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Biochemistry

A STUDY OF SERUM TRACE ELEMENT LEVEL IN PATIENT OF CORONARY ARTERY DISEASE

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ABSTRACT The present study was undertaken to determine the status of trace element in coronary artery disease. The samples were analysed by atomic absorption spectrophotometer. The findings revealed that the values of Serum Zinc and serum Selenium decreased in CAD patient in comparison to normal subject was found to be statistically significant P< 0.01. Whereas, the value of Serum iron increased in CAD patient in comparison to the control subjects which was statistically Significant P<0.01. The level serum Copper also increased in the CAD patient but the difference is not statistically significant P>0.05.

KEYWORDS : Coronary artery disease, Atomic absorption spectrophotometer, Zinc, Copper, iron, Selenium.

INTRODUCTION:

Coronary artery Disease (CAD) is the most common health problem in developed and developing countries. The disease continues to be the principal cause of death in the United States and Asia (1).

In Indian subcontinent there has been a rapid rise in CAD prevalence. In 1959, Padmawati reported the prevalence of CAD at 1% and this rose to 4.5% in the year 1975 and 7.9% in the year1996 in subjects age 20 years and above (2).

Coronary heart disease (CHD) are the generic designation for the four forms of cardiac diseases, i.e., angina pectoris, sudden cardiac death, myocardial infarction and chronic ischemic heart disease (3). A high saturated fat diet tends to raise the LDL cholesterol, and the oxidation of LDL cholesterol increases the free radical production and LDL gets converted to atherogenic oxidized form with a continued high level of oxidized lipid. Blood vessel damage to the reaction process continues and can lead to the generation of foam cells and plaque which ultimately leads to the symptoms of atherosclerosis (4, 5).

To prevent this mechanism our cells possesses antioxidant system. Many antioxidant such as copper-zinc superoxide dismutase (Cu-Zn SOD), glutathione peroxidase (GSH-Px) & catalase are present in the cell (6).

All these antioxidants are designed to prevent the occurrence of free radicals injury under normal condition. These antioxidants contain trace elements such as selenium, copper, zinc and iron (6).

So the trace element being increasingly recognized as essential mediators of development and progression of heart disease such as Keshan disease, heart failure, cardiomyopathy and atherosclerosis. Therefore, the trace element may play an important role in etiopathogenesis of the disease (9).

Some researcher speculate that Zinc and selenium decreased and iron is increased in coronary artery disease.(16,35).Some revealed that serum copper decreased in CAD but other findings speculate that serum copper is increased in CAD (9,32).

So, the present study has been designed to evaluate the status of trace element in patient of coronary artery disease and to confirm previously documented changes in the trace element status in coronary artery disease.

MATERIALAND METHOD:

Study design and Patients

The study was Case- Controlled in design, it included two groups, one with the 30 patients suffering from coronary artery disease and admitted in cardiology and intensive care Unit of S.M.S. hospital and medical college, Jaipur. Another group included 30 healthy volunteers. These control subjects were free from any abnormality of the vital organs. They were not taking any drugs or antioxidant

Sample Collection And Preparation:

Venous blood sample was systematically collected from the CAD patients shortly after their admission to the hospital and from the controls after an overnight fast.

The serum was separated and was directly used for studying the lipid profile. For the trace element study the serum was diluted with the deionized water, keeping the dilution factor to be 1:10 for Zinc and copper and 1:5 for selenium. But for iron, the blood samples were first diluted with a 20% w/v TCA solution and then were heated and cooled and were centrifuged, this precipitates plasma protein and removes 95% of any hemoglobin. The supernatant was then separated for estimation of iron.

Investigations Done

Biochemical investigations included the whole lipid profile in which triglycerides, phospholipids, HDL-C done by Loga tech reagent, Total Cholesterol done by Anamol and Total lipids were done by GPL kits. All these parameters were run on auto analyzer (Au400)/ Kopran. Zinc, Copper, iron and selenium levels were estimated on Atomic Absorption Spectrophotometer (hollow Cathode Lamp) ECIL AAS 4129/AAS4139/AAS4141.

RESULTS:

Table no.1: Comparative analysis of the trace element status between the Control and CAD patient.

	Control N=30 Mean ± S.D.	CAD patient N=30 Mean ± S.D.	P value
Zinc µg/L	618.8±12.98	551.82±12.19*	p<0.01
Copper µg/L	955±16.3	972±14.3	p>0.05
Selenium µg/L	144±10.5	124.7±8.5*	p<0.01
Iron µg/L	102.9±19.4	156.96±27.90*	p<0.01

- p≤0.05vs control is significant & * p≤0.01vs control is highly significant

Table 1 summarizes data on serum concentrations of selenium, zinc, iron and copper elements in the patients with coronary artery disease and in the controls. Patients with coronary artery disease had lower serum selenium and zinc concentrations than the controls (p<0.01). However, serum copper concentrations in coronary artery disease patients were higher but not significant than in the control group (p>0.05). Also, the serum concentration of iron were significantly higher in the patients of CAD than the control group (p≤0.01).

Table 2: Comparative analysis of the lipid profile between the Control and CAD patients

	Control group N=30 Mean ± S.D.	CAD group N=30 Mean ± S.D.	P value
TG mg/dl	121.2±21.79	176.3±16.99*	p<0.01
Total cholesterol mg/dl	192.1±16.69	225.9±24.25*	p<0.01
LDL cholesterol mg/dl	128.24±16.33	145.82±23.37*	p<0.01
HDL cholesterol mg/dl	44±8.06	41.95±4.47	p>0.05

- p≤0.05vs control is significant & * p≤0.01vs control is highly significant

Table 2 summarizes data on serum lipid profile in the patients with coronary artery disease and in the controls. Patients with coronary artery disease had significantly higher triglycerides, total cholesterol and LDL cholesterol compared to the controls (p<0.01).However, serum HDL concentrations in coronary artery disease patients were lower but not significant than in the control group (p>0.05).

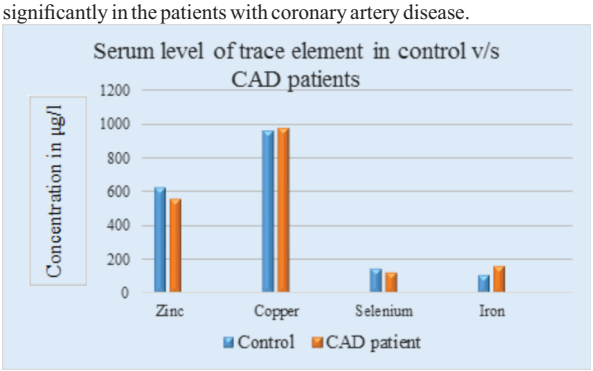
Statistical Analysis

All values are presented as mean±SD. Statistical significance was analyzed by Student t-test. The level of significance was set at P<0.05.

DISCUSSION:

In this study, the status of various trace elements and the lipid profile of the coronary artery disease patients have been compared with the normal healthy controls.

From the results it is evident that the trace element status changes significantly in the patients with coronary artery disease.



The Zn and Se levels decreased significantly in the Coronary Artery Disease patients compared to healthy controls. Zinc is mainly required for DNA synthesis, cell division and protein synthesis (8). Decrease in serum Zn levels have been found by several other workers, however the cause and mechanism resulting in decreased serum zinc concentration in CAD remain unexplained yet. Hypoxic damage of myocardial tissue may increase the activity of some endogenous mediators, which help the hepatic uptake and sequestration of circulating trace element (9). The excess release of steroids due to the release of leucocyte endogenous mediators which redistributes the body Zn from serum and may cause a drop in serum Zn and also due to elevated levels of macroglobulin which is a transport protein containing large amounts of Zn.(10). A decrease in the levels of Zn and HDL-C strongly suggests the role of Zn in cholesterol and lipid metabolism as observed by Wen Yong(10,11). In addition to its antioxidant and anti inflammatory properties (12-14).

In animal experiments, a diet low in copper and high in zinc leads to hypercholesterolemia and cardiovascular complications (16). Zinc-deficient diet increases concentrations of cholesterol and triglycerides in the VLDL and HDL fractions. Zinc supplementation decreased these lipid variables. Higher concentrations of glutathione reductase mRNA in the thoracic aortae were detected in the zinc-deficient mice. Furthermore, inflammatory markers, such as nuclear factor-kappaB and vascular cell adhesion molecule-1, were significantly increased in zinc-deficient mice. Thus, zinc deficiency induces proinflammatory events in an atherogenic mouse model and adequate zinc amount may also be a critical component in protective PPAR signaling during atherosclerosis(13,15,17). Zinc supplementation alone on amelioration of lipid peroxidation is more effective than that of copper supplementation alone in this atherosclerotic animal model. The effect of zinc nutriture on the antioxidant enzyme activities is unclear; nonetheless, the primary role of copper was also shown in cellular antioxidant capacity in rats treated with high dietary zinc intake (18,19) and with combined copper deficiency and iron overloading (20). It seems that zinc itself may exert 2 possible mechanisms to protect the lipid peroxidation, including: it may induce some compounds with antioxidant property such as metallothionein (21) shown to be in favor with the endothelial cell barrier functions that also may decrease the risk of LDL oxidation by inhibition of ceruloplasmin entry (22) and it may lower the serum iron concentration (23), possibly because of indirect effects reducing the activities of ceruloplasmin and hephaestin. Banik S and et al. elucidated the association of serum zinc level and coronary artery disease and The findings of the meta-analysis suggested that relatively low levels of Zn might have a potential role in the pathogenesis of CAD. Furthermore, large-scale observational studies are highly recommended in order to fully understand the association between Zn status and CAD (53).

The results also showed an increase (P> 0.05) in Cu concentration in CAD patients as compared to control group though not significant. Increased levels of Cu may be due to a rise in the copper-binding capacity of ceruloplasmin. The elevation in Cu concentration is described by other workers such as Leon et al (24,25) who studied zinc, copper and magnesium and risk cause for all cancer and cardiovascular mortality and Metwalli studied the serum metals and lipids profile in patients with acute myocardial infarction (26) and Issa N.M. studied serum levels of Zn, Cu, Cr, Ni in iranian subjects with athero sclerosis. The elevation in Cu concentration may be due to a rise in the copper – binding capacity of ceruloplasmin and the increase in Cu may be also due to injury and subsequent necrosis of myocardial cells (10). Serum Cu was positively related to TC, TG and LDL-C, which suggests that high serum Cu levels may induce the development of CHD (10). High serum copper may be connected with the development of the atherosclerotic process in the arterial wall.(27). In a population-based prospective study, high serum copper was found to be associated with an increased carotid artery tunica intima-media thickness evaluated by ultrasonography (28). High serum copper may function as a pro-oxidant and thus a free radical-promoting factor (27). It is equally likely that high serum copper and high serum ceruloplasmin may be associated with other, largely unknown, processes leading to vascular attacks. Many unspecific factors, such as intrinsic and extrinsic stress factors causing tissue injury, may contribute to an increase in ceruloplasmin as an acute-phase reactant protein (29). In other epidemiological studies, serum copper has been shown to be raised in patients with a history of acute myocardial infarction ,whereas serum zinc was found to be normal or decreased (30). On the contrary to this some studies also mention the role of reduced copper in hypercholestolemia (31,32). Experimental evidence suggests that the dietary Zn/Cu ratio may be a significant factor in coronary heart disease (10). The interaction between copper and zinc may modulate the genesis of atherosclerosis by influencing cellular antioxidant capacity and serum lipid profiles and that the inhibitory effects of zinc on atherogenesis are in association with copper (18). The exact mechanism of alteration of these trace elements is not known. Some factors such as dietary deficiency, anorexia and possible use of drugs may be major components (10). Some study revealed that Copper deficiency creates or impacts virtually every risk factor of IHD, such as hypercholesterolemia and glucose tolerance, and can increase chronic inflammation and oxidative stress, which are also thought to be important factors in cardiovascular disease risk (52).

Iron levels have been found to be significantly high in the CAD patients compared to the control groups. Because of the considerable iron-binding capacity of the carrier protein transferrin, free low-molecular-weight iron cannot be detected in plasma under physiologic conditions (25). Iron is stored in the ferrite form that is bound to apoferritin, a protein widely distributed throughout the body. To exert its catalyzing activity, the ferrite ion must first be released from the protein moiety and reduced to the ferrous form. Superoxide anions produced by oxidative stress and reducing agents have been found to be capable of mobilizing iron from ferritin (33,34). Iron could increase the risk of myocardial infarction through the elevation of blood haematocrit and hemoglobin levels. This in turn increase the viscosity of blood and has a direct trombogenic effect (35). Also the Experiments with iron chelators or iron overloading in animals (36) provided indirect evidence that iron is involved in reperfusion injury after an ischemic insult. During ischemia, the amount of iron in the low molecular weight pool was observed to increase (37), and iron accumulated in the perfusate when circulation was restored (38). Entailed iron-catalyzed redox reactions within the cardiomyocyte might be sufficient to elicit contractile dysfunction and lethal cell disruption (39). This possibility suggests that iron-catalyzed reaction involving partially reduced oxygen might exacerbate reperfusion injury, which would imply that high iron stores may affect the fatality rate of myocardial infarction apart from their effect on the incidence of myocardial infarction (36). The same findings also reported bysreekant k.and etal .The presence of iron in cytochrome, catalase, hydroxylase, peroxidase, lipoxygenases suggest iron has an important role in various metabolic events related to lipids, such as the oxidative degradation of fatty acid, prostaglandins and plasminogen (35).

Oxydation of LDL-cholestrol is catalyzed by iron present in atherosclerotic gruel. Iron may stimulate the oxidation of LDL cholesterol by catalyzing production of tissue-damaging free radicals (33,34). Lipid peroxidation of LDL cholesterol can be enhanced by metal-catalyzed reactions, resulting in highly reactive hydroxyl radicals. Recently, hemin also has been shown to promote LDL cholesterol oxidation very efficiently in vitro (40). Further evidence suggesting that heme iron plays a role in lipid peroxidation comes from the results of a nested case-control study showing a positive association between blood hemoglobin concentration and the titer of autoantibodies against malondialdehyde-modified LDL cholesterol (41) which indicates its role in the risk for myocardial infarction.

Selenium is important in the active centre of the Se dependent GSH-Px. This enzyme has four subunits and each contains one selenium atom. It was suggested that selenium protects cell by inhibiting free oxygen radical production. Moreover, an important antioxidant Vit. E is transported by selenoproteins (8,42). Statistical analysis has shown that low plasma selenium levels was an independent risk factor for CAD, this is in accordance with other studies which reported that low concentrations of selenium (Se) are associated with coronary risk factors, including body mass index, serum HDL-C and CRP and high concentrations of serum Se predict reduced levels of oxidative stress and subclinical cyclooxgynase-mediated inflammation (43). The associations between Se, oxidative stress and inflammation, respectively, might be related to the proposed cardiovascular protective property of Se (43,44). The low antioxidant levels could be attributed to different mechanisms. Endothelial damage and the increase in polymorphonuclear leukocyte activity which occur during atherogenesis lead to an overproduction of reactive forms of oxygen, which in turn exhaust the anti-oxidative pool of the organism. Moreover, the decreased physical activity of CAD patients has further influence on SOD and GPx activities. GPx was shown to inhibit 5-lipoxygenase in monocytic cells which is induced in monocytes and macrophages within progressing atherosclerotic lesions (45). GPx deficiency leads to increase cell-mediated oxidation of LDL and endothelial dysfunction combined with structural vascular abnormalities, such as increased periadventitial inflammation, neointimal formation, and collagen deposition surrounding the coronary arteries (46). Some workers reported reduced levels of GSH and GPx in CAD patients (47,48). Blankenberg reported that low erythrocyte glutathione peroxidase activity identifies patients with coronary artery disease who are at the highest risk for cardiovascular events (49). Also according to some other studies the patients suffering from AMI exhibit lower plasma concentration of selenium. The data showed that selenium deficiency not only increases the risk of coronary artery diseases, but also increases the risk of acute events (50). The association between serum selenium concentration and the risk of cardiovascular disease remains uncertain but these changes may also be attributed to inadequate dietary intake of trace element and concomitant therapy agent such as diuretics or by plasma expansion due to water ingestion (12). Oruc M, Mercan S studied 35 patients (male, 60%) with a mean age of 45.7 ± 10.4 years and 35 controls . CHD patients showed significantly lower levels of selenium and uranium and higher cadmium (Cd), cobalt, lithium, manganese, nickel, lead, platinum, tin, strontium, and thallium levels compared to controls. Thus, Trace elements are known to have a key role in myocardial metabolism.

CONCLUSION:

In conclusion, this study confirmed that coronary artery disease is associated with decreased serum Se and Zn element concentrations and increased serum Fe and Cu element concentrations.

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