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Evaluation Of Effect Of Trace Elements And Antioxidants Levels In Patient With Ischaemic Heart Disease

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Abstract:

Cardiovascular Disease, one of the chronic, non-communicable diseases, has become a major public health problem in many developing countries. Of the CVD, ischaemic heart disease is the predominant entity contributing to mortality and morbidity. Therefore, rapid diagnosis and treatment as well as assessment of the prognosis are very essential and challenging in ischaemic heart disease. Oxidative stress is implicated in the etio pathogenesis of ischaemic heart disease. Measurement of lipid peroxidation is of value in estimating oxidative stress. Therefore, the aim of this study was 1) To assess oxidative stress by determination of MDA as a lipid peroxidation marker in ischaemic heart disease and controls. 2) To measure trace elements iron, copper and zinc 3) To assess antioxidants enzymes i. e. erythrocyte superoxide dismutase and erythrocyte catalase levels have been determined in 30 patients with ischaemic heart disease & 30 controls. We observed that significantly increased levels of serum iron (P< 0. 001), copper (P<0. 001) and lipid peroxidation (P<0. 001) whereas the levels of serum zinc and activities ofenzymes like erythrocyte SOD and catalase were significantly decreased (P<0. 001) in ischaemic heart disease as compared to controls. Iron /zinc ratio, copper/zinc ratio were elevated in IHD patients. Significantly positive correlation was observed between MDA and iron as well as MDA and copper levels. Negative correlation between MDA & zinc levels was found. This study indicated that the antioxidant status of IHD patients was low; suggesting the role of free radical mediated oxidative stress in IHD. Iron /zinc ratio, copper/zinc ratio may provide an additional index for prognosis of the IHD.

Key words: Cardiovascular Disease (CVD), Erythrocyte Catalase. Ischaemic Heart Disease (IHD), Malondialdehyde (MDA), Superoxide Dismutase (SOD)

Introduction

Cardiovascular Disease (CVD) is the most frequent cause of death in adult life in developed countries, and is increasingly becoming important in developing countries like India [1]. According to World Health Report 2002, cardiovascular diseases will be the largest cause of death and disability by 2020 in India. In 2020 AD, 2. 6 million Indians are predicted to die due to coronary heart disease which constitutes 54. 1% of all CVD deaths. Nearly half of these deaths are likely to occur in younger and middle aged individuals (30-69 years). Currently, major Indians experiencing are CVD deaths at least a decade earlier than their counterparts in economies (EME). The Global Burden of Disease (GBD) study estimates that 52% of CVD deaths occur below the age of 70 years in India as compared to 23% in EME, resulting in a profound adverse impact on its economy.

The contributing factors for the growing burden of CVDs are increasing prevalence of cardiovascular risk factors especially hypertension, dyslipidemia, diabetes, overweight or obesity, physical inactivity and smoking etc. [2, 3]. Other factors such as micronutrients may be involved in lipid peroxidation. Thus there is scope for other dependent and independent risk factors which may contribute o the onset of CVD.

In the development of IHD and evolution of atheroma (atherogenesis) emphasis is given on oxidative stress and damage caused by it. Trace elements and free radicals play an important role in the pathogenesis of atherosclerosis and also affect the lipid profile in patients with IHD [4]. All trace elements are harmful to human body beyond a certain level. Copper and iron ions are powerful promoters of free radical damage, accelerating lipid peroxidation and causing formation of hydroxyl radicals [5]. Zinc acts as a biological antioxidant by a decreasing lipid peroxidation and stabilizing the membrane [6]. Thus trace elements may play a vital role resulting in either harmful or beneficial effects by damaging or protecting the vessel wall and altering the lipid profile.

"Lipid peroxidation" is the most intensively studied process for assessment of free radical mediated oxidative stress [7, 8].

Hence it was thought worthwhile, to study the levels of iron, copper, zinc & lipid peroxide, correlation among them, and to assess the levels of antioxidants in IHD.

Materials and methods

The present study was carried out in the Dept. of Biochemistry, Govt. Medical College, Miraj.

Inclusion Criteria:

- 30 patients with IHD
- Cases of IHD were diagnosed by clinicians

- Age ranging from 35-70 years.
- 30 healthy controls with age and sex matched were included in this study.

Exclusion Criteria:

- Patients with liver disease, renal disease, chronic debilitating illnesses (cancer, AIDS) and diabetic patients were excluded from this study.
- Below 35 yrs and above70 yrs, age of patients and controls were excluded.
- Patients and controls that were not willing to participate in this study were excluded.

The Institutional Ethical Committee approved the plan of study and informed consent was obtained from each participant in the study.
Blood samples were collected after an overnight fast, by taking aseptic precautions. Heparinised blood samples were centrifuged, and after separation of plasma, RBC's were washed thrice with normal saline, Hemolysate was prepared by adding 4 volumes of D/W & mixing. Haemoglobin concentration was adjusted to 10 Gm/dl. Erythrocyte SOD activity was determined by Winterbourn C. C. method [9]. Erythrocyte catalase activity in the hemolysate was determined by the spectrophotometric method of Hugo Aebi [10]. Lipid peroxide in serum was determined by estimating malondialdehyde (MDA) produced using thiobarbituric acid (Kei Satoh method) [11]. Serum iron, copper and zinc were estimated by atomic absorption spectrophotometer (Perkin-Elmer Model 3030) [12]. The serum samples were diluted to 1:10 dilution using deionized water, and then aspirated directly into burner of atomic absorption spectrophotometer. The wavelength was set at particular wavelength for different element. For example – for iron the selected wavelength is 248. 3nm for copper 324. 8nm and for zinc is 213. 9nm. Zero absorbance was adjusted using deionized water. The instrument was standardized by using standard solution of element in measure; the value of absorbance was checked. Finally the absorbances of diluted unknown samples were measured and concentration of element was calculated.
All data were expressed as mean $\pm$ SD. Statistical significance was analyzed by using "Z" test

Results and discussion

Serum iron, copper and lipid peroxide levels were significantly increased ($p < 0.001$) in IHD patients as compared with control (Table no 1). The ratios of iron/zinc, copper/zinc was increased in IHD patients as compared with healthy controls ratios (Table no 2). Significant positive correlation between MDA & iron, copper were found in IHD. Iron ions themselves free radicals, and ferrous ions can take part in electron transfer reaction in presence of oxygen in the process of changing from ferrous to the ferric state, an electron is transferred from iron to oxygen to generate a superoxide [13]. Kelley MK [14] showed that the xanthine oxidase activity is depending upon iron. In case of ischaemia, iron level is elevated with increasing

xanthine oxidase activity causing generation of superoxide (FIG. 1) and also increased oxidative stress, which in turn increases lipid peroxidation, could increase myocardial vulnerability to ischaemia. In reperfusion of an ischaemic area of myocardium, superoxide ($O^{\cdot}_2$) and hydroxyl radicals ($OH^{\cdot}$) may play a role in reperfusion injury. Hydroxyl radicals are particulary reactive. They can be generated by myocardial cell or by circulating blood cells; they damage cells by causing lipid peroxidation.

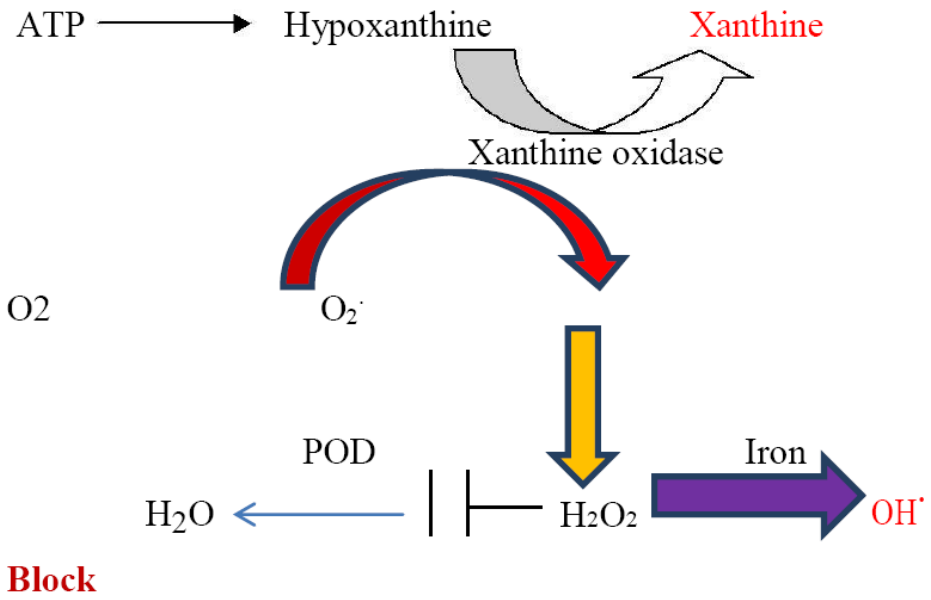

Figure 1

In Fenton chemistry, reaction between iron salts and H_2O_2 that caused oxidative damage to organic molecules [13].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot} \text{ (Fenton reaction)}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\cdot} + H^+$$

$$O_2 + H_2O_2 \rightarrow O_2 + OH^- + OH^{\cdot} \text{ (Iron catalysed Haber-Weiss reaction).}$$

The addition of an iron complex to lipids will stimulate peroxidation by peroxide decomposition, generating very reactive alkoxyl ($LO^{\cdot}$) and peroxyl ($LO_2^{\cdot}$) radicals. Hydroxyl and peroxyl radicals are responsible for the damage caused to the bio-molecules, such as PUFA, which affect the antioxidant system, by increasing lipid peroxidation, rendering the myocardium more susceptible to oxidative injury. IshwarlalJialal et al. [15] reported that oxidation of LDL is a key step in atherogenesis. This LDL can be modified oxidatively in the presence of transition metals such as copper and iron, which can promote rapid lipid peroxidation.

Guttridge [13] and Sushamakumari S. et al. [16] showed that an alteration in the metabolism of lipid peroxides is closely and strongly associated with myocardial damage as indicated by increasing malondialdehyde in the heart tissue on induction of myocardial infarction. Reasons for this increased peroxidizability of damaged tissues, includes inactivation of some antioxidants, leakage of antioxidants from the cells and the release of metal ions (especially iron and copper) from storage sites and from metalloproteinshydrolyzed by enzymes released from damaged lysosomes. Increased lipid peroxidation leads to multiple membrane damage and disturbance in cardiac metabolism, structure and function. Hence measurement of lipid peroxidation may therefore, be excellent marker of cardiovascular diseases.

Serum zinc levels were significantly decreased (p ‹ 0. 001) in IHD patients as compared with controls (Table No1). Findings of Comar CL and Chvapil M (4, 17) support our result.

Zinc can "interfere with metal catalyzed peroxidation". Zinc diminishes the weakening effect on the membrane caused by the peroxidative damage. Significant negative correlation between MDA & zinc were found in IHD. In our study, erythrocyte superoxide dismutase and erythrocyte catalase levels were significantly lowered (p ‹ 0. 001) in IHD as compared with controls (Table no 1). Rao et al. [18] and Meerson et al. [19] reported that the accumulation of lipid peroxides in myocardial tissue during ischaemia induced injury, along with simultaneous loss of myocardial SOD, catalase and glutathione peroxidase.

Table 1: Serum iron, copper, zinc, MDA, erythrocyte SOD & catalase levels in controls and ischaemic heart disease.

Sr. No	Biochemical Parameters	Healthy Controls Mean ± SD		IHD Mean ± SD	"P" value
1	Iron (µg/dl)	119. 2 ± 37. 698		166. 63 ± 53. 71	P<0. 001
2	Copper (µg/dl)	95. 40 ± 15. 70		127. 63 ± 9. 80	P<0. 001
3	Zinc (µg/dl)	91. 200 ± 19. 99		73. 90 ± 6. 472	P<0. 001
4	MDA ("n" moles/ml)	3. 700 ±	0. 273	6. 073± 1. 838	P<0. 001
5	SOD (U/G Hb)	4. 08 ±	0. 307	2. 958± 0. 513	P<0. 001
6	Cat (U/G Hb)	497. 43±58. 79		346. 94± 45. 20	P<0. 001

Table 2: Iron / Zn ratios and Copper / Zn ratios in controls and ischaemic heart disease.

Sr. No	Ratios	Healthy controls	IHD
1	Iron / Zn ratio	1. 2	2. 2
2	Copper / Zn ratio	1. 0	1. 7

Our study supports the hypothesis that defective antioxidant status, low levels of zinc and increased levels of iron and copper increases oxidative stress. These trace elements help in generation of free radicals. This in turn increases free radical mediated oxidative stress which is confirmed by increased levels of oxidative stress

marker (MDA) and decreased levels of antioxidants. Our findings will definitely help the clinicians in the determination of oxidative stress in IHD.

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