EFFECTS OF TRACE ELEMENTS AND ANTIOXIDANT STATUS IN SUBJECTS WITH CORONARY HEART DISEASE

Chikkanna Da,*, Thammanna Gowda SSb, Dinesha Rb, Harsha Rb, Chethan kumar Mc

a - Department of Biochemistry, Adichunchanagiri Institute of Medical Sciences and Research Center, B.G. Nagara, Mandya, Karnataka -571 448.
b – Adichunchanagiri Biotechnology and Cancer Research Inst, B.G. Nagara, Mandya, Karnataka -571 448.
c – J.S.S college of arts, commerce and science, Ooty rd, Mysore, Karnataka.

Summary

Oxidative damage is known to cause deleterious effects on cellular function leading to a number of disease conditions. Involvement of oxygen radical has been suggested in many clinical conditions including Coronary Heart Disease (CHD). The present investigation describes the lipid profile, trace element profile, SOD, catalase activity and lipid peroxidation in CHD patients in the age group between 40-60 years. The concentration of cholesterol, LDL, TG, and VLDL were found to be increased when compared to the control, where as the levels of HDL decreased, when compared to the control. Concentration of Iron, Copper and Albumin Cobalt binding Absorbance (ABSU) were elevated in CHD as compared to the control. Total Iron Binding Capacity (TIBC) and Zn were found to be decreased in CHD. Lipid peroxidation as measured by the level of MDA was increased, where as SOD and catalase activities was decreased in CHD condition.

Key words: Trace elements, Lipid peroxidation, Coronary Heart Disease, Superoxide dismutase, Catalase.

Abbreviations: ROS, Reactive Oxygen species; LDL, Low Density Lipoprotein; SOD, Superoxide Dismutase; CHD, Coronary Heart Disease; HDL, High Density Lipoprotein; TG, Triglycerides; VLDL, Very Low Density Lipoproteins; LPO, Lipid Peroxidation; MDA, Malonaldehyde; TIBC, Total Iron Binding Capacity; ABSU, Albumin-Cobalt Binding Absorbance Units.
**Introduction**

Oxidative stress occurs due to either increased production of reactive oxygen species (ROS) or decreased levels of antioxidants (enzymatic or non enzymatic) or both\(^1\). Oxidative stress is implicated in many etio patho-genetic diseases like Cancer, Atherosclerosis, Cataract, Diabetes mellitus, Alzheimer’s disease, Parkinson’s disease and so on\(^2\). Free radicals arise under both normal and pathological circumstances from a number of source and cellular mechanisms. The cells possess highly efficient protective mechanisms, including antioxidants such as \(\alpha\)-tocopherol, ascorbate, \(\beta\)-carotene, Glutathione and ceruloplasmin and enzymes such as manganese superoxide dismutase (Mn SOD), Copper-Zinc superoxide dismutase (Cu-Zn SoD), Seleno enzymes, Glutothione peroxidase (GSHPX) and Iron containing enzyme catalase\(^3\).

The process of LDL oxidation has been repeatedly demonstrated to be dependent on high concentrations of transition metals such as copper, Iron, etc in-vitro\(^4,5\). Copper mediated oxidation is frequently used to assess the susceptibility of LDL to oxidation in vitro, which is known as a possible risk factor for atherosclerosis. Copper is also a constituent of Cu-Zn SOD, which is involved in preventing oxidative injury\(^6\). Other than copper, Iron is also an strong oxidant, catalyses LDL oxidation in vitro. Iron might contribute to coronary artery disease\(^7,8\).

Zinc, an essential component of Cu-Zn SOD, plays a crucial role in CHD. The deficiency of Zn could induce an increase in tissue oxidation damage. Zn deficiency is also associated with an increase of Cu and Fe due to the antagonistic relationship between these metals\(^9\). An imbalance between Cu and Zn may be an important factor in the etiology of CHD.

The acute CHD shows a reduced Co binding capacity to albumin in the serum\(^10,11\). This decreased binding reflects changes to the NH\(_2\) terminal of albumin, binding site for the transition metals as Co (II), Cu (II) and Ni (II)\(^10\). Trace elements are required in small amount as an essential components of antioxidative enzymes\(^12\), while cytoplasmic Cu-Zn SOD enzyme contains copper and zinc metals as cofactor, whereas GSH-Px and catalase enzyme contain selenium and Iron respectively\(^13\).

Chromium is an essential micro nutrient, complexes with niacin and glutamic acid termed as glucose tolerance factor, where it acts as a cofactor of insulin in all insulin dependent system\(^14\) and also plays a very important role during lipid metabolism. The association of Chromium deficiency with abnormal carbohydrate metabolism has been the subject of study in assessing the chromium status in diabetes mellitus.

Manganese is an essential trace element in biological systems. Manganese dependent enzyme is found within diverse locations in the cell including the golgi, mitochondria and cytoplasm. However, high concentrations of manganese are potentially toxic. Exposure at higher concentration may lead to Manganism, Parkinson’s disease\(^\text{15, 16}\). Manganese is most important mineral when trying to stabilize blood sugar level, particularly in hypoglycemic individuals, and for lowering total cholesterol (Cholesterol lowering drugs actually raise manganese).

Nickel chloride is considerably less toxic. Nickel (II) chloride induces coronary vaso constriction in the dog heart in situ and in isolated perfused rat heart\(^17\). In sufficient levels of nickel, cobalt, vitamin B 12, Vitamin B6, folate and other factors may lead to increased levels of
homo cysteine that can damage blood vessels and lead to an increased risk of heart disease and stroke. Nickel is a well-established carcinogen both in humans and in experimental animals\textsuperscript{18, 19}. It is also toxic towards the pancreas. Acute injection rats produce hyper glycemia and hypo insulinemia\textsuperscript{20}, which may be caused by the induction of nitric oxide synthase. Most plasma nickel is a constituent of the circulating proteins nickeloplasmin and albumin and it is also thought to be a factor in hormone, lipid and cell membrane metabolism Insulin response is increase after ingesting nickel, which may be related to its activation of enzymes associated with the breakdown or utilization of glucose.

Cobalt specifically affects the right coronary artery, resulting in vasodilation with low levels, and vaso constriction with high levels, while nickel exerts the same effect on the left coronary artery\textsuperscript{21}.

In humans, the serum levels were found to be elevated in patients with unstable angina or with acute myocardial infarction, but not in patients with atherosclerosis\textsuperscript{22}. Cobalt appears to exert mixed effects on the cardiovascular system of mammals. In humans, elevated Cobalt exposure resulted in elevated mortality from ischemic heart disease, partially prevented the cardiovascular lesions caused by Co and or Vit B12 deficiency Co is essential components of vitamin B\textsubscript{12}. Therefore studies were undertaken to assess the levels of trace elements and antioxidant status in normal and CHD patients.

**Methods**

Cholesterol, thiobarbituric acid, dithiothreitol, SOD, Catalase, Cobalt, Batho phentroline disulfonic acid, Sodium diethyl dithio carbonate, Dithizone, Dithiothreitol, Nitro blue tetrazolium and Riboflavin were purchased from Sigma Chemical. All other chemicals used were of analytical grade.

**Experimental design**

The experiment were carried out in two groups, one is control and the other being experimental group. The number in each group is 40 (n=40). The control group consisted of normal male individuals in the age group of 40-60 years, selected from the nearby villages of B.G.Nagara, Nagamangala Taluk, Mandya dist, Karnataka, India. Male patients in age group, 40-60 years admitted as inpatients to Adichunchanagiri Institute of Medical Sciences and Research Centre, clinically diagnosed were selected for the studies with their consent. Information regarding general history of the patients with their daily diet and occupation was recorded. Blood samples both from the control group and the experimental groups were collected for the determination of Trace element like Iron, Copper and Zinc, Total Iron binding capacity (TIBC), Lipid profile (Total cholesterol, LDL cholesterol, HDL cholesterol and Triglycerides), Lipid peroxides (Malondialdehyde), Antioxidant enzymes like SOD and Catalase, and also binding capacities of the elements such as Cobalt, Nickel, Chromium and Manganese with albumin.
Estimations

Total cholesterol was measured by the method of Zalkits et al\textsuperscript{23}. Serum Tri glycerides was estimated according to the methods of Gowan et al\textsuperscript{24}. HDL Cholesterol was measured by the method of Burstein et al\textsuperscript{25}, Copper and Zinc were determined according to the methods of Eden et al\textsuperscript{26}. Cobalt – albumin binding assay as described by David Bar–or et al\textsuperscript{10}. Serum Lipid peroxidation is measured by malondialdehyde (MDA) as per the method of Okawa et al\textsuperscript{27}. Hemoglobin content of the erythrocytes was determined by cyanmethemoglobin methods of Drabkin et al\textsuperscript{28}. SOD and Catalase activity of the hemolysate were determined by the method of Mc Cord and Fridovich\textsuperscript{29} and Brannan et al\textsuperscript{30} respectively.

Statistical analysis

The values obtained from 40 subjects in each group were expressed as mean ± standard deviation (S.D). The significant difference between control and experimental subjects were determined by student’s Newman Keuls Test. One way analysis of Variance (ANOVA) was done to compare the mean level between control and experimental subjects.

Results and Discussion

Total cholesterol, LDL, TG, HDL, VLDL and Iron, Copper, Zinc and Cobalt albumin binding capacity, Lipid peroxidation were measured in two groups consisting of 40 individuals aged between 40 – 60 years, one group consisting of healthy individuals served as control and the other group consisting of individuals with coronary disease of the same age group served as a experimental group.

In the present study the concentration of cholesterol, LDL, TG, and VLDL are found to be increased as compared to the control, where as concentration of HDL decreased compared to the control (Table 1). Increase in the level of cholesterol in particular LDL cholesterol undergoes oxidation resulting in oxidized LDL in atherosclerosis. According to Kathy et al\textsuperscript{31} the level of oxygen free radicals is increased in hypercholesterolemia and would cause endothelial damage leading to the development of atherosclerosis.

Table 1 – Serum level of cholesterol, HDL, LDL, TG & VLDL in normal and coronary heart disease patients.

<table>
<thead>
<tr>
<th></th>
<th>Control Mean ± SD (40-60 years n=40)</th>
<th>Coronary heart disease Mean ± SD (40-60 years n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol mg/dl</td>
<td>182.00 ±16.65</td>
<td>275.1 ± 5.45</td>
</tr>
<tr>
<td>HDL mg/dl</td>
<td>40.00 ± 2.28</td>
<td>29.1 ± 1.79</td>
</tr>
<tr>
<td>LDL mg/dl</td>
<td>113.97± 17.66</td>
<td>206.1 ± 7.06</td>
</tr>
<tr>
<td>TG mg/dl</td>
<td>141.60 ± 9.53</td>
<td>199.8 ± 37.0</td>
</tr>
<tr>
<td>VLDL mg/dl</td>
<td>28.04 ± 1.84</td>
<td>39.89 ± 4.35</td>
</tr>
<tr>
<td>SOD units/gm Hb</td>
<td>4736.4 ± 1210.13</td>
<td>2150.60±552.01</td>
</tr>
<tr>
<td>Catalase units/gm Hb</td>
<td>4604.60±191.84</td>
<td>3161.70±426.8</td>
</tr>
<tr>
<td>LPO in μmole (MDA)</td>
<td>3.07 ± 0.24</td>
<td>6.43 ± 1.54</td>
</tr>
</tbody>
</table>

P < 0.05 significant. HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, TG: Triglycerides, VLDL: Very Low Density Lipoprotein.
Lipid peroxidation is increased in coronary heart disease when compared to control. SOD and catalase activities are decreased in subjects with coronary heart disease compared to the control (Table 1). In the present study, it is observed that the activity of antioxidant enzymes SOD and Catalase are lower in Coronary heart disease than the normal subjects (Table 1).

It is well known that reactive oxygen metabolites such as hydrogen peroxide and super oxide anion increases in atherosclerosis and hydrogen peroxide inactivates SOD and Superoxide anion radical inactivates Catalase. A decrease in SOD activity in patients with coronary heart has been reported earlier\textsuperscript{40}. This may due to increased production of Reactive oxygen species as MDA. Buczynski et al\textsuperscript{41}, have also showed an increase in platelet MDA and Thromboxane A\textsubscript{2} and decrease in SOD and Catalase activities. This decrease in SOD activity as observed in the present studies may be explained with the effect of increased oxygen derived free radicals on SOD. It is known that, lower oxygen concentrations induces the SOD activity while higher oxygen concentrations inhibits SOD. Furthermore Catalase is activated in higher H\textsubscript{2}O\textsubscript{2} concentrations while SOD is inhibited. Absorbance units of cobalt is higher compared to Nickel, Chromium and Manganese in CHD when compared to normal may be due to increased free radicals in the presence of transition metals which causes structural changes in intact human serum albumin with reduced cobalt binding capacity\textsuperscript{4}.

Hypercholesterolmia, increased oxygen free radicals, increased LPO, decreased antioxidant enzyme and endothelial dysfunction acting in tandem causes atherosclerosis. Concentration of Iron, copper and Albumin Cobalt binding Absorbance (ABSU) are elevated in coronary heart disease when compared to the control, whereas concentration of TIBC and Zn were found to be decreased in coronary heart disease (Table 2).

### Table 2 – Serum level of Iron, Total iron-binding capacity, Copper and Zinc in normal and Coronary Heart Disease

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Coronary heart disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron µg/ dl</td>
<td>157.67 ± 5.40</td>
<td>252.64±52.76</td>
</tr>
<tr>
<td>TIBC µg/ dl</td>
<td>223.44± 27.82</td>
<td>193.82± 53.24</td>
</tr>
<tr>
<td>Copper µg/ dl</td>
<td>139.57 ± 3.63</td>
<td>178.88 ± 13.59</td>
</tr>
<tr>
<td>Zinc µg/ dl</td>
<td>83.78 ± 5.60</td>
<td>45.87 ± 5.71</td>
</tr>
</tbody>
</table>

Table shows the level of Iron, Total iron-binding capacity, Copper and Zinc in blood serum in control and CHD patients. All the patients were between the age group of 40 – 60 years.

Divergent information is available on the relationship between body Iron and Coronary heart disease\textsuperscript{32}. It has been shown that the concentration of body Iron store attributed as a strong predictor of Coronary heart disease in eastern Finnish men\textsuperscript{33}. Increased levels of Copper may be due to rise in the copper binding capacity of ceruloplasmin. In present investigation, serum copper was positively related to TC, TG, LDL-C, which suggests that high serum Copper levels.
may induce the development of Coronary heart disease. The increase in Cu may also be due to injury and subsequent necrosis of myocardial cells\textsuperscript{34}. The status of Zinc has been shown to have an important role in the metabolism of cholesterol and HDL, two important molecules involved in the disease\textsuperscript{35}. Animal experimental studies suggest that the dietary Zn/Cu ratio may be a significant factor in coronary heart disease\textsuperscript{36}. The exact mechanism of alteration of these trace elements is not clear. Some factors such as dietary deficiency, anorexia and possible use of drugs may be major components. Besides, these factors, low serum Zn level has been related to excess release of steroids due to the release of leucocytes endogenous mediators which redistributed in the body Zinc from serum and may cause a decrease in serum Zn and also due to elevated levels of macroglobulins a transport protein containing large amounts of Zn\textsuperscript{37}. Elevated serum copper levels have been suggested as a risk factor for cardiovascular disease\textsuperscript{38}. Reunanen et al\textsuperscript{39} have reported that high serum copper and low serum Zn are associated with cardiovascular mortality.

In case of subjects with coronary heart disease the findings on metal binding capacity is shown in Table 3. Cobalt has more minding capacity to albumin in CHD patients than other Trace elements like nickel, chromium and manganese. Cobalt binding capacity of albumin has been reported by several research workers\textsuperscript{10} and it is taken as an index of lipid peroxidation and it is known to cause several pathological disorders.

Table 3 – Albumin binding capacity of trace elements in Normal and Coronary Heart Disease (CHD)

<table>
<thead>
<tr>
<th>Trace elements</th>
<th>Control</th>
<th>Coronary Heart Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td></td>
<td>40-60 yrs</td>
<td>40 – 60 yrs</td>
</tr>
<tr>
<td>Cobalt (Results expressed as optical density), \textit{P}&gt;0.05 Not Significant</td>
<td>0.211 ± 0.013</td>
<td>0.824 ± 0.082</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.246 ± 0.040</td>
<td>0.306 ± 0.061</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.239 ± 0.032</td>
<td>0.203 ± 0.055</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.192 ± 0.012</td>
<td>0.185 ± 0.052</td>
</tr>
</tbody>
</table>

Binding of albumin to different trace elements like Co, Ni, Cr, Mn were checked in both control and CHD patients, Cobalt was found to be more affinity towards albumin in CHD.

It is observed from the above findings that the albumin binding capacity of trace elements, activity of antioxidant enzymes and lipid peroxidation can be used as markers in coronary heart disease.

Acknowledgement

The authors wish to thank Sri Adichunchanagiri Shikshana Trust for the financial support. Dr. Rajagopal, Principal., AIMS, B.G. Nagara for their support. Authors also acknowledge, Director, ABCRI, B.G.Nagara, Karnataka For kind help and support.
References

42. David AM, James AL, Marc SS, Elliott MA. The Search for a Biomarker of Cardiac Ischemia , Clinical Chemistry 2003;49:537-539.