

## Measurement and Evaluation of Nutritional Antioxidant Status in Atherosclerotic Cardiovascular Disease

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*Cardio vascular Disease (CVD) is one of the most important degenerative diseases of arteries. Free radicals have been implicate in the aetiology of aging and disease relate to aging such as cancer, CVD and arthritis. Diet variety of antioxidant food play essential in the prevention and treatment of many diseases in the middle age people. The human body has several mechanisms to counter out damage by free radicals and other reactive oxygen species. The oxidative modification is through to play a crucial role in objective of this study is the inhibition of atherosclerosis. We propose to estimate the effects Zinc, Copper, Magnesium And Vitamin C on Cardio vascular Disease patient were investigated. Body's antioxidant system depends on the nutritive antioxidant vitamins that can protect or may reduce the risk of atherosclerotic disease by protecting from oxidative modification of early atherosclerotic lesions.*

*Keywords: Cardio Vascular Disease, Atherosclerotic, Nutritive antioxidant*

### INTRODUCTION

Cardiovascular Disease is the most prevalent Complication of diabetes Mellitus. A large proportion of diabetic subjects continue to have uncontrolled risk factors of coronary disease such as hyperglycemia, obesity, hypertension and Dyslipidemia (1, 2). Antioxidant plays a protective role in the pathophysiology of diabetes and cardiovascular disease. People with metabolic disorder have high risk for developing diabetes and Cardiovascular Disease. (3, 4, 5).

Native Low Density Lipoprotein cholesterol is a vitally important substance and is not in any way Atherogenic. Statin drugs, the only Low Density Lipoprotein lowering agents shown to have clinical benefit in reducing the incidence of heart disease, that have been shown to exert benefits via mechanisms totally unrelated to Low Density Lipoprotein cholesterol reduction (6).

Triglycerides levels are associated with at least four pathogenic conditions such as serum High Density Lipoprotein cholesterol levels increased remnant lipoproteins, increased small dense Low-density lipoprotein and increased

thrombogenesis, all of which are believed to expedite atherosclerosis (7)

Oxidation of Low-density lipoprotein is a free radical-driven lipid per oxidation process, as a Disturbance in the balance between the production of reactive oxygen and Nitrogen species and antioxidant defense. The defense against oxidative stress Includes enzymatic systems (Superoxidedismutase, Catalase, Glutathione peroxidase) and Non-enzymatic systems. (Vitamin E, C, Glutathione, flavonoid) (8, 9). Antioxidants that protect against lipidperoxidation in arterial cells and Lipoproteins can significantly attenuate the development of atherosclerosis. (10).

People with diabetes can work with their health care, to develop an action plan to reach their goals. An action plan can help people to reach and stay at a healthy weight being overweight or obese is a risk factor for heart attack and stroke. (11, 12).

Eat foods that are low in saturated fats, cholesterol, salt, added sugars, choose lean meats, poultry, fish, nuts (in small amounts), fat-free or low-fat milk, and milk products. Eat more fiber, whole grains, fruits, vegetables, and dry peas and beans. Smoking is one of the major risk factors

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associated with heart attack and stroke. (13,14). Take medications as directed and Ask family and friends to help them manage their diabetes. This support can help people reach their goals. (15).

Thus in the present study we have measured the levels of Zinc, Copper, Magnesium and Vitamin C in Cardiovascular Patients and Compare with age, Sex matched healthy persons serving as control.

## MATERIALS & METHODS

Ten male patients with cardio vascular disease confirmed by physician who came to Thanjavur Medical College, for treatment was selected. The age of the patients were ranging in 45-55 years. An equal number of age and sex matched subjects were served as controls. Simultaneously the information regarding dietary and nondietary habits was also collected from the patients. The basal characteristic of the normal & CVD patients is subjected for investigation.

### Collection of Sample

Blood samples were collected from diabetes patients at fasting condition. Their blood samples were collected from anti cubical vein. 2ml of whole blood with anticoagulants were taken to prepare plasma. Plasma was used for estimation of vitamin C. 2ml of whole blood without anti coagulant were also collected and was allowed to clot for 30 minutes. The sample was centrifuged and the clear supernatant serum was separated and analyzed for zinc, copper and magnesium.

### Estimation of Serum Zinc Level

Then 0.5ml of serum sample is taken in a test tube and add 2ml of de-ionized water immediately and mix the solution. Establish instrumental & gas flow setting and aspirations rate precisely to optimize signal and minimum background noise. The aspiration rates optimized with 10 ml aliquots of water lock the nebulizer flow adjustment aspirate glycerol water solution (5/95 volume) into the luminescent flame and set base line 0.000 absorbance. Then take the base line reading before and after each sample and reset the base line, as it is required. The sample was aspirated & the absorbance was monitored & recorder. Serum zinc

was determined by Atomic Adsorption Spectroscopy (AAS) using wavelength: 213.8nm & zinc intention lamp.

### Estimation of Serum Copper Levels by Sodium Diethyl Dithiocarbonate Method

0.5 ml of serum add 1 ml of 0.1N HCl and warm in boiling water, stirring continuously, until the mixture begins to cool. Then add 1.5 ml of 6N HCl and stand for 10 minutes. Add 3 ml. If 20% TriChloroAceticacid and centrifuge. Remove and wash the precipitate with 3ml of 5% TriChloroAceticacid. Centrifuge again and combine the supernatant field. Add 1 ml of 0.4% Sodium diethyl dithiocarbonate and shake well for about 2 minutes with 5ml of the amyl alcohol either mixture to extract the copper. Remove the amyl alcohol layer and dry by shaking with a little powdered anhydrous sodium sulphate. Read using a violet fitter or transmission at 440 millimicrons. Treat 5ml of standard in the same way as the serum and carry through a complex blank against which both standard and unknown are read (Eden and green 1940 Ventura and King 1951).

### Estimation of Serum Magnesium

Prepare 1 ml of Chromogen containing (calmagite-0.35mM/lit, EGTA, DSMO and Preservatives) and 1 ml working reagent to each tube. Pipette 0.1 ml of sample to respective and mix well. Incubate all the tubes at room temperature at 10 minutes. After incubation mix by solution inversion containing (complex agents pH -11.6, Buffer and surfactants of ethyl amino ethane, EGTA of 11.8mM and Potassium cyanide of 0.01%). After 10 minute measure the specimen optical density at 545nm against the reagents as blank solution without sample. Values are expressed as µg/dl

### Estimation of Vitamin C

1.0 ml of the plasma was mixed thoroughly with 1.0ml of ice cold 10% TriChloroAceticacid and centrifuged for 20 minutes at 3500rpm. To 0.5ml of the supernatant, 0.1 ml of DTC reagent was added and mixed well. The tubes were incubated at 37 °C for 3 hrs. 0.75 ml of ice cold 65% H<sub>2</sub>SO<sub>4</sub> was added and the tubes were allowed to stand at room temperature for additional 30 minutes. Likewise a set of standard containing 10-50ml of ascorbic acid

was processed similarly along with a blank containing 0.5 ml of 10% TriChloroAceticacid and the colour developed was read at 520 nm (16).

All result was expressed as the mean value + SD mean statistical comparisons were performed by student t- test and millimicrons matched pair signed rank test respectively. The null hypothesis were rejected for  $P < 0.01$ .

**RESULTS**

- Case studies of the subject investigated Table: I
- Level of serum Zinc in normal & CVD patients Table: II
- Level of serum Copper in normal & CVD patients Table: III
- Level of serum Magnesium in normal & CVD patients Table: IV
- Level of Vitamin C in normal & CVD patients Table: V

**RESULT AND DISCUSSION**

In the present study we have monitored the level of zinc, copper, magnesium in serum and vitamin C in plasma of 10 cardiovascular patients. The results were compared with an equal number of age and sex matched normal subjects.

The concentration of zinc, magnesium and vitamin where found to be significantly decreased while the concentration of copper was found to be significantly elevated in all cardiovascular patients. The analysis in the present study is compared with normal subjects.

Vitamin C Deficiency was associated in this study with raised blood pressure, that evidence in favour the role of oxidative stress (16). Free radicals are oxidants and higher toxic to all types of biological molecules including DNA, lipid, protein

and carbohydrate. Increased production of reactive oxygen species have absorbed in many human diseases including cancer and cardiovascular disease. Dietary factors like zinc, copper and vitamin as nutritive antioxidant are especially important in protecting against human diseases activated with free radical damage (17, 18). Free radicals have been implicated in the aetiology of aging and disease such cancer, atherosclerosis and arthritis (18). Health consequences of Zn-induced Cu deficiency can be quite serious (19).

In the present study the patients analyzed are associated with decreased level of zinc and elevated levels of copper that have long been suspected potential risk factor for cardiovascular disease.

In the absence of copper supplementation, Vitamin C also been reported to impair copper metabolism (21). The dietary antioxidant zinc and copper are served as a cofactor for the activity of enzyme super oxide dismutase, which involved in the bodies antioxidant defense system. Zinc deficiency severely impass endothelial cells function that result hypertension and cardiovascular disease (19, 20, 21). In the increase of Mg level will impressive decrease of angial attacks (22).

In this study serum copper has been mostly shown to be raised in patients with CVD, whereas serum zinc has been normal are decreased. Elevated level of copper in the present study may be breakdown of bodies Cu-SOD defends system in the Cardio Vascular patients. In the present study we observed a significant decrease level of magnesium in all patients and compared with normal subjects.

Magnesium has cardio protective effect by improving energy production, inhibiting plate

**Table I**  
**Case Studies of the Subject Investigated**

| S. No. | General characteristic   | Normal patients             | CVD patients                |
|--------|--------------------------|-----------------------------|-----------------------------|
| 1.     | Total number of subjects | 10 Nos                      | 10 Nos                      |
| 2.     | Sex                      | Male                        | Male                        |
| 3.     | Age                      | 45 - 65 yrs                 | 45 - 65 yrs                 |
| 4.     | Habits / Dietary         | Vegetarian & Non Vegetarian | Vegetarian & Non Vegetarian |
| 5.     | Others                   | -                           | Alcohol, smoking, meat      |
| 6.     | Duration                 | -                           | 10 yrs                      |

**Table II**  
**Level of Serum Zinc in Normal & CVD Patients**

| Parameter    | Normal         | Abnormal       |
|--------------|----------------|----------------|
| Zinc mg / dl | 101.31 + 16.14 | 59.72 + 6.30 * |

\* Data represents mean + SD from 10 subjects in each groups

\* Significantly different from normal P < 0.01

**Table III**  
**Level of Serum Copper in Normal & CVD Patients**

| Parameter    | Normal        | Abnormal        |
|--------------|---------------|-----------------|
| Copper mg/dl | 95.05 + 23.61 | 129.11 + 29.62* |

\* Data represents mean + SD from 10 subjects in each groups

\* Significantly different from normal P < 0.01

**Table IV**  
**Level of Serum Magnesium in Normal & CVD Patients**

| Parameter       | Normal     | Abnormal    |
|-----------------|------------|-------------|
| Magnesium mg/dl | 1.8 + 0.25 | 1.2 + 0.26* |

\* Data represents mean + SD from 10 subjects in each groups

\* Significantly different from normal P < 0.01

**Table V**  
**Level of Vitamin C in Normal & CVD Patients**

| Parameter       | Normal       | Abnormal      |
|-----------------|--------------|---------------|
| Vitamin C mg/dl | 1.23 + 0.173 | 0.90 + 0.194* |

\* Data represents mean + SD from 10 subjects in each groups

\* Significantly different from normal P < 0.01

period aggregation, reducing vascular resistance, promoting clot breakdown dilating blood vessels, improving the functions of the heart. So the deficiency made increased the risk of CVD in several days. Microscopic change in the heart atleast and the development of atherosclerosis have been absorbed in animal with many deficiency (22, 23). Both Zinc and copper changes were more likely non-specific secondary manifestation of inflammatory or injurious process in the body (23).

Results from the Atherosclerosis Risk in Communities (ARIC) support that the association between low serum and dietary magnesium and various types of CVD including high blood pressure and we also analysed the same concept in our studies also (24). Ascorbate is considered to be the most effective antioxidant in human plasma and vitamin C inhibits the oxidation of low-density lipoprotein invitro (25).

In the present we noted that all the patients with CVD associated with vitamin C deficiency. The dietary vitamin is a well-known utility antioxidant

in humans. A major culture in the development of Cardio Vascular Disease is oxidized by Low Density Lipoprotein Level, which caused endothelial cell damage. Vitamin C protects against the Low Density Level Lipoprotein oxidation. There is also evidence that vitamin C increases High Density Levels lipial and may also lower total cholesterol in the blood, thus reducing the risk of Cardio Vascular Disease (25,26).

Vitamin C concentration and risk infraction or the statistical control for confounding may not have been perfect because of imprecision's is measured. Thus this study shows Vitamin C deficiency may associated with an increased risk of myocardial infraction this finding also provide additional support for the role of oxidation stress and lipid peroxidation in coronary risk. This study does provide evidence in favour of the benefit of Vitamin C supplements (26).

Serum Magnesium and zinc level were significantly decreased in Cardio Vascular Disease patients and those of copper level is significantly increased when compared to normal patients likewise the level of vitamin C is significantly decreased in Cardio Vascular Disease patients as compared to normal subjects.

In this study we analyzed are associated with decreased levels of Zinc and elevated levels at copper that have long been suspected as potential risk factor of Cardio Vascular Disease. Elevated level of Copper in the present study may be breakdown of bodys Cu-SOD defense system in the Cardio Vascular patients. A significant decreased level of Mg in all patients when compare with normal subjects.

## CONCLUSION

Results of Atherosclerotic Risk In Communities (ARIC) support that the association between low serum & dietary Mg and various types of Cardio Vascular Disease including high Blood Pressure. And this study noted that all the patients associated with Cardio Vascular Disease with Vitamin C deficits. Dietary vitamin C is well known antioxidant in human protects against Low Density Lipoprotein oxidation Vitamin C, High Density Level and many also lower total cholesterol in blood, thus reducing the risk of Cardio Vascular Disease.

Free radicals are oxidant and higher toxic to all types of biological molecules including DNA, lipid, Protein & Carbohydrates. Production of Reactive Oxygen Species have absorbed in many human diseases including Cardio Vascular Disease & cancer. Dietary factors links Zinc, Copper & Vitamin C as nutritive antioxidant are especially important in protecting against human diseases activated with free radical damage.

Thus the study concluded that lifestyle with such as balanced level of nutritive antioxidant Zinc, Copper, Magnesium & Vitamin C are essential Cardio Vascular homeostatic and may be beneficial to prevent may degenerative disease developed by free radicals in middle aged people.

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