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"Zinc Deficiency A Cause or A Consequence of Oxidative Stress" A Case Control Prospective Study in Patients of Ischemic Heart Disease

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Abstract

Zinc has antioxidant and anti-inflammatory properties. Zinc deficiency can induce a state of endothelial activation which is characterized by a pro-inflammatory, proliferative and procoagulatory milieu that favors all stages of atherogenesis. Much less attention has been paid to micronutrients, and particularly to zinc. The aim of the study was to determine the protective functions of zinc in the pathogenesis of atherosclerosis. The study included 50 non diabetic Chronic Ischemic heart disease patients and 50 normal healthy individuals, in the age range of 40- 75 years. All the subjects were evaluated for serum zinc, super oxide dismutase(SOD),Malondialdehyde(MDA), Total Cholesterol, triglycerides, very low density lipoprotein (VLDL), low density lipoprotein(LDL) and High density lipoprotein (HDL) cholesterol concentration estimations. The results obtained were compared with the normal healthy control subjects. As compared to the control group, the patient group showed lower serum zinc levels ($p<0.001$), while serum MDA as well as SOD levels were found to be high ($p<0.001$) in the patients under study. Serum total cholesterol, triglycerides, VLDL, LDL, were significantly higher but the HDL was low significantly in the patient's group as compared to normal control subjects. Statistically insignificant variations of serum zinc levels were observed with the age of the patient and the duration of the disease. Zinc deficiency induces oxidative stress and can progress to atherosclerosis. Timely supplementations of zinc and anti-oxidants are required for the prevention and. progression of atherosclerosis.

Key words: Super oxide dismutase (SOD), Malondialdehyde (MDA), Total Cholesterol, triglycerides, very low density lipoprotein (VLDL), low density lipoprotein (LDL), High density lipoprotein (HDL), Chronic Ischemic heart disease.

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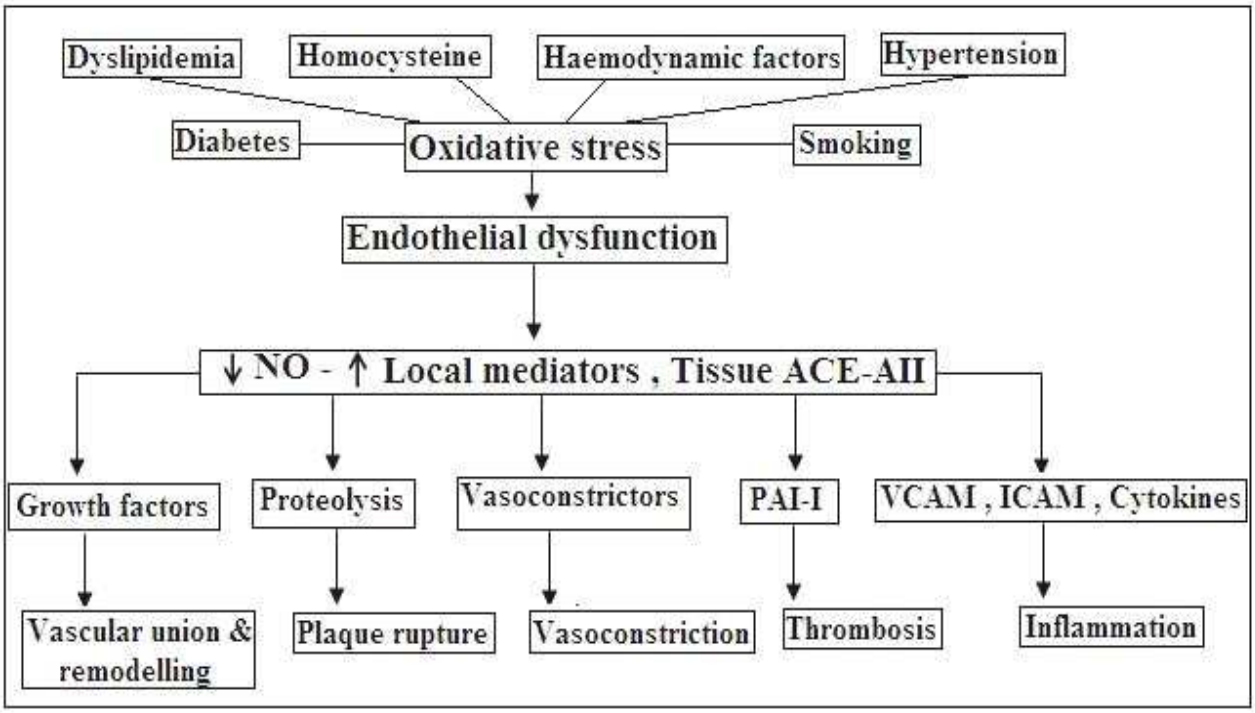
Introduction

Atherogenesis is a complex process involving mechanical, chemical and biological factors. [1] Recent insight into the basic mechanisms involved in Atherogenesis indicate that deleterious alterations of endothelial physiology , also referred to as endothelial dysfunction , represents a key early step in the development of atherosclerosis and is also involved in plaque progression and the occurrence of atherosclerotic complications .

Figure1: Showing the central role of oxidative stress in the causation and progression of Atherosclerosis



Most, if not all risk factors that are related to atherosclerosis and cardiovascular morbidity and mortality, including traditional and nontraditional risk factors, are also found to be associated with endothelial dysfunction. [2]

Many of these risk factors, including hyperlipidemia, hypertension, diabetes and smoking are associated with overproduction of reactive oxygen species or increased oxidative stress.[3] Hence increased oxidative stress is considered a major mechanism involved in the pathogenesis of endothelial dysfunction and may serve as a common pathogenic mechanism of the effect of risk factors on the endothelium.[4] [Figure – 1] There is evidence that certain antioxidant nutrients and adequate antioxidant enzyme activities may protect against atherosclerosis by preventing metabolic and physiologic derangements of the vascular endothelium [5]. Of particular interest is zinc, because it may function as an antioxidant and membrane stabilizer [6]. Epidemiological studies suggest that in some population groups lower serum levels of zinc are inversely associated with coronary artery disease [7]. Mechanisms of the protective function or functions of zinc in the pathogenesis of atherosclerosis, including vascular cell injury or dysfunction and the inflammatory response, are not clear. [8]

Zn is required for structural and functional integrity of more than 2000 transcription factors and 300 enzymes. Therefore, almost every signaling and metabolic pathway is in some way dependent on at least one, and often several, Zn-requiring proteins.[9] There is evidence suggesting that zinc can act as an endogenous protective factor against atherosclerosis by inhibiting the oxidation of LDL by cells or transition metals [10]Because of its antioxidant and membrane-stabilizing properties, zinc appears to be crucial for protection against cell-destabilizing agents such as inflammatory cytokines and polyunsaturated lipids.[11]

Because zinc is a micronutrient with indirect antioxidant activity, it might be relevant to assess its role in the Atherogenesis. The objective of the present study was

- To determine the correlation between serum zinc levels and the degree of oxidative stress in patients of Ischemic heart disease.
- To determine the potential use of serum zinc level as predictor of underlying endothelial dysfunction in predisposed individuals**.**

Materials and Methods

The study included 50 non diabetic Chronic Ischemic heart disease patients and 50 normal healthy individuals, in the age range of 40- 75 years. All the subjects were evaluated for serum zinc, Serum total cholesterol (TC), serum Triglycerides(TG), Serum High density lipoprotein cholesterol (HDL-C), serum very low density lipoprotein cholesterol (VLDL-C), serum low density lipoprotein cholesterol and (LDL-C) concentration, All the subjects were also evaluated for Superoxide dismutase, Malondialdehyde and Glutathione peroxidase enzyme estimations to assess the degree of oxidative stress. The comparisons were made between the healthy normal individuals and the ischemic heart disease patients.

All blood specimens were drawn at 8:00 A.M. after a 12-h fasting. Samples were centrifuged within 1 hour. The concentrations of serum total cholesterol, triglycerides, high density cholesterol (HDL) levels were estimated by Human Diagnostics Reagent (Max-Planck-Ring 21 D-65205 Wiesbaden Germany) adapted to 550 express plus auto analyzer (Ciba corning diagnostics corporation, 63 north street, Medfield, MA 02052-9990, USA). Low density cholesterol (LDL) was measured by enzymatic method[12].

Patient Group	Serum Zn Level (µg/dl) Mean ± SD	Serum SOD Levels (U/ml) Mean ± SD	Serum MDA Levels (U/ml) Mean ± SD
Control (n = 50)	99 ± 8.3	4.08 ± 0.31	2.39 ± 0.13
A (n = 10)	42.9 ± 7.87*	2.83 ± 0.38*	5.84 ± 1.72*
B (n = 30)	62.5 ± 4.88*	2.78 ± 0.47*	5.47 ± 1.58*
C (n = 10)	77.3 ± 5.19*	2.67 ± 0.36*	5.14 ± 1.75*

TABLE – 1: Distribution of patients into groups as per serum zinc levels and correlation of serum SOD and MDA levels among patient groups.
Range of Serum zinc levels of patient groups A = 30 to 50 µg/dl, B = 51 to 70 µg/dl, C = >70 µg/dl.
*p value is < 0.001 highly significant

The estimation of MDA in serum was done by the method of Kei Satoh. [13] The color produced by the reaction of thiobarbituric acid with MDA was measured at 530 nm with the help of spectrophotometer. The results were expressed as nmol/ml . SOD was assayed by the method of Marklund and Marklund [14] modified by Nandi *et al* . [15] This method is

TABLE – 2 Comparison and Distribution of serum ZINC, SOD and MDA levels according age group
Age range of subjects in group – I = 40 to 50years, group – II = 51 to 60 and group – III = 61 to 70 years.
*P < 0.001

Age Group	ZINC Levels (µg/dl) Mean ± SD		SOD Levels (U/ml) Mean ± SD		MDA Levels (U/ml) Mean ± SD	
	Controls	Patients	Controls	Patients	Controls	Patients
Group – I	118 ± 8.1	76.4 ± 11.1 (n = 10)	3.77 ± 0.40 (n = 4)	2.65 ± 0.47 (n = 10)*	2.26 ± 0.38 (n = 4)	5.02 ± 1.62 (n = 10)
Group – II	96 ± 9.2	63.8 ± 8.4 (n = 19)	4.09 ± 0.99 (n = 18)	2.78 ± 0.39 (n = 19)*	2.40 ± 0.20 (n = 18)	5.34 ± 1.88 (n = 19)
Group – III	83 ± 6.5	58.4 ± 7.6 (n = 21)	4.39 ± 1.36 (n = 28)	2.87 ± 0.38 (n = 21)	2.52 ± 0.50 (n = 28)	5.74 ± 1.70 (n = 21)

based on the ability of SOD to inhibit auto-oxidation of pyrogallol under specific conditions. Reading was taken at 420 nm and expressed as units/ml. GPx was measured by the method of Paglia and Valentine [16]. Serum Zn measurement was performed by flame atomic absorption spectrophotometery with deuterium background correction (Perkin-Elmer model 5000) [16]. A complete clinical examination of the patients was recorded. The case history was recorded and informed consent was taken from all the subjects under study. All the results were compared with those of normal healthy individual and these results were expressed as mean ± SD. The comparisons were done by using student 'T' test on the number of variables for each parameter.

Results

In the present study majority of the patients (52%) were females and were in the age group of 61 -70 years implying thereby that menopause opens the road to atherosclerosis. Serum zinc amongst patients was significantly lower (Table 1) as compared to control group ($p<0.001$).All the subjects were distributed in to three groups based on the age, Group I, II and III (Table – 2). In both the study subjects serum zinc level was found to be falling with the advancing age signifying there by the decreased anti oxidative defense against oxidants and a rising predisposition for atherosclerosis even in the control subjects.

Based on serum zinc level the study subjects were classified in to three groups, A , B and C with the values ranging between 30-50, 51-70 and above 71 µg/dl .Majority of the patients were having serum zinc level ranging between 51-70 µg/dl, signifying the loss of protection with the onset of zinc deficiency .The least levels observed were between 30 -50µg/dl in some of the patients.(Table 1)

Discussion

There are several mechanisms by which zinc might be capable of providing protection against atherogenesis. The enzyme superoxide dismutase, an endogenous antioxidant enzyme loses its functional potential upon loss of its zinc atom [17], zinc protects sulfhydryl groups against oxidation and inhibits the production of reactive oxygen by transition metals, in addition to its function as a membrane stabilizer [18]. Dietary zinc deficiency is also reported to lower lymphatic absorption of vitamin E [19] and decrease concentrations of this vitamin in plasma [20] and selected organs [21], suggesting that dietary zinc deficiency may increase the nutritional requirement for vitamin E necessary to maintain adequate plasma and tissue concentrations thus predisposing more for oxidative stress induced cell injury.

As an antioxidant, zinc has membrane-stabilizing properties and is said to preserve endothelial function .[22] zinc can protect endothelial cells against tumor-necrosis-factor-(TNF)-induced cell injury [23], and one of the underlying mechanisms could be the ability of zinc to down-regulate oxidative stress-sensitive transcription factors. The fact that zinc can also in part block genes encoding for inflammatory cytokines, such as IL-6 or IL-8, in endothelial cells strongly supports the hypothesis that adequate zinc nutrition may protect against inflammatory diseases such as atherosclerosis by inhibiting the activation of oxidative stress-responsive transcription factors, as well as expression of inflammatory cytokines. [24]

In the present study, an inverse relationship was observed between the severity of the disease and the serum zinc levels .Although statistically insignificant but certain variations were also observed in terms of decreasing serum zinc levels and increasing duration of the disease, implying the loss of protective effects of zinc with the progression of the disease.

In the age matched subjects variations of Serum superoxide dismutase (SOD) levels were observed among patients and control subjects. Serum superoxide dismutase (SOD) level was found to be lower amongst patients (2.0-3.3 U/ml) as compared to control subjects (2.8 to 6.6U/ml) .The difference was significant statistically in group I and II while it was statistically insignificant in group – III subjects (Table 2). This might be due to advancing age, since as the age advances the oxidative stress also increases.

Superoxide dismutase (SOD) is the most important antioxidant enzyme synthesized in response to oxidative stress. It protects the cells from damages caused by superoxide anion (O_2-.) and H_2O_2[25]. The decrease in activity of SOD could be due to decreased Zinc levels and this explains the indirect antioxidant role of zinc [17]. The lowered serum SOD activity amongst such patients could also be explained by the fact that initial increased SOD activity leads to excessive production of H2O2 which is inhibitory to the activity of SOD. H_2O_2 rapidly reduces Cu (in Cu-Zn-SOD) at the active site and then more slowly inactivates the reduced enzyme [26].

Statistically insignificant variations of serum SOD levels were observed amongst males and females of study subjects; signifying the equal predisposition of each group against oxidative stress.

Hydroxyl radicals produced as a result of metal catalyzed reactions are highly reactive and can oxidize lipids giving rise to lipid peroxidation. Malondialdehyde (MDA) is a major end product and an index of lipid peroxidation. A significant increase in serum MDA level ($p<0.001$) was observed in patients as compared to controls (Table - 2). The increase in MDA levels observed could be due to increased oxidative stress or decrease in anti oxidant defense mechanisms.[27] The serum SOD activity and the serum MDA levels were found to be rising amongst both the study groups (controls as well as patients) with advancing age. The maximum concentration was observed in group 3 (of the age range of 61-70 years) suggesting that oxidative stress increases with the advancing age. Upon comparison, the difference between the levels of serum SOD and MDA in age matched normal individuals and patients was statistically highly significant ($p<0.001$) (Table - 2).

A parallel trend of serum SOD activity was observed with serum MDA levels. With the rise in serum SOD activity, serum MDA level

was also found to be rising proportionately signifying excessive production or decreased quenching of free radicals. An inverse relationship was observed with the serum zinc levels and the parameters of oxidative stress. (Table - 1) Lesser the zinc levels more were the levels of SOD and MDA indicating the presence of oxidative stress.

All the patients were having statistically high serum total cholesterol, high serum triglycerides, high serum VLDL and LDL but low serum HDL in comparison to the control subjects. The ratios of Serum total cholesterol/serum HDL and Serum triglyceride/ serum HDL were also significantly higher than the normal control subjects. (Table - 3) Dyslipidemia is also well-known accelerating risk factors for atherosclerosis. [28] .This compromised state of oxidative stress in the presence of zinc deficiency produces low resistance to chemically induced oxidant injury, and produces high vulnerability of lipoproteins to oxidation thereby enhancing the risk for I.H.D. Although it is difficult to prove whether zinc deficiency is a cause or a consequence but it can be said based on the observations that zinc deficiency is certainly correlated with oxidative stress and that is the main culprit behind the pathogenesis of atherosclerosis.

S.N.	Lipid profile	Control (Range)	Control (Mean ± SD)	Patients (Range)	Patients (Mean ± SD)
1	Serum total cholesterol(mg/dl)	155-210	177.4±16.98	172-275	226.4±20.42
2	Serum Triglycerides(mg/dl)	110-162	142.7±11.48	109-240	174.29±25.22
3	Serum VLDL (mg/dl)	22-32.4	28.5±2.26	21.8-48	35.2±7.07
4	Serum LDL (mg/dl)	67.9-115	96.9±14	99-204	150±22.8
5	Serum HDL (mg/dl)	40-58	52.1±6.09	33.5-55	40.76±4.8

TABLE – 3: Comparison of lipid profile in different study subjects

Conclusion

As it's evident now that the oxidative stress is the major mechanism involved in the pathogenesis of endothelial dysfunction and its progression to atherosclerosis.

The administration of antioxidants would be expected to be a reasonable strategy to treat this disorder. Zinc can exert a number of indirect antioxidant functions. Increased zinc intake will protect against oxidant stress in persons with tendencies for both moderate zinc deficiency and high oxidant stress.

Zinc supplementation is expected to decrease plasma lipids also below control levels. The adult Recommended Dietary Allowance (RDA) for zinc is about 15 milligrams a day for adult men and women; slightly more for women that are pregnant or nursing.

A timely supplementation with the required amount of zinc will prevent the onset of oxidative stress induced endothelial dysfunction and its progression to atherosclerosis. A close attention is required to be paid for this 'mineral of life.' Thus to conclude-

1 The simple, highly economical and highly relevant estimation of serum zinc level

can be used as predictor of underlying endothelial status.

1 The screening of the predisposed or high risk individuals can be done by the set of Serum Zinc, SOD and MDA levels. These investigations can be considered the early and the best markers of oxidative stress and the related endothelial dysfunction.

1 The treatment of zinc deficiency will directly affect the endothelial health and its functions.

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