found in the males. Higher blood pressures were observed in both genders of the AVSc patient group, compared with the healthy group ($p < 0.05$). On the other

Table 2. Descriptive analyses of the study groups.

	Gender	Patient (mean + SD)	Control (mean + SD)	*p*
Age	Female	56.63 ± 7.66	57.43 ± 7.07	0.676
	Male	56.80 ± 6.21	53.40 ± 7.58	0.287
	Total	56.67 ± 7.25	56.42 ± 7.32	0.878
BMI	Female	34.37 ± 6.51	29.47 ± 5.86	0.003
	Male	28.71 ± 2.95	28.73 ± 3.40	0.992
	Total	32.96 ± 6.30	29.28 ± 5.32	0.006
Systolic blood pressure (mmHg)	Female	145.06 ± 15.91	121.73 ± 9.02	0.000
	Male	136.70 ± 15.36	120.00 ± 8.28	0.007
	Total	142.97 ± 16.00	121.30 ± 8.77	0.000
Diastolic blood pressure (mmHg)	Female	74.56 ± 11.71	64.76 ± 7.80	0.000
	Male	72.30 ± 9.60	63.80 ± 5.92	0.028
	Total	74.00 ± 11.14	64.52 ± 7.32	0.000
Diabetes	Female	1.43 ± 0.50	1.00 ± 0.00	0.000
	Male	1.40 ± 0.51	1.00 ± 0.00	0.025
	Total	1.42 ± 0.50	1.00 ± 0.00	0.000
Iron (µg/dL)	Female	56.60 ± 24.02	67.80 ± 34.97	0.154
	Male	62.90 ± 32.18	81.00 ± 28.99	0.203
	Total	58.17 ± 26.00	71.10 ± 33.71	0.059
Copper (µg/dL)	Female	96.88 ± 27.51	85.19 ± 21.48	0.072
	Male	84.08 ± 25.14	92.77 ± 22.10	0.423
	Total	93.68 ± 27.21	87.08 ± 21.60	0.233
Selenium (µg/L)	Female	61.71 ± 11.36	59.76 ± 11.33	0.509
	Male	58.30 ± 12.68	66.66 ± 10.18	0.122
	Total	60.85 ± 11.63	61.48 ± 11.33	0.807
Zinc (µg/dL)	Female	72.91 ± 9.04	101.23 ± 24.17	0.000
	Male	73.71 ± 14.70	98.86 ± 25.61	0.015
	Total	73.11 ± 10.52	100.64 ± 24.23	0.000

$p < 0.05$.
BMI, body mass index.

hand, 42% of the AVSc patient group had diabetes, whereas none of the control group had diabetes. Also, there was no significant difference in the prevalence of diabetes between different genders in the AVSc patients group ($p > 0.05$). (See more detail in Table 2.)

The values of the AVSc patient and values of the studied elements in the serum were found to be in the normal ranges (100–250 µg/dL Fe; 16–71 µg/L Se; 20–70 µg/dL Cu; and 65–140 µg/dL Zn).

The AVSc group had lower Fe and Se serum concentrations than those of the healthy group. However, there was no significant difference in the Fe and Se values of the patient and control groups ($p > 0.05$). The serum Zn levels were found to be significantly lower in the AVSc patient group compared with the control group ($p < 0.01$).

The correlations between the studied variables in the AVSc patient group are given in Table 3. Systolic blood pressure had a significantly positive correlation with diastolic blood pressure ($r = 0.517$, $p < 0.01$), age ($r = 0.399$, $p < 0.05$), and BMI ($r = 0.442$, $p < 0.01$). Moreover, Fe positively correlated with Se ($r = 0.374$, $p < 0.05$) and negatively correlated with Cu values ($r = -0.423$ in the AVSc patient group, $p < 0.01$).

Discussion

AVSc is a type of aortic valve disorder[32] and is the most common valve disorder in developed countries.[6] AVSc is defined echocardiographically by focal regions of valve thickening usually located in the centre of the leaflet with commissural sparing and normal cusp motility.[33] The present study is the first study to evaluate serum trace element levels (Zn, Fe, Cu, and Se) in AVSc patients in comparison with a control group. The main findings of this study indicate that there was a significant difference between the prevalence of diabetes and blood pressure levels in the AVSc patient group compared with the healthy group ($p < 0.05$), as shown in Table 2. The results of a previous study[34] found that there was a significantly higher prevalence of diabetes mellitus and hypertension in patients with obstructive coronary artery disease than in the subjects in the control group ($p < 0.05$), which are in line with the results of our study. Additionally, this present study indicated that there was a significantly positive correlation between the patient's systolic blood pressure

Table 3. Correlation between serum peroxynitrite, Fe, Cu, Se, and Zn, and various clinical parameters in aortic valve sclerosis patients.

	Age	BMI	SBP	DBP	Dia.	Fe	Cu	Se	Zn
Age									
BMI	0.036								
SBP	0.399*	0.442**							
DBP	0.079	–0.002	0.517**						
Dia.	0.235	0.070	–0.151	–0.274					
Fe	0.080	0.142	0.043	0.186	–0.114				
Cu	–0.159	0.154	–0.027	–0.253	–0.167	–0.423**			
Se	–0.042	0.167	0.106	0.216	–0.169	0.374*	0.089		
Zn	0.233	0.204	–0.026	–0.083	0.167	0.180	0.019	0.303	

*and ** correlations are significant at the 0.05 level and 0.01 level (two-tailed), respectively.
BMI, body mass index; Cu, copper; DBP, diastolic blood pressure; Dia, diabetes; Fe, iron; SBP, systolic blood pressure; Se, selenium; Zn, zinc.

values and BMI values ($p < 0.01$), as shown in Table 3.

Trace element and mineral deficiencies are widespread and common among populations[35] and lead to nutritional problems.[19,36] There is also a noticeable relation between this and adverse cardiovascular endpoints.[37] An excess presence can lead to obesity[36] and the resulting toxicity.[19]

In the present study, we also found an imbalance in some of the trace elements among our population. For instance, although the ratios were not significantly different between the two groups, a slight accumulation of Cu was found in the AVSc subjects when compared with the normal serum ranges. A bit increase in serum Cu levels more likely reflects the increased or ongoing inflammation process in this disorder or might be related to the imbalance in the other trace elements such as Zn and Fe. The Cu values in the AVSc patient group were found to be higher in the present study than those of patients with rheumatic heart disease[38] and heart failure,[39] and lower than those of patients with obstructive coronary artery disease[34] and sclerotic heart valves,[15] as shown in Table 4. The micronutrient Cu has physiological capacities linked with bone development, heart function, cellular respiration, the processes of keratinization and pigmentation, and myelination of the spinal line.[40] The excess aggregation of Cu may impact the metabolism of other trace elements, such as Fe and Zn.[41] Excessive Cu can cause several disorders such as headaches, nausea, diarrhoea, and dizziness, a metallic taste in the mouth, weakness, vomiting, cramps, and abdominal pain.[42] Furthermore, our study detected a significant inverse correlation between serum Cu concentrations and serum Fe concentrations ($p < 0.01$, $r = –0.423$) in the patient group (see Table 3). Our study also recorded low Fe values in the AVSc group compared with the control subjects; however, these were not statically significant ($p > 0.05$) (see Table 2). The Fe levels in patients with AVSc were found to be lower in the present study than those of patients with sclerotic heart valves,[15] heart failure,[43] and obstructive coronary artery disease,[34] as shown sequentially in Table 4. The essential trace element Fe is required for the growth and survival of nearly all organisms[44] and plays a role in host defence mechanisms in our bodies.[45] Fe deficiency (ID) leads to a complication of chronic disorders (e.g. Parkinson's disease, inflammatory bowel disorder, chronic renal failure, rheumatoid disease), irrespective of concomitant anaemia.[25,27,46] ID is known to be linked with some cardiovascular diseases involving heart failure, coronary artery disease, and pulmonary arterial hypertension. These disorders have been shown to improve with Fe supplements, proving the hypothesis that ID is a common problem in cardiovascular patients.[37] In addition, a significantly positive

Table 4. Previous studies on serum zinc, copper, selenium, and iron levels in patients from different countries diagnosed with different diseases.

	Country	Disease	Serum levels	*p*-value	Reference
Copper	Sweden	Sclerotic heart valves	1258 ± 412 ng/mL	$p < 0.05$	Nyström-Rosander *et al.*[15]
	Turkey	Rheumatic heart disease	1.93 ± 0.59 µg/L	$p < 0.001$	Kosar *et al.*[38]
	Turkey	Heart failure	880 ± 185 µg/L	0.000	Kosar *et al.*[39]
	Netherlands	Fatal cases of cardiovascular disease	1.32 ± 0.31 mg/L	$p > 0.05$	Kok *et al.*[55]
	Turkey	Aortic valve sclerosis	93.68 ± 27.21 µg/dL	$p = 0.233$	The present study
	Sweden	Sclerotic heart valves	1357 ± 481 ng/mL	$p > 0.05$	Nyström-Rosander *et al.*[15]
Iron	Poland	Heart failure	79 ± 44 µg/dL	–	Tkaczyszyn *et al.*[43]
	Turkey	Aortic valve sclerosis	58.17 ± 26.00 µg/dL	$p = 0.059$	The present study
	Sweden	Sclerotic heart valves	99.9 ± 13.8 ng/ml	$p < 0.001$	Nyström-Rosander *et al.*[15]
	Turkey	Rheumatic heart disease	136 ± 11 µg/l	$p < 0.05$	Kosar *et al.*[38]
Selenium	Finland	Coronary heart disease	51.8 ± 13.82 µg/L	–	Salonen *et al.*[48]
	Turkey	Heart failure	121 ± 5 µg/L	0.000	Kosar *et al.*[39]
	France	Cardiomyopathy	69 ± 2 µg/L	–	Auzepy *et al.*[49]
	Finland	Acute myocardial infarction	48 ± 12 µg/L	–	Westermarck[47]
	Turkey	Aortic valve sclerosis	60.85 ± 11.63 µg/L	$p = 0.807$	The present study
	Sweden	Sclerotic heart valves	752 ± 216 ng/mL	$p < 0.05$	Nyström-Rosander *et al.*[15]
	Turkey	Rheumatic heart disease	0.41 ± 0.16 µg/L	$p < 0.001$	Kosar *et al.*[38]
Zinc	Turkey	Heart failure	555 ± 104 µg/L	<0.01	Kosar *et al.*[39]
	Netherlands	Fatal cases of cardiovascular disease	0.71 ± 0.19 mg/L	$p > 0.05$	Kok *et al.*[55]
	Turkey	Aortic valve sclerosis	73.11 ± 10.52 µg/dL	$p = 0.000$	The present study

Concentrations are shown in their mean ± SD or median form.

correlation was found between the serum Fe values and the serum Se values in the patient group (Table 3). Moreover, the present study reported imperceptibly lower levels of serum Se in the AVSc patient group compared with the healthy group ($p > 0.05$) (Table 2). A similar pattern of lower levels was found in studies,[47–49] which observed lower serum Se values in patients with acute myocardial infarction, coronary heart disease, and cardiomyopathy, respectively, in comparison with healthy subjects. In contrast, the results of Nyström-Rosander *et al.*[15] were not consistent with our data as they demonstrated significantly higher serum Se values in the blood of patients with sclerotic heart valves, compared with plasma from normal valves, as shown in Table 4. Se is a trace mineral with both enzymic and structural roles that are fundamental for normal physiology.[50] Se deficiency has been linked with thyroid disease, infertility and adverse reproductive

outcomes,[51] cancer, and different heart diseases,[52] including Chagas[15] and Keshan diseases,[53] and can also lead to Kashin–Beck disease, a type of osteoarthropathy.[54]

The present study also indicated that serum Zn concentrations in AVSc patients were significantly lower ($p < 0.05$) than those of the control group, as shown in Table 2. This was in line with the results of some studies[15,34,38,39] which found that serum Zn concentrations were significantly lower in patients with coronary artery disease, sclerotic heart valves, rheumatic heart disease, and heart failure, respectively, compared with healthy subjects (see Table 4). Zinc deficiency has been linked with acrodermatitis enteropathica, malabsorption, malignancy, sickle cell disorder, chronic renal disorder,[42] type 2 diabetes mellitus, chronic liver disorder,[41] cardiovascular disorder,[37] and Parkinson's and Alzheimer's diseases.[56] Changes in blood Zn and Cu values might be indicative of a low-grade infectious/inflammatory process, and any condition linked with increased oxidative stress or inflammation may be expected to decrease Zn and Se values. Because AVSc is an inflammatory state, it is not surprising that low Zn and Se values were observed in our patients. In other words, we can speculate that the alterations in the trace element levels in these patients might be the result of either an ongoing inflammatory process or the inadequate dietary intake of trace elements.

The trace element levels in these patients with various diseases are given in Table 4. With respect to both serum Cu and Zn concentrations in fatal cases of cardiovascular disease, Brewer *et al.*[56] showed slightly higher levels for these parameters in fatal cases of cardiovascular disease in comparison with their matched controls.

Limitations

There are several limitations of this study. First, we studied a limited number of patients. The study was also performed as a single-centre study. A multicentre trial would reflect real-world data more accurately. There was also a difference in BMI between females in patient and control groups. Finally, a prospective follow-up of these patients might show the different rates of progression to clinical aortic stenosis between patients with normal and lower levels of these trace elements.

Conclusion

The present study indicated that patients with AVSc have an imbalance of trace elements in their serum compared with healthy subjects. Patients with AVSc had a higher prevalence of hypertension, diabetes, and obesity. In the context of the declared goal and the results obtained, it can be assumed that the Zn content was a biomarker of metabolic disorders – obesity and diabetes, not AVSc. Regularly measuring serum Fe, Cu, Se, and Zn in patients with AVSc will be beneficial to prevent any associated risks. In accordance with the results of our study, patients with AVSc should consume foods rich in Fe, Se, and Zn in order to receive the recommended intake of and preserve the normal levels of these minerals stored in the body.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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