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Serum Zinc and Copper Levels in Ischemic Cardiomyopathy

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Abstract Changes in the copper (Cu) and zinc (Zn) concentrations have been reported previously in ischemic cardiomyopathy (ISCMP). Due to controversial results, the aims of this study were to compare levels of Cu, Zn, and Zn/Cu ratio of ISCMP patients with healthy volunteers and also to investigate the possible relationship between trace elements status in ISCMP patients with the severity of clinical disease based on the New York Heart Association (NYHA) classification. The subjects of this study consisted of 30 ISCMP and 27 healthy volunteers. ISCMP was diagnosed with a history of previous myocardial infarction and also coronary artery disease was confirmed by coronary angiography. Exclusion criteria were renal or hepatic insufficiency, alcohol usage, and intake of supplements containing Cu or Zn within 1 week. Cu and Zn levels have been assayed with atomic absorption spectrophotometry. Statistical analysis was performed with the SPSS 10 software using independent sample *t* test for comparing the levels of Cu and Zn between ISCMP and normal subjects. The mean Cu level of the ISCMP group (1.54±0.52 mg/L) was significantly more than the Cu levels of the healthy volunteers (1.31±0.24 mg/L; $p=$

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0.048). The mean Zn levels of the ISCMP and healthy volunteers were 1.05±0.28 and 1.12± 0.42, respectively, without any significant difference between groups. There was a trend for higher Cu level, lower Zn level, and lower Zn/Cu ratio in NYHA III patients in comparison with NYHA II group. Considering the results of this study, Cu may have a role in the development of ISCMP. Interventions such as administration of Cu chelators to relieve the symptoms or to decrease the progression of ISCMP is needed to be examined in large clinical trials. In this study, the Zn level of ISCMP patients was not significantly different in comparison with the healthy volunteers.

Keywords Ischemic cardiomyopathy · Copper · Zinc

Introduction

Congestive heart failure (CHF) is an important disease which affects 1.5–2% of the population and is demonstrated when the ability of blood circulation in preparing metabolic demands of the body is reduced [1]. Despite rapid diagnosis and proper treatment of the disease, patients have poor prognosis and the typical symptoms of CHF such as edema, fatigue, weakness, and dyspnea can affect the quality of patient's life; also, it can cause death in 30–50% of patients with advanced stages of disease within 1 year and also 70% of them during 3 years [2, 3].

Cardiomyopathies are a group of heart diseases which influence cardiac muscles directly and are not caused by hypertension, congenital, valvular, and pericardial heart diseases. The most common type of cardiomyopathy is dilated cardiomyopathy that is presented with symptoms such as systolic failure of one or both ventricles. Ischemic cardiomyopathy (ISCMP) results following severe coronary artery disorders and usually angiography must be performed for proper diagnosis. The cardiomyopathy with no specific reason is called idiopathic dilated cardiomyopathy (IDCMP) [3]. Although increased preload and afterload and decreased contractility are three major mechanisms for CHF, studies showed that alteration of body content of trace elements causes myocardial metabolic disorders and dilated cardiomyopathy [4]. Furthermore, studies showed protective and pathologic effects of some elements on cardiomyopathy. Thiol oxidation and lipid peroxidation are mentioned as factors of structural and functional disorders [5, 6]. It appears that CHF has a relationship with increased oxidative stress [7] and decreased content of antioxidative elements such as zinc (Zn), selenium (Se), and manganese (Mn). Moreover, increased content of some elements including copper (Cu), cobalt (Co), and arsenic (As) may elevate the oxidative stress leading to cardiac functional disorders [4, 7, 8]. There are paradoxical results regarding the role of Cu and Zn contents in various types of cardiomyopathy.

Animal studies showed that Cu deficiency results in the appearance hypertrophic cardiomyopathy (HCMP), myocardial hardness, increased interstitial collagen, and systolic and diastolic failure [9]. Heart failure induced by Cu deficiency is related to decreased activity of superoxide dismutase enzyme and decreased myocardial resistance against oxygen free radicals [10]. Furthermore, administration of antioxidative substances can subside cardiac hypertrophy and mitochondrial damages in animals with cardiomyopathy induced by Cu deficiency [11]. It has been reported that high Cu serum level is associated with high mortality rate in ISCMP patients [12]; however, no significant relationship was observed between Cu serum level and function of the left ventricle [13].

Zn is another vital element which is essential for structural and functional stability of the cellular membrane, and regarding its antioxidative and membrane stabilizing effects, it can

protect membrane against unsaturated lipids and inflammatory cytokines [14]. It should be noted that both decreased and increased Zn serum level were reported in ISCMP patients [15, 16]. In addition, Cu and Zn levels were not determined together in some studies [12, 13, 17].

According to the mentioned paradox in the role of Cu and Zn concentrations in ISCMP and the lack of studies regarding this subject, this study was performed for the assessment of Cu and Zn serum levels including Zn/Cu ratio in ISCMP patients in comparison with healthy volunteers. Furthermore, the possible relationship between trace element status in ISCMP patients with the severity of their clinical disease based on the New York Heart Association (NYHA) classification has been addressed.

Materials and Methods

This study was performed as a descriptive–analytical study on 57 subjects in Fatemeh Zahra Heart Center during 2007. The first group included 30 ISCMP patients and the second group included 27 healthy volunteers.

Clinical Evaluation and Inclusion/Exclusion Criteria All patients and healthy subjects were informed about the purpose of the investigation and procedure. Patients were classified into four classes based on the New York Heart Council criteria [18].

The diagnosis of ISCMP was based on history, physical examination, electrocardiogram, radiography, and echocardiography. The ischemic etiology of heart failure was confirmed by angiography in all patients. For healthy volunteers, in addition to history, physical examination and common laboratory tests (fasting blood sugar, urea, creatinine, hepatic enzymes, triglycerides, cholesterol), echocardiography was performed to assure the cardiovascular health.

Exclusion criteria for patient group were hepatic and renal failure, alcohol usage, and intake of vitamin supplements containing mineral and therapeutic multivitamin or zinc sulfate within 1 week of taking blood samples. Also, exclusion criteria for healthy volunteers were any disease or intake of any drug or supplement within 1 week of the assessment.

Clinical profile of patients, including risk factors, clinical severity of heart failure based on NYHA, results of echocardiography, and prescribed drugs, were established in query forms.

Sampling and Determination of Cu and Zn Serum Level A 15-mL blood sample was taken from patients and healthy volunteers, then the samples were moved to special tubes, and were heated in water bath (37°C) for 1 h. The samples were centrifuged (1,500 rpm) and freezed at −20°C after serum isolation.

To determine Zn and Cu concentrations in serum samples, standard Zn and Cu solutions were prepared. Four standard Zn solutions (0.1, 0.2, 0.3, and 0.4 ppm) and four standard Cu solutions (0.5, 1, 2, and 2.5 ppm) were made. After defreezing, 1.5 mL of serum sample for Zn assessment and 2.5 mL of serum sample for Cu assessment were isolated. The sera were moved to 5 mL volumetric flasks, and then glycerol solution 5% and 10% were used for determination of Zn and Cu concentration, respectively. Zn and Cu serum levels were assayed by flame atomic absorption spectrophotometry with λ_{max}=324.8 nm for Cu level assessment and λ_{max}=213.9 nm for Zn level determination. Then, the concentrations were determined following the preparation of calibration curves and evaluation of line equation [19].

Table 1 Demographic Profile and Results Echocardiography of ISCMP Patients and Healthy Volunteers

Parameters	Healthy volunteers (n=27)	ISCMP patients (n=30)
Age (years)	42.3±8.99	57.17±8.88
Sex (female %)	55	47
Severity of heart failure (NYHA)	–	
Class 1		1
Class 2		17
Class 3		10
Class 4		2
LVESD (mm)***	27.71±2.82	47.77±4.58
LVEDD (mm)***	47.54±3.87	56.07±5.04
Ejection fraction	Normal	26.33±5.71

ISCMP ischemic cardiomyopathy, *NYHA* New York Heart Association, *LVESD* left ventricular end systolic diameter, *LVEDD* left ventricular end diastolic diameter

***$p<0.001$

Statistical Analysis Statistical analysis was performed by the SPSS 10 software followed by independent sample t test for comparing the Cu and Zn level of IDCMP patients with normal subjects and chi-square test for qualitative data. Pearson coefficient was used to study the correlation between Cu and Zn level and age. $p<0.05$ was considered to be significant.

Results

Demographic profile and results of the laboratory tests of ISCMP patients and healthy volunteers are illustrated in Table 1. Forty-seven percent of the patients and 55% of the healthy volunteers were female. The majority of patients were in class 2 and 3 NYHA functional class. The mean blood sugar of the ISCMP patients was above normal limits. On the other hand, mean urea and creatinine, hepatic enzymes, triglycerides, and cholesterol of both ISCMP patients and healthy volunteers were within normal range.

Table 2 shows the mean Zn and Cu concentrations and Zn/Cu ratio in ISCMP patients and healthy volunteers. Patients with ischemic heart failure had higher Cu serum level than that of sample subjects (mean division, 0.23; 95% confidence limit, −0.26 to 0.11; p= 0.048). Zn serum level and Zn/Cu ratio showed no significant difference in patients and healthy volunteers.

Figure 1 presents the Zn, Cu, and Zn/Cu ratio of patients based on their NYHA classification. Since there was only one patient in class 1 and two patients in class IV, the

Table 2 Zn and Cu Concentrations and Zn/Cu Ratio in ISCMP Patients and Healthy Volunteers

Parameters	Healthy volunteers (n=27)	ISCMP (n=30)	95% Confidence limit of mean division	p value
Zn (mg/L)	1.12±0.42	1.05±0.28	0.002 to 0.44	0.42
Cu* (mg/L)	1.31±0.24	1.54±0.52	−0.26 to 0.11	0.048
Zn/Cu ratio	0.91±0.43	0.77±0.33	−0.34 to −0.007	0.19

ISCMP ischemic cardiomyopathy

*$p<0.05$

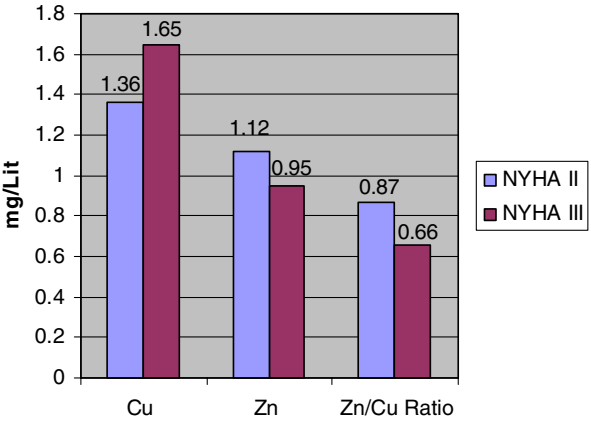

Fig. 1 Comparison of zinc, copper, and zinc/copper ratio of patients based on their NYHA classification

data was provided for patients who were in the NYHA II and III groups. The trend indicates higher Cu levels and lower Zn levels in patients who had a severe clinical heart failure (e.g., NYHA III) in comparison with those who had a milder form of the disease (e.g., NYHA II) (for Cu, 1.65±0.54 vs. 1.36±0.35, p=0.111; for Zn, 0.95±0.27 vs. 1.27±0.29, p=0.14).

Discussion

In this study, Cu serum concentration in the patient group was higher than that of the healthy volunteers (p=0.036). In addition, the mean Cu serum level in the two groups were not significantly different. Various studies were performed to determine the role of Cu and Zn contents in ISCMP which is shown in Table 3.

Cu and Cardiomyopathies The results of studies regarding the role of Cu content in different types of cardiomyopathy have been controversial. Our study supports the result of Kosar et al. who found a higher Cu level in ISCMP compared to healthy volunteers [15]. In that study, they reported a higher Cu serum level in 26 ISCMP patients when compared to 30 healthy subjects [15]. Similar results were found in children with heart failure [20, 21], and also, there exist some evidence that the higher Cu level of ISCMP patients is associated with a higher mortality rate [12, 17]. In addition, we investigated the trace elements status of patients based on their NYHA classification. Although differences were not statistically significant, there was a trend for higher Cu and lower Zn in NYHA III patients when compared to the NYHA II group.

It appears that alteration of Cu serum level is a stimulatory factor for heart failure and causes extended cardiac muscle damage. Although the predominant reason for increased Cu level in cardiomyopathy patients is still unclear, it may be related to oxidative damages or inflammation [15]. In addition, Cu accumulation was mentioned as a causative mechanism of left ventricular hypertrophy, and in some investigations, administration of specific chelating agents such as trientine was suggested [22].

Zn and Cardiomyopathies Reactive oxygen species hold an important role in oxidative damages and may cause myocardial functional disorders by reaction with proteins or lipids of membrane [23]. Zn is presented in several enzymes, such as superoxide dismutase and

Table 3 Published Human Studies Regarding the Role of Zn and Cu Level in Ischemic Cardiomyopathy

Study	Disease	Studied person	Results		Reference
			Zn	Cu	
Kosar et al. (2006)	ISCMP IDCMP	28 ISCMP patients, 26 IDCMP patients, and 30 sample subjects	Zn concentration was decreased in both patient groups in comparison with healthy volunteers	Cu concentration was increased in both patient groups in comparison with healthy volunteers	[15]
Malek et al. (2003)	ISCMP	64 patients	–	Cu concentration was increased in patient group and related to yearly death	[12]
Malek et al. (2001)	ISCMP	40 patients	–	Cu concentration was increased in patient group	[17]
Malek et al. (2002)	ISCMP	38 patients	–	No relation between Cu serum level and LVEF was observed	[19]
Atlihan et al. (1990)	ISCMP	15 patients	No significant differences were observed in comparison with patient and healthy volunteers	Cu concentration was increased in patient group in comparison with healthy volunteers	[20]
Cooper et al. (2004)	ISCMP	20 patients	–	Cu accumulation in cardiac damages induced by Diabet	[21]
Bialkowska et al. (1987)	Athrosclorosis and MI	29 patients and 23 sample subjects	Zn concentration and Zn/Cu ratio were increased in patient group in comparison with healthy volunteers	–	[22]
Present study	ISCMP	30 ISCMP patients and 27 sample subjects	No significant differences were observed in patient and healthy volunteers	Cu concentration was increased in ISCMP patients in comparison with healthy volunteers	–

ISCMP ischemic cardiomyopathy, *IDCMP* idiopathic dilated cardiomyopathy

glutathione peroxidase [24]. Anionic groups of superoxide react with hydrogen peroxide in the absence of superoxide dismutase and release hydroxide radicals, which cause cellular membrane lipid peroxidation [16]. In addition, following atrial natiuretic peptide activation in heart failure, increased urinary Zn excretion could result in secondary decreased Zn concentrations of plasma and red blood cell. Primary and secondary Zn deficiency can cause myocardial structural and functional disorders [25]. Although in some previous studies diminished Zn concentration in ISCMP patients were reported in comparison with healthy volunteers [15, 20], no significant difference was observed in our study in this regard (1.05 ± 0.28 vs. 1.12 ± 0.42; $p = 0.42$).

Comparison of Zn/Cu Ratio Between the Patients and Healthy Volunteers In some clinical conditions, determination of Zn/Cu ratio may be more justified than the determination of Zn and Cu alone. For instance, decreased Zn concentration, increased Cu concentration, and decreased Zn/Cu ratio were observed in rheumatoid arthritis [26, 27]. Moreover, decreased Zn content and Zn/Cu ratio were mentioned as risk factors of some cancers, such as thyroid carcinoma [28]. In this investigation, in addition to Zn and Cu concentrations, Zn/Cu ratio was calculated in ISCMP patients and healthy volunteers, which showed no significant difference (Table 2).

Conclusion

Higher Cu serum level in ISCMP patients may inform the role of Cu in cardiomyopathy, or Cu serum level may secondarily increase as a result of cardiomyopathy. Using Cu chelating agents to decrease signs or to slow the progression of ISCMP needs to be further studied in large clinical trials. Although Zn content in healthy volunteers was higher than that of ISCMP patients, the difference was not statistically significant.

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