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Study of Serum Metals and Antioxidant Enzymes in Patients with Coronary Artery Disease

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Abstract

Trace elements and antioxidant enzymes play a vital role in health and diseases, and have been the subject of several investigations on their role in the etiology of coronary artery disease (CAD). Our study including 45 patients with CAD and 25 controls was randomly selected. The mean level of serum copper (Cu), ceruloplasmin (Cp), iron (Fe), Ferritin(Fer), transferin (Trans) was higher and there was significant difference detected between the patients with CAD and controls ($p \leq 0.001$) whereas zinc (Zn), chromium (Cr), total iron binding capacity (TIBC) levels were decreased significantly in patients with CAD than controls. Analysis of levels of lipids of triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL) showed higher levels in patients with the exception of high-density lipoprotein (HDL). Siginificant decrease of the mean levels of antioxidant enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and vitamin E activity in patients with CAD and controls was found. Catalase (CAT) activity was moderately decreased. However, thiobarbituric acid reactive substances (TBARS) levels were significantly increased. Trace elements also decreased in patients in CAD than controls. The results obtained indicated that deficiency or suboptimal levels of trace metals and antioxidant enzymes may contribute to the increased susceptibility to CAD development that can be substantiated

with the above results which show a significant difference of the levels detected between in patients and controls.

Keywords: Trace elements; Antioxidant enzymes; Coronary artery disease.

Introduction

CAD is one of the major health problems responsible for increasing mortality and morbidity in Indian subcontinent as well as in ethnic Indian communities all over the world [1]. In the U.K, which has one of the highest mortality rates of CAD, the prevalence of symptomatic CAD in Asian Indians is similar to Whites (8.5% versus 8.2%), but the asymptomatic or silent CAD is high. In US, the prevalence of CAD in Asian Indians is 4-fold higher than Whites (10% versus 2.5 %) [2]. In recent years, awareness has created on the very important roles of trace elements that play either beneficial or harmful in human health and disease. The search for its etiology has led to some theories that dietary intake of minerals; in particular, trace elements may have a role in the progress of atherosclerosis [3-4]. The deficiency or excessive intake and uncontrolled homeostasis of some of these elements may lead to cardiovascular mortality.

Some authors have reported low Zn and high Cu levels in patients with atherosclerosis [5]. Many research evidences have linked low dietary chromium with disturbances in lipid metabolism that has led to the development of arteriosclerosis [6]. In another report, chromium levels were found significantly lower in the serum of individuals with CAD when compared to healthy controls [7]. In the Finnish cohorts, high amounts of copper in the drinking water were found to be associated with 10-year mortality rates due to CAD [8]. The first report on correlation between caeruloplasmin and cardiovascular disease was shown by Adelstein et al (1956). in patients after suffering from heart attacks that there was an increase in serum caeruloplasmin. Similar reports have been found in patients with a multitude of cardiovascular disorders such as atherosclerosis, abdominal aortic aneurysms, angina and peripheral arterial disease. Many studies have showed that an elevated level of serum LDL was associated with accelerated atherosclerosis which was found only in the subjects with higher levels of serum copper [9].

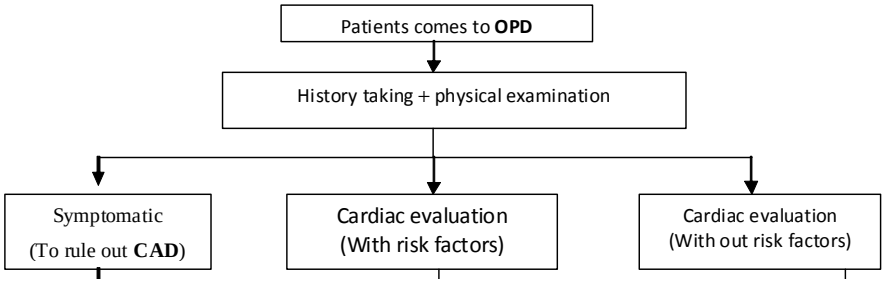
Sullivan (1981) [10] proposed that storage of systemic iron is positively related to inhibition of coronary heart disease (CHD) risk. A report stated that the subsequent production of free radicals modified the low density lipoprotein cholesterol, which was important in the development of atherosclerosis. In this disease prognosis, iron helps to catalyze the oxidation reactions that produce free radicals [11]. Trace elements like Se, Zn and Cu have an antioxidant role in many essential enzyme systems [12]. Antioxidant nutrients are believed to play a role in the prevention and treatment of a variety of chronic diseases ranging from asthma to CVD and cancer. The proposed mechanism by which antioxidants detering cells from oxidative stress is by scavenging free radicals and halting lipid peroxidation chain reactions which can cause DNA damage [13].

Several researchers have indicated that the development of atherosclerosis is related to free radical processes, lipid peroxidation and oxidative modifications of LDL. Free radicals may arise from a number of sources and cellular mechanisms and their over production occurs under pathological circumstances. Fortunately, the cell possesses highly efficient protective mechanisms that include antioxidants, metal-binding proteins and enzymes which combat the effects produced by oxidative stress. [14]. All these mechanisms are designed to prevent the occurrence of free radical-induced injury under normal conditions. However, it has been argued that these protective mechanisms may be overwhelmed and severe free radical-mediated injury may occur under pathological circumstances. In the present investigation, we studied the levels of serum metals such as copper, zinc, chromium, iron, ferritin, transferin and its relation with the serum lipid profile. We also studied the enzymatic antioxidant capacity in human erythrocyte cells' superoxide dismutase, catalase and glutathione peroxidas activities. We determined the extent of vitamin-E in order to verify the possible correlation between the cellular enzymatic antioxidant capacity and the degree of membrane lipid peroxidation. The aim of this study was to evaluate the status and the interrelationships of the different metals and antioxidant enzymatic activities in CAD patients in comparison with controls.

Subjects and Methods

Study sample

The present study was prospectively selected 45 CAD cases (33 males and 12 females) and 25 control (17males and 8 females) subjects with ages ranging from 35-65 years. The mean percentage of stenosis was then calculated and the patients were divided into two groups based on stenosis percent (> 50% and < 70%). All of our patients had lesions with > 50% stenosis showing in the study design (figure 1) and all patients had CAD confirmed by coronary angiography. Angiography reports were studied and stenosis percentages of the four major vessels, right coronary artery (RCA), left circumflex coronary artery (LCX), left anterior coronary descending aorta (LAD), and left main coronary artery (LM) were evaluated. The control group subjects were instructed not to take any cholesterol lowering drugs and to stop taking β-blockers or calcium channel blocker 3 days before the exercise test. Five ml of venous blood sample were taken immediately before and half an hour after finishing the exercise test.



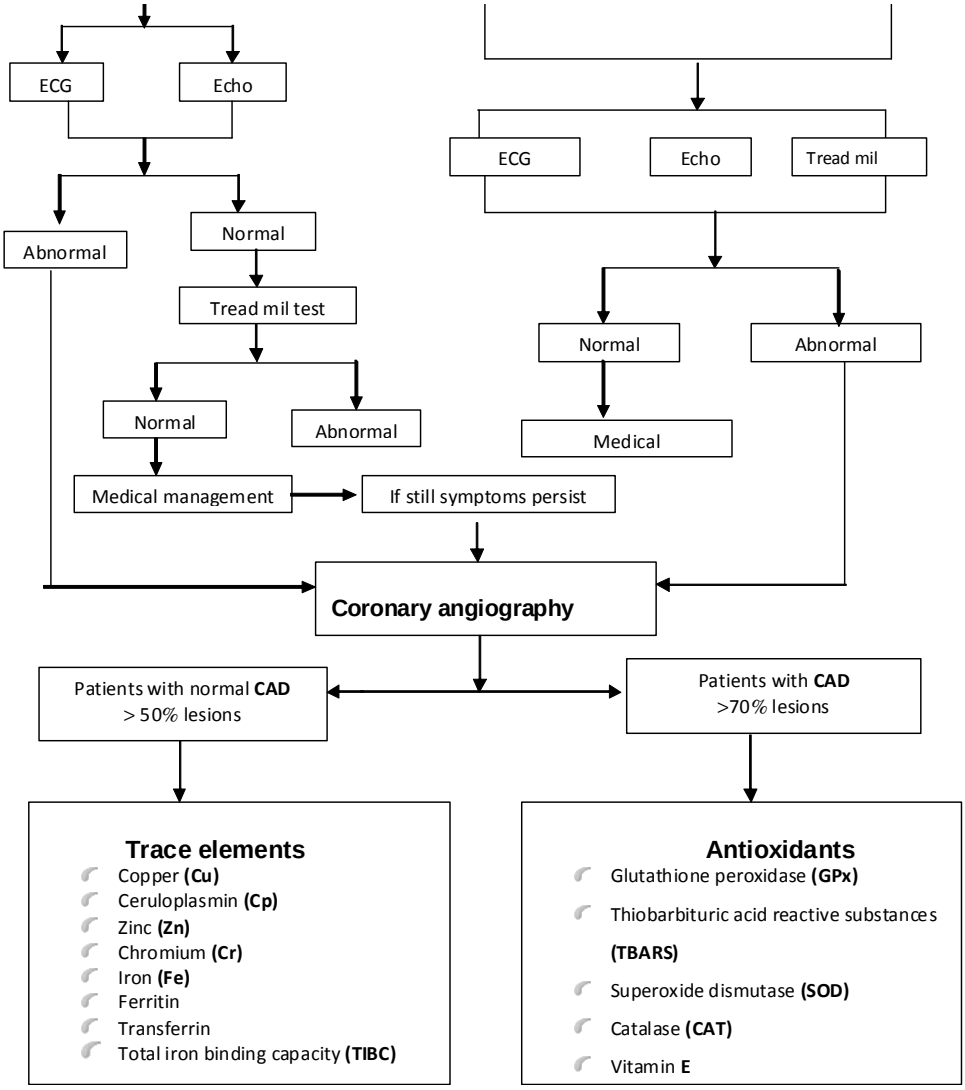


Figure 1: Study Design.

Data Collection

We collected information in regard to the presence of categoric cardiac risk factors in every patient. Risk factor data were derived from patient interview, referring physician contact and existing medical record data. Systemic arterial hypertension was defined as documented history of high blood pressure or treatment with medication, diet, and/or exercise. A history of current smoking or cessation of smoking within 3 months before testing was defined as positive smoking status. Hypercholesterolemia was determined on the basis of the answers to the following questions: “Has your physician ever told you that you need medications for high cholesterol?” “Are you currently taking cholesterol medications?” Answers to these questions often identified patients who were currently receiving cholesterol-lowering medications. Individuals were classified as having diabetes mellitus if they had

received a previous diagnosis of diabetes mellitus that was determined with blood glucose levels or if they had received treatment with insulin or oral hypoglycemic agents.

Trace elements analysis

Blood samples were collected in metal-free tubes from patients and controls after an overnight fast. Sterile, disposable plastic syringes were used. Blood samples were transferred immediately and carefully to clean trace element polypropylene tubes, left to clot and the serum was separated by centrifugation. Serum was divided into several polypropylene tubes, stoppered, labeled and stored at –86 ºC until analyzed. Care was taken to protect the samples from light. Serum samples were diluted with deionized water and then, added 3-5 ml of conc nitric acid. This was allowed to digest in the boiling water-bath until the contents of the tube were almost dry. The remaining organic matter was destroyed by heating with 1 ml of conc sulphuric acid and perchloric acid (about 3 ml). The contents of the tube were diluted to 10 ml with distilled water and then added 5 ml of 4 % sodium pyrophosphate solution. The solution was treated with ammonia until slightly alkaline to litmus and then placed in the waterbath at 80ºC for 15 minutes. After cooling, 5 ml of amyl alcohol were added followed by 0.5 ml of 2 % aqueous sodium diethyldithiocarbamate and the whole was well shaken. Serum copper, zinc and chromium levels were determined by atomic absorption spectrophotometry (AAS-UNICAM 929). Serum iron, total iron-binding capacity, transferrin and ferritin were measured by the modified automated Technico AAII-25 Method, which was based on the procedures[15].

Biochemical Analysis

Five ml of venous blood were withdrawn from each subject just before the exercise test. The samples were centrifuged for 15min at 3000 rpm in order to provide an appropriate amount of serum to use in measuring the biochemical tests which included: TC, TG, HDL and LDL. Serum TC and TG were measured by methods routinely used in medical-biochemical laboratories (Enzymatic PAP-method) in the Olympus AU 600 Analyser (Olympus Mishima Co., Shizuoka, Japan). High-density lipoprotein cholesterol (HDL-C) was measured with the direct method based on selective inhibition of the non-HDL fractions by means of polyanions.

Antioxidant enzyme analysis

Venous peripheral blood samples (10 ml) were collected in EDTA-coated tubes and centrifuged for 20 min at 3000x g. The supernatant diluted in the buffer was used as the enzymatic extract. RBC was washed three to four times with isotonic saline. Centrifugation for each wash was done at 1400x g at 4°C. After the final wash, the red blood cells were lysed by hypotonic shock [16] and different dilutions were used as hemolysate. Analysis of GPHx, SOD, TBARS, catalase, and vitamin-E were also carried out. The absorbance of the mixture was determined using a spectrophotometer (Shimadzu UV-160 A) against a blank without the sample.

Statistical analysis

All data analyses were performed using the SPSS Version 12.0. Data were double entered and the resulting data sets were compared and checked for completeness and accuracy of entry. The significance of differences between the mean for the two groups was assessed with Student's t-test and the significance of differences in proportions was tested with the chi-square statistic. Differences in Vessel diseases were tested using ANOVA. A p value <0.05 was considered to be statistically significant Descriptive statistics were applied for calculating the distribution of various characteristic estimate.

Results

Level of serum metals and lipid profile in coronary artery disease

Trace elements, which play a vital role in health and disease, have been the subject of several investigations concerning their role in the etiology of CAD in the recent years. Our study showed the increase of total serum trace elements such as Cu, Fe, Ferritin and transferin in patients than controls. However Zn, Cr and TIBC decreased in patients than normal subjects. Thus, the results of our study explored significance of the trace elements in CAD condition. Lower dietary consumption of Cu, Cr and Zn was associated with an increased risk of CAD and diabetes as well as associated risk factors including hypertension, hypertriglyceridemia and other factors.

An elevated level of TC is the strongest risk factor for CAD. We significantly ($p<0.001$) observed the lower serum concentration of HDL and higher concentration of LDL in patients (Table 1). This suggested that trace elements, nutritional and environmental factors can further increase the risk of CAD by virtue of acquired dyslipidemia in these genetically predisposed individuals. Our findings emphasized the importance of lipids and metals measurements in CAD patients.

Table 1: Serum metals and lipids in patients with CAD.

	Cu (µg/dl)	Cp (µg/dl)	Zn (µg/dl)	Cr (µg/dl)	Fe (µg/dl)	Fer (µg/dl)	Trans (µg/dl)	TIBC (µg/dl)	TG (mg/dl)	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Controls (N =25)	68.05 ± 17.26	396.6 ± 83.2	114.3 ± 4.07	1.97 ± 0.68	86.5 ± 15.85	144.0 ± 82.3	23.5 ± 13.2	296.25 ± 18.8	93.5 ± 39.2	157.7 ± 30.1	93.1 ± 4.69	44.4 ± 5.61
CAD Patients (N =45)	90.75 ± 30.46	425.0 ± 96.0	59.6 ± 16.3	1.22 ± 0.56	132.9 ± 23.6	216.0 ± 175.8	43.2 ± 30.46	260 ± 67.25	213.7 ± 120.0	203 ± 72.8	118 ± 5.68	32.0 ± 7.90
P value	0.001	0.05	0.001	0.001	0.001	0.001	0.01	0.01	0.001	0.01	0.001	0.001

Values are expressed as mean ± SD, n = 25(control) n = 45 (CAD) P values difference between the control and CAD.. Statistical significant difference in two groups P < 0.001. n= number of cases; Cu: copper; Cp: ceruloplasmin; Zn: zinc; Cr: chromium; Fe: iron; Fer: ferritin; Trans: transferin; TIBC: total iron binding capacity; TG: triglyceride; TC: total cholesterol; LDL-C: low-density lipoprotein; HDL-C: high - density lipoprotein.

Table 2: Antioxidant enzymes in CAD patients and control.

Parameters	Normal subjects (n =25)	CAD patients (n = 45)	*P value*
Glutathione peroxidase (U/g Hb)	35.77 ± 16.60	27.96 ± 8.44	P ≤ 0.001
Thiobarbituric acid reactive substances (U/g Hb)	27.52 ± 0.81	35.28 ± 13.15	P ≤ 0.01
Superoxide dismutase (U/g Hb)	574.1± 173.2	244.5 ± 57.4	P ≤ 0.001
Catalase (U/g Hb)	131.10 ± 13.82	102.47 ± 24.03	P ≤ 0.001
Vitamin E (U/g Hb)	15.3 ± 2.8	11.5 ± 2.3	P ≤ 0.001

Values are expressed as mean ± SD, n = 25 (control) n = 45 (CAD) P values difference between the control and CAD statistical significant difference in two groups $P < 0.01$ and $P < 0.001$.

Effects of antioxidant enzymes on CAD

In this study, we investigated the possible involvement of antioxidant enzymes in the regulation of CAD. The level of GPx was found to be 27.96 ± 8.44 (U/g Hb) and 35.77 ±16.60 (U/g Hb) in patients and controls respectively and GPx significantly decreased in CAD patients compared to control. CAT activities were 131.10 ± 13.82(U/g Hb) and 102.47 ± 24.03(U/g Hb) in patients and controls respectively. The levels of SOD in patients and controls were 244.5 ± 57. (U/g Hb) and 574.1± 173.2 (U/g Hb) respectively. These antioxidants significantly ($p<0.001$) decreased. TBARS levels were 35.28 ± 13.15(U/g Hb) and 27.52 ± 0.81(U/g Hb) in patients and controls respectively and TBARS significantly ($p \leq 0.001$) increased in CAD patients compared to healthy subjects. Significant lower levels of vitamin E in patients 11.5 ± 2.3 (U/g Hb) than in controls 15.3 ± 2.8 (U/g Hb) were observed. The association of vitamin E with CAD remained inversely significant even with other coronary risk factors (sex, age, smoking, diabetes, family history, TC,, TG, HDL cholesterol and LDL cholesterol).

Discussion

Relationship between trace elements and lipid profile in CAD

Trace elements are being increasingly recognized as essential mediators of the development and progression of heart diseases. On theoretical grounds, trace elements may be protective against oxygen free radicals in the development of cardiovascular disease [17]. Dietary factors such as inadequate intake of vitamins, minerals and trace elements may contribute to the development of heart diseases. In addition, adding the adequate nutritive supply can prevent these diseases. The hypothesis of the current study is that elevated levels of antioxidant enzymes are associated with increased cardiovascular risk in patients with stable CAD.

Klevay [18] stateed that relative deficiency of dietary copper may be a factor in the development of CAD. It would therefore be important to define the copper status of populations that have large differences in CAD mortality. In the present study, serum copper levels showed significant increase in patients with CAD compared to control. Increased level of Cu may be due to rise in the copper-binding capacity of ceruloplasmin. In our study, serum Cu was positively related to TC, TG and LDL-C,

which suggested that high serum Cu levels may induce the development of CVD. The increase in Cu may also be due to injury and subsequent necrosis of myocardial cells [19]. In the present study serum TG, TC, LDL, levels showed significant increase in CAD patients than control. Serum HDL level decreased in patients. In general increased TG levels may have a genetic basis. Nutritional habits may increase serum triglycerides as high consumption of saturated fats and/or sucrose. Moreover, inherited or acquired abnormalities of lipoproteins especially very low-density lipoprotein cause alterations in triglyceride levels [20].

A decrease in the levels of Zn and HDL-C strongly suggested the role of Zn in cholesterol and lipid metabolism as observed by Wen *et al* [21]. Animal experimental evidence suggested that the dietary Zn/Cu ratio may be a significant factor in coronary heart disease [22]. Low Cr dietary has been shown to increase aortic lesions and should also be considered as one of several nutritional factors that influence these recognized risk factors for public health problems of cardiovascular disease, impaired glucose tolerance, elevated circulating insulin levels and elevated serum cholesterol [23]. In the present study CAD patients showed a decrease in serum zinc ($p \leq 0.001$) and Cr levels ($p \leq 0.001$) compared to control values.

Several epidemiological studies had shown that the level of body iron stores is positively correlated with the incidence of coronary heart disease in human populations (Lee-young chau, 2000). . Serum ferritin levels were significantly higher in patients with CAD compared with control groups (105 - 65 ng/ml versus 83 - 71 ng/ml) ($p < 0.01$). TIBC was lower in patients with CAD (333 - 62 7g/dl) versus 348 - 48 7g/dl), ($p < 0.05$). However, high ferritin concentrations may also be unhealthy [24]. There was a higher risk of myocardial infarction in those with serum ferritin concentrations > 200 mg/L and the risk related to ferritin may be increased in those with elevated cholesterol.

In the present study, it is clear that all the patients groups serum iron , ferritin and transferin levels of CAD was significantly different than that of control group. Total iron binding capacity levels in patients was also decreased. This decrease may be explained by infarction inducing a shift of serum iron into the reticuloendothelial system, which may initiate ferritin synthesis [25].

Nutrients with an antioxidant role

Sufficient levels of antioxidants are important to live with healthy condition for human being and most important for the prevention of diseases such as cancer and CAD. Low plasma levels of antioxidants as well as low intake of dietary antioxidants have been associated with an increased risk of atherosclerotic heart disease [26]. More recently scores of epidemiological studies have noted a lower risk of cancer and cardiovascular disease among persons whose diets included relatively large amount of vegetables and fruits [27]. A popular explanation, both within the scientific community and among members of the public, is that antioxidant vitamins in vegetables and fruits prevent carcinogenesis and atherogenesis by interfering passively with oxidative damage to DNA and lipoproteins [28].

Very recent studies indicated that higher intakes of vitamin E may reduce the risk of coronary heart disease in both women and men [29] prompting serious consideration of recommending the use of supplemental vitamin E and other so-called

'antioxidant vitamins' (vitamin C and p-carotene) as a therapeutic strategy for prevention of atherosclerosis . Low levels of vitamin E were associated with increased risk of CHD [30]. Significant inverse association of vitamin E and CHD was observed in studies conducted in India and abroad [31]. Other studies have shown that there is a decline in glutathione (GSH) concentration with increasing erythrocyte vitamin E concentration and this may indicate an increased utilization of GSH in the reductive regeneration of the vitamin E its peroxyl radical [32].

The antioxidants, which counter the attack of oxygen free radicals, include enzymes like SOD, GP and catalase and nonenzymic chain breaking antioxidants like vitamins A, E & C, glutathione and carotenoids. There are varying reports on the erythrocyte activities of the enzymes SOD and GP [33] reported an increased erythrocyte SOD activity in haemodialysis patients whereas Tomas *et al*., [34] reported a decrease. The present study showed that Glutathione peroxidase, vitamin E level was significantly decreased and thiobarbituric acid reactive substances, Superoxide dismutase, Catalase level was significantly increased in CAD patients compared to control.

Deficiency or suboptimal intake of micronutrients may play a role in the etiology of CHD. Although there have been some reports that trace elements do not play a role in atherosclerotic changes of arteries, this conflict may be due to differences in the analytical or methodological techniques. One recent study confirmed that heart failure is associated with decreased serum selenium and zinc element concentrations and increased serum copper element concentrations. These changes in certain trace elements (Se, Zn, Cu) may play an important role in the pathogenesis of myocardial damage in chronic heart failure regardless of their etiologies [35]. However, more investigation is needed to clarify the extent of changes in these essential elements associated wth the activity of antioxidant enzymes and it,could be a reliable diagnostic aid in CAD and the extent of changes having prognostic implications. In view of these findings, the most prudent and scientifically supportable recommendation for the general population is to consume a balanced diet with emphasis on antioxidant rich fruits and vegetables and whole grains, which is consistent with the current dietary guidelines of the American Heart Association [36].

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