A Pilot Study on Correlation Between Serum Selenium and Copper Concentrations and Coronary Slow Flow Phenomenon

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Abstract- The pathophysiology of coronary arteries slow flow phenomenon is related to the microvascular and endothelial disorder. The role of copper and selenium in the development of microvascular and endothelial disorders, as well as atherosclerosis, has been proven in previous studies. The aim of this study was to evaluate the serum copper and selenium concentrations in patient with coronary slow flow phenomenon to find a probable relationship between them. In this study, 125 patients who referred to Ghaem Hospital in Mashhad for angiography were selected based on entry and exit criteria. Patients were divided into 5 groups according to the results of angiography. Blood samples of these patients were evaluated about the levels of copper and selenium. Finally the correlation between these levels and the intensity of coronary arteries slow flow (based on TIMI scores) was evaluated. In the present study, serum selenium and copper concentrations did not show a significant correlation with the intensity of coronary arteries slow flow (P>0.05). In the present study, no significant correlation was found between copper and selenium serum concentrations and coronary arteries slow flow based on TIMI. Further studies are recommended to investigate this association.

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Introduction

Microvascular angina refers to a condition in which the patient experiences angina pains, but his/her epicardial arteries are normal (1). As yet, the exact pathophysiological mechanism of this coronary phenomenon has remained indistinctive. However, microvascular disorders, endothelial disorders, subclinical atherosclerosis, inflammation, and anatomical factors are known to be involved as factors leading to this disease (2). Although the microvascular angina pathogenesis is not well known, probably there are two simultaneous mechanisms cause pain in patients; myocardial ischemia (due to the dysfunction of the microvascular coronary artery) and increased sensitivity to chest pains (3,4). The vascular endothelial disorder is an important etiology. It may be attributed to an imbalance in the production and consumption of nitric oxide (NO) resulted from its altered metabolism caused by increased oxidative stress and inflammation (5,6). Moreover, there is a defect in endothelin-1, resulting a dysfunction of vascular dilatation or constriction in patients with vascular endothelial disorder (7). On the other hand, endothelial disorders provide the condition for activating platelets, leukocytes, and cytokines. These could cause increasing the permeability of the arteries to oxidized lipoproteins, which ultimately induces vascular wall damage and formation of atherosclerotic plaques (7). The findings from the angiography of patients with microvascular angina indicate the Coronary Slow Flow

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Phenomenon (CSFP). This means that the contrast agent in the coronary epicardial artery has a slow progressive movement, while no obstruction is found in these arteries (2,8). It has been reported in about 1-7% of patients underwent coronary angiography.

Currently, counting the TIMI frames as a quantitative index of blood flow in vascular angiography is the only diagnostic and evaluator instrument (2). In this process, full passage speed of the injected contrast agent is checked in coronary arteries. Copper (Cu) is an essential element for human life that is available in the human body at an average concentration of 1.4-2.1 mg per kg of body weight, and the minimum daily human requirement for this element is 0.7 mg (9,10). Copper is considered as an oxidizing/reducing agent in the body. On the other hand, it has been observed in previous studies that consuming food supplements containing copper reduces the amount of oxidized low-density lipoprotein cholesterol in the blood, thus, prevents atherosclerosis in the patients (11). Serum copper concentrations have been found to be lower in patients with atherosclerosis than those without, which confirms the effect of copper and its deficiency on atherosclerosis. In addition, it has been found that copper can lead to inhibition of platelet adhesion and oxidative stress to prevent atherosclerosis (12,13).

Copper is an effective mediator for the Lysyl oxidase enzyme to maintain the integrity of endothelial and preventing microvascular and endothelial disorders in the arteries. It also has an antioxidant role besides its effects on superoxide dismutase and nitric oxide to affect vascular tone and prevents damages caused by oxidative stress (12,13).

It has been observed in previous studies that serum level of copper is associated with the incidence of ischemic heart disease, and that copper deficiency in the human body can lead to the higher incidences of these and coronary artery diseases. Selenium (Se) is the main element involved in the activity of the glutathione peroxidase function (14). The recommended amount of Se available in daily diet is 55 μg, and the total amount of Se available in the human body has also been reported between 13-20 mg (15). One of the most important roles of Se in the human body is its cofactor function for the revival of antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase. These enzymes accelerate the reactions in which free oxygen species are eliminated, thereby acting as potent antioxidants in the human body (16). Se has previously been found to prevent the early onset of heart failure by its antioxidant activity, which is possibly mediated by glutathione peroxidase enzyme (17). Se prevents damages to the arterial endothelium by inhibiting the oxidative stress due to thioredoxin reductase and glutathione peroxidase enzymes. Se also affects the vascular tone through an effect on NO; in addition, it helps to maintain its integrity by affecting the adhesion of vascular endothelium cells. Another Se activity implemented by selenoproteins is the regulation of endothelial cells' apoptosis (13). Se prevents the appearance of atherosclerosis by affecting the adhesion of vascular endothelial cells. Such enzymes as cyclooxygenase being involved in this function (18). Previous studies suggest a reverse relationship between Se levels and the incidence of myocardial infarction (19).

As briefly reviewed above, endothelial dysfunction, inflammation, and oxidative stress play an important role in CSFP pathogenesis, and serum levels of Se and Cu are effective on these factors. But to the best of our knowledge none of the previous studies are focused on relationship between Se and Cu levels with appearance of CSFP. So, the present study is a novel experimental attempt, which aims to do so.

Materials and Methods

Study population

We conducted this study from July 2016 until November 2017. Based on the inclusion and exclusion criteria, the patients attending the Ghaem Hospital, affiliated to Mashhad University of Medical Sciences, Mashhad, Iran, for angiography were the subjects of the present study. Patients who were suspected of having coronary artery disease were included in study. Patients with coronary artery aneurysm, hyperhomocysteinemia, myocarditis, pericarditis, cardiomyopathy and sitagliptin use history or who were on hemodialysis were excluded from the study. All of the participants signed a written informed consent before the beginning of the study.

This research was approved by the Ethics Committee of Mashhad University of Medical Sciences (code number: IR.MUMS.REC.1395.599).

Determination of serum concentration of Se and Cu and coronary blood flow speed

The existence or absence of coronary obstructive artery disease, number of involved arteries, and coronary slow flow arteries were evaluated as the visual findings during the angiography by a cardiologist.

Different definitions for the slow blood flow of coronary arteries are available that the one is used in this

study is "the corrected TIMI frame counts more than two standard deviations from the normal range in the absence of an obstruction in the coronary arteries" (2). According to the result of the angiography, the patients were divided into 5 groups as mentioned in table 1.

Table 1. Classification of the study population

Group's Acronym	The group of patients
CAD(-) , Slow Flow(-)	Patients with no coronary artery disease and no epicardial arteries slow flow
CAD(-) , Slow Flow(+)	Patients with no coronary artery disease and with the slow flow of epicardial arteries
CAD(+) , (<50%),Slow Flow(+)	Patients with coronary artery disease (less than 50%) having no slow flow of epicardial arteries
CAD(+) , (>50%, <90%),Slow Flow(-)	Patients with coronary artery disease (between 50 to 90%) having no slow flow of epicardial arteries
CAD(+) , Slow Flow(+)	Patients with coronary artery disease having the slow flow of epicardial arteries

Twenty milliliters of patients' whole blood were collected from the brachial vein, transferred to the Ghaem hospital emergency ward laboratory, and centrifuged at 3000 rpm for 10 min. The plasma fraction was isolated and stored at -80° C until required for analysis. Serum concentration of Cu (AA240FS, Varian, Australia) and Se (GTA120, Varian, Australia) were determined using the atomic absorption flame spectrophotometry. Patients' demographic data including gender, age, and body mass index (BMI), past medical history (e.g. hypertension, dyslipidemia, and diabetes Mellitus), smoking and family history were collected using patients' records in a pre-designed checklist.

Statistical methods

Statistical analysis was carried out by SPSS 19, Results have been shown as mean±standard deviation or median (interquartile range) for normally and non-normally distributed continuous variables, respectively, and number (percentages) for nominal variables. Kolmogorov-Smirnov test was used to assess the normality of the distributions of the variables. Independent sample t-test and Mann-Whitney U-test were used respectively to compare normally and non-normally distributed variables between the two groups. For comparison of more than two groups in normally and non-normally distributed variable one way ANOVA and Kruskal–Wallis tests were used, respectively.

Besides, the Pearson correlation test (in the case of normal distribution) and Spearman's correlation test (in the case of non-normal distribution) were employed to examine the intensity and correlation between the two quantitative variables. All the tests were conducted at the significant level of 0.05.

Results

A total of 125 patients undergoing angiography were included in the study. Of these, 48 subjects (38.4%) were males, and the mean age of the studied population was 54.58±10.23-year-old (Table 2).

Table 2. Demographic data

Parameter	Mean ± SD
age	54.58±10.23
BMI(Kg/m^2)	27.90±4.38
Fasting blood sugar(mg/dL)	116.93±61.01
Serum Selenium concentration(µg/L)	101.55±18.40
Serum Copper concentration(µg/dl)	91.93±24.40

As mentioned in table 3, most of patients had no coronary artery disease and were without slow flow epicardial artery (30.4%), followed by those with coronary obstructive disease less than 50% and slow flow of epicardial artery (20%).

Table 3. Distribution of patients in the studied groups.

Group	Frequency (%)	Distribution (N)
CA D(-),Slow Flow(-)	0.4	38
CAD(-),Slow Flow(+)	17.6	22
CAD (+)(<50%),Slow Flow(-)	20	25
CAD (+)(>50%,<90%),Slow Flow(-)	18.4	23
CAD(+), Slow Flow(+)	13.6	17
Total	100	125

Most patients with coronary obstruction rate of 50-90% had one artery involvement (39.1%), followed by two vessels (34.8%) and three vessels (26.1%), respectively.

Serum selenium concentrations (Table 4) were not significantly different in the studied groups (*P*=0.49). However, the mean serum concentrations of selenium in patients with the coronary obstructive disease and slow flow epicardial artery were lower than the other groups.

In addition, the mean serum levels of copper had no significant differences among the groups (*P*=0.847).

Table 4. Comparison of mean serum selenium concentrations and serum copper concentration in the studied groups

Group	Serum copper level (µg/dl) (mean±SD)	Serum Selenium level (µg/l) (Mean±SD)
CAD(-),Slow Flow(-)	04/24±59/92	102.24±17.67
CAD(-),Slow Flow(+)	98/22±52/93	106.44±15.86
CAD(+)(<50%),Slow Flow(-)	77/30±07/95	98.64±18.19
CAD(+)(>50%,<90%),Slow Flow(-)	93/21±39/87	102.45±20.99
CAD(+), Slow Flow(+)	54/19±43/90	96.68±19.96
P	0.847	0.49

Statistical test: ANOVA
CAD: Coronary Arterial Disease

Table 5. The relationship between serum levels of selenium and copper and the coronary artery flow rate based on TIMI score

Parameter	*P**	The correlation coefficient
Serum selenium concentration	0.19	-0.224
Serum copper concentration	0.73	0.061

*: Pearson correlation test

In addition, the mean serum levels of copper had no significant differences among the groups (*P*=0.847).

TIMI score was measured in various coronary arteries separately (LCX, LAD, and RCA) in patients with a slow flow coronary artery. Considering that two or three vessels had a slow flow in some patients, mean TIMI values were calculated individually. Table 3 indicates that both serum levels of selenium (*P*=0.19) and copper (*P*=0.73) had no significant relationships with the severity of the slow flow of the coronary arteries based on TIMI scores. (Figure 1 and 2).

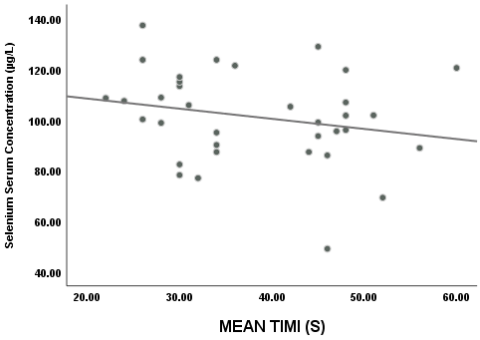

Figure 1. The relationship between serum selenium concentration and the mean of the flow of coronary arteries based on the TIMI

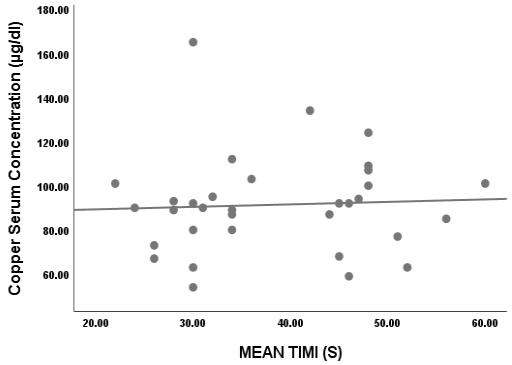

Figure 2. The relationship between serum copper concentration and the mean of the flow of coronary arteries based on the TIMI

Discussion

Previous studies have shown that copper and selenium are effective in the development of microvascular and endothelial disorders, as well as atherosclerosis. Since there is a relationship between CSFP pathophysiology and the above disorder and no study examined selenium and copper contributions to CSFP, it seems necessary to investigate the levels of copper and selenium in patients with a CSFP. Selenium affects the factors that influence the pathophysiology of the slow flow of the coronary arteries. Therefore, it was assumed that a relationship might be observed between serum selenium concentration and CSFP.

Our results show that there is not any relationship between serum selenium concentration and CSFP. It's probably due to the sample size limitation in the present study. However, further studies are needed to evaluate this issue. The effect of selenium on the slow flow of coronary arteries has been examined very limitedly in previous studies. These studies also somewhat confirm the results of present study. For example, Hawkes et al. observed that patients with high serum selenium levels were not significantly different from those with low levels of selenium in terms of obstruction's degree and also the diameter of brachial artery (20). Bleys *et al*., in a meta-analysis, reported that selenium intake and high serum levels of this element did not have a preventive effect on the development of atherosclerosis (21). Flores-Mateo *et al*., also demonstrated that selenium intake could not reduce the incidence of atherosclerosis and cardiovascular diseases (22). In a study performed in Germany, the mean normal serum selenium concentration in humans has been reported to be about 95.5 µg/L (23).

In another study conducted in Tehran, the mean serum selenium concentration was estimated to be 100.6 µg/L in healthy individuals over 16-year-old (24). Considering that mean serum levels of selenium in all examined patients in our study were very close to the normal values, this can be one of the reasons that we couldn't find this relation between serum selenium concentration and CSFP. Moreover, there are findings that show significant and inverse relationships between serum selenium concentrations and the incidence of cardiovascular diseases (25-27). For example, it was observed in a meta-analysis by Zhang *et al*., (28) that serum selenium concentrations ranged between 45-145 µg/L showing a significant and inverse relationship with the incidence of coronary artery diseases (28). So, as in our research the mean serum level of selenium was significantly close to each other in various studied groups; it was expected to find no difference regarding cardiovascular disease occurrence and CSFP.

Our findings on copper as another examined element in this study showed no significant relationships between copper serum concentrations and the severity of the slow flow of coronary arteries based on TIMI. Mean serum copper concentrations were not significantly different among the studied groups. Since serum level of copper also seems affective on different aspects of the slow flow of coronary arteries pathophysiology, it was assumed that a relationship might be observed between serum copper concentration and coronary artery slow flow phenomenon, but such a relationship was not found in this study.

The relationships between serum copper concentrations and cardiovascular diseases have been examined in previous studies. For example, it has been reported by Klevay that copper is an important chemical element for prevention of cardiovascular disease pathogenesis, and especially cardiac ischemic diseases (29). On the other hand, Bagheri *et al*., (30), noticed that serum copper concentrations were higher in patients with ischemic heart disease than disease-free patients, which is contrary to that of Klevay (30). Lutfi *et al*., (31) also examined 140 patients suffering from coronary artery disease compared with healthy individuals and detected no significant relationships between serum copper concentrations in two groups (31). It seems that further studies in this field are needed to define the relationship between serum copper concentrations and cardiovascular diseases. Regarding the high prevalence of these micronutrients' deficiency in the community and considering the pathophysiology of the slow flow of coronary arteries, it seems necessary to conduct further studies in this field.

The present study is the first study to examine

selenium and copper elements in patients with coronary artery slow flow phenomenon and those with coronary obstructive disease. Our findings could be the prospect for future study with a similar purpose. This study was confronted with various limitations, including relatively small sample size. Moreover, in the current study, coronary obstructive disease and the slow flow of the epicardial microvessels were measured visually and dependent on the physician. However, it was tried to carry out all angiographies by only one cardiologist to avoid errors and bias in the results.

Considering the above limitations, conducting a study including more patients to compare the incidence rate of coronary artery slow flow in patients with selenium and copper deficiency and those with the normal level of these elements is recommended.

Based on findings from the TIMI score in the present study, the serum concentrations of the selenium and copper have not a significant relationship with the intensity of coronary slow flow. Further studies are recommended.

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