



ROLE OF IRON AND COPPER IN DIABETICS

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ABSTRACT

Diabetes mellitus is an endocrine disorder affecting millions of people every year. Diabetes remains latent and its secondary complications leads to the morality and morbidity. Iron and copper are essential minerals that are required for a variety of molecules to maintain their normal structures and functions and for cells to live, grow, and proliferate. The homeostasis of iron and copper results from a tightly coordinated regulation by different proteins involved in uptake, excretion and intracellular storage/trafficking. Although it is essential, iron can also be toxic once in excess amounts. Through Fenton reaction, iron as a transit mineral can generate various reactive oxygen or nitrogen species; therefore, abnormal metabolism of iron can lead to several chronic pathogenesis. Oxidative stress is one of the major causative factors for diabetes and diabetic complications. Increasing evidence has indicated that iron overload not only increases risks of insulin resistance and diabetes.

KEY WORDS: Copper, Iron, FRAP (Ferric Reducing Ability of Plasma), AGE(Advanced Glycation End products), DM(Diabetes Mellitus).

INTRODUCTION

Diabetes mellitus, an endocrine disorder, is a health problem affecting millions of individuals worldwide. It is a major source of morbidity in developed countries. The World Health Organization (WHO) predicts^[1] that 300 million people will have diabetes mellitus by the year 2025. Diabetes mellitus affects people of all ages and ethnic groups. It was estimated that 2.8% of the world's population was

diabetic in 2000 and this figure would climb to be as high as 4.4% of the world's population by 2030^[2]. Diabetes affects about 5% of the global population and the management of diabetes without any side effects is still a challenge to the medical system^[3]. Diabetes is not a single disease but rather a heterogeneous group of disorders that lead to an elevation of glucose in the blood. Chronic hyperglycaemia and the risk of developing complications are the two

unifying properties which have held the notion of diabetes together.

Trace elements function primarily as catalysts in enzyme systems; some metallic ions, such as iron and copper, participate in oxidation-reduction reactions in energy metabolism. Iron, as a constituent of hemoglobin and myoglobin, also plays a vital role in the transport of oxygen. All trace elements are toxic if consumed at sufficiently high levels for prolonged periods. The difference between toxic intakes and optimal intakes (to meet physiological needs) for essential trace elements is great for some elements but is much smaller for others.

Trace elements are also found in organic and inorganic combinations in food. In the body only 5% of the human body weight is mineral matter, vital to all mental & physical processes and for total well-being. They are most important factors in maintaining all physiological processes, are constituents of the teeth, bones, tissues, blood, muscle, and nerve cells.

Acting as catalysts for many biological reactions within the human body, they are necessary for transmission of messages through the nervous system, digestion, & metabolism or utilization of all nutrients in foods.

Micronutrients, minerals and trace elements are very important for the human body. They have various roles in metabolism and body functions. They are essential for the proper function of cells, tissues, and organs. Some minerals, such as iron, make up part of many proteins and enzymes in the body. Minerals also play a role in the building up of muscle and bone and are important for normal body growth. It is well established that several trace elements are of great importance in a number of biological processes, mostly through their action as activators or inhibitors of enzymatic reactions, by competing with other elements and proteins for binding sites, by influencing the permeability of cell membranes, or through

other mechanisms. It is therefore reasonable to assume that these minerals would also exert an action, either directly, or indirectly, on the pancreas. Disorders of mineral metabolism are sometimes passed from parents to their children through genes. Other medical conditions, such as starvation, diarrhoea, or alcoholism, can cause mineral metabolism problems. Minerals and trace elements may exert protective or scavenging effects, as well as being essential components of several key enzymes in intracellular antioxidant defense^[4]. Their deficiency, or excess, may contribute to derangement of the pro-oxidant/anti-oxidant balance, and hence to the progressive appearance of secondary complications as the disease advances. Both type I and II diabetes are accompanied by alterations in micronutrient absorption, tissue uptake, and excretion, some in a time-dependent fashion. A major effect of these changes may be a worsening of the oxidative balance, with declining capability to combat endogenously produced free radicals.

Macro and microelements are involved in the complex processes of development of the secondary complications of diabetes mellitus affecting many organs. They may be integral components of antioxidant enzymes (e.g., Cu, in the case of the superoxide dismutase, and Se for Glutathione peroxidase), cofactors in a variety of enzymatic processes of importance in glucose and lipid metabolism (e.g., Cu), or potential pro-oxidant catalysts (e.g., Cu, Fe).

According to Djoussé et al. (2011)^[5], the etiology of diabetes and its complications still is not clear, however several factors as aging, obesity and oxidative damage have been implicated. Several micronutrients have beneficial effects in healthy subjects and also in diabetes^[6]. Copper, iron and manganese are important components of metalloenzymes such as Se-cys containing glutathione peroxidase, Cu/Fe cytochrome C oxidase and/or different types of superoxide dismutases, all of them

imperative in intra- and extra-cellular antioxidant defense^[7].

Copper is found in the liver, gallbladder, lungs and heart. It is essential primarily for the absorption and metabolism of iron. A deficiency in copper results in the same effects as an iron deficiency, such as retarded hemoglobin production, general debility, limited growth, etc. No official recommendations are made for copper allowances. Some sources have estimated about 2 milligrams per day. Very few cases of copper depletion have been observed in humans. Copper is needed for synthesis of hemoglobin, proper iron metabolism, and maintenance of blood vessels. Copper is an integral part of the enzyme copper-zinc superoxide dismutase (CuZn SOD); also present in other enzymes, including cytochrome oxidase, ascorbic acid oxidase, and tyrosinases. It is usually found in the red blood cells, and in blood plasma. The chief supplementary sources of copper are seafood, nuts, legumes, green leafy vegetables. Insufficient copper has been associated with: changes in hair colour & texture, and hair loss; disturbances to the nervous system; bone diseases. Serious deficiency is rare but can lead to: Menke's syndrome.

Copper has been shown to be elevated in experimentally diabetic rats^[8]. Iron status is little affected by diabetes per se; however, because of its role as a catalyst in free radical generation, and the given state of increased oxidant stress in diabetes, it is probably advisable for diabetic individuals to avoid excess iron. Hence, this present research is mainly focused on the role of the following essential trace elements in Type I DM and Type II DM conditions: iron, selenium, copper, chromium, vanadium and molybdenum. Interactions between these trace elements and hyperglycemia are also briefly considered. Epidemiologic data on the relationship between many of the trace elements and the incidence of diabetes and hypertension are incomplete.

Most such studies have focused on cadmium, chromium, and selenium. Furthermore, most of the evidence is not related to dietary exposure but focuses, for example, on inhalation exposure in the workplace. Data from animal feeding experiments are also incomplete. The present research identifies such gaps in knowledge and suggests directions for research. The main objective of the present study is to identify the alterations in the blood levels of iron and copper and their effect in diabetics.

The biochemical role for copper is primarily catalytic, with many copper metalloenzymes acting as oxidases to achieve the reduction of molecular oxygen. Many copper metalloenzymes have been identified in humans^[9]. Ferroxidases are copper enzymes found in plasma, with a function in ferrous iron oxidation ($\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$) that is needed to achieve iron's binding to transferrin^[10]. Ferroxidase I, also called ceruloplasmin, is the predominant copper protein in plasma and may also have antioxidant functions. Defects in ceruloplasmin function produce cellular iron accumulation, a result that supports its ferroxidase role⁹. Ferroxidase II is found in human plasma, but it may have a role in iron metabolism in specific cellular sites. A transmembrane copper-containing protein (hephaestin) with ferroxidase activity has been described^[11]. Cytochrome c oxidase is a multisubunit enzyme in mitochondria that catalyzes reduction of O_2 to H_2O . This establishes a high energy proton gradient required for adenosine triphosphate (ATP) synthesis. This copper enzyme is particularly abundant in tissues of greatest metabolic activity including heart, brain, and liver. Dopamine β monooxygenase uses ascorbate, copper, and O_2 to convert dopamine to norepinephrine, a neurotransmitter, produced in neuronal and adrenal gland cells. Dopa, a precursor of dopamine, and metabolites used in melanin formation are oxidatively produced from tyrosine by the copper enzyme tyrosinase. α -amidating

monooxygenase, also called peptidylglycine alpha amidating monooxygenase, uses copper and ascorbate to remove two carbons from a C-terminal glycine of peptides, thus generating an amide. A number of peptide hormones are posttranslationally modified by alpha amidating monooxygenase^[9].

Copper/zinc superoxide dismutase (Cu/Zn SOD) uses two copper atoms for the conversion of the superoxide anion (O_2^-) to H_2O_2 and O_2 . Zinc atoms have a structural role in the enzyme^[9]. The enzyme is localized in the cytosol and, along with the mitochondrial manganese-containing form, provides a defense against oxidative damage from superoxide radicals that, if uncontrolled, can lead to other damaging reactive oxygen species. Mutations in the Cu/Zn SOD gene, which alter the protein's redox behavior, produce amyotrophic lateral sclerosis (Lou Gehrig's disease).

These are the principal copper metalloenzymes found in humans. There is substantial documentation from animal studies that diets low in copper reduce the activities of many of these copper metalloenzymes. Activities of some copper metalloenzymes have been shown to decrease in human copper depletion^[12]. Physiologic consequences resulting from copper deficiency include defects in connective tissue that lead to vascular and skeletal problems, anemia associated with defective iron utilization, and possibly specific aspects of central nervous system dysfunction^[9]. Studies suggest that immune and cardiac dysfunction occurs in experimental copper deficiency and the development of such signs of deficiency has been demonstrated in infants¹³.

In spite of the great amount of work that has been done on the relationships between trace elements and diabetes, the evidence is still fragmentary. The nature of the correlations—whether it is a cause-to-effect relationship or simply a statistical association—is still unknown. The mechanisms of action are

also poorly understood. Further clinical investigations are needed to elucidate these problems, and hence the present study has been taken as a contribution to the research activities in this field with special emphasis on role of minerals in influencing the metabolic homeostasis in Type I and Type II diabetes.

MATERIAL & METHODS

Eighty five patients each of type I and type II diabetes of not more than 3 years duration were selected for the study. Eighty five individuals with no diabetes (whose 75 g oral glucose tolerance test in the last one year were normal) and absence of any systemic illness comprised the control group. Samples were collected from diabetic patients and normal individuals as per the guidelines of ICMR, New Delhi, India. Informed written consent was obtained from all subjects after explanation of the nature, purpose and potential risks of the study. History of presenting complaints was obtained from each patient.

Exclusion criteria: Other endocrinological dysfunctions, infectious diseases, malignancy, any other systemic illness, patients with history of cerebrovascular event or myocardial infarction, patients with a serum creatinine more than 1.5 mg/dl and those who received antihypertensive and/or diuretic drugs within the last one month.

The case history of the patients was obtained using a standard data collection sheet. The blood samples collected from diabetics and non diabetic controls were analysed for mineral contents using atomic absorption spectrophotometer- Iron, and Copper, at Central Institute of Fisheries Technology, Cochin, Kerala.

Chemicals: All the chemicals were purchased from National scientific suppliers, Puducherry, all of them were analytical grade.

Estimation of minerals using Atomic Absorption Spectrophotometer

Iron and copper were estimated according to the method of the AOAC (1980).

Reagents

1. Concentrated Nitric acid
2. Perchloric acid-70%
3. Nitric acid & Perchloric acid in 9:4 ratio
4. Stock solution of Iron and copper.

Iron: Dissolve 1000mg of iron granules in 20 ml of 5M hydrochloric acid. Dilute to 1 litre in a volumetric flask with deionised water. Working standard is prepared by diluting 1 ml of stock solution to 1000ml with deionised water(1 ppm).

Copper: Dissolve 1000mg of copper metal in 50 ml of 5M nitric acid. Dilute to 1 litre in a volumetric flask with deionised water. Working standard is prepared by diluting 1 ml of stock solution to 1000ml with deionised water(1 ppm).

STATISTICS:

Results were expressed as mean \pm SD. Multiple comparisons of the significant ANOVA were performed by Duncan's multiple comparison test. A P-value <0.001 was considered as statistically significant. All data were analyzed with the aid of statistical package program SPSS 10.0 for Windows.

RESULTS AND DISCUSSIONS

Iron plays a pathogenic role in diabetes and its complications such as microangiopathy and atherosclerosis^[14]. The importance of protein glycation is well known in the pathogenesis of diabetic vascular complications.

Transition metals also play a role in protein glycation induced by hyperglycemia. Glycated proteins possess a substantial attraction for the transition metals, and the bound metal retains redox activity and contributes to catalytic oxidation processes^[15]. Thus, should similar glycochelates form *in vivo*, reactions mediated by the iron chelates could be involved in the vascular complications of diabetes^[16]. During superoxide-dependent formation, more reactive radicals such as hydroxyl radical (OH^{\bullet}) requires the presence of transition metal ions such as copper or iron^[17]. Although OH^{\bullet} is highly reactive, its *in vivo* formation is contingent upon the availability of physiological iron. Interestingly, in the present study, a parallel increase in the amount of iron with the malondialdehyde release (an index of oxidative stress) was observed in Type I and Type II diabetic patients as compared to healthy normal individuals (Fig .1). Also as shown in the table.1, there is a significant increase in the levels of iron in type 1 DM subjects (Mean \pm SD, 93.21 \pm 6.67) when compared to healthy control subjects (Mean \pm SD, 70.38 \pm 6.25) at $p<0.001$. There is significant increase in the levels of iron in type 2 DM subjects (Mean \pm SD, 84.45 \pm 6.64) when compared to healthy control subjects (Mean \pm SD, 70.38 \pm 6.25) at $p<0.001$. Also, there is significant increase in the levels of iron in type 1 DM subjects (Mean \pm SD, 93.21 \pm 6.67) when compared to type 2 DM subjects (Mean \pm SD, 84.45 \pm 6.64) at $p<0.001$.

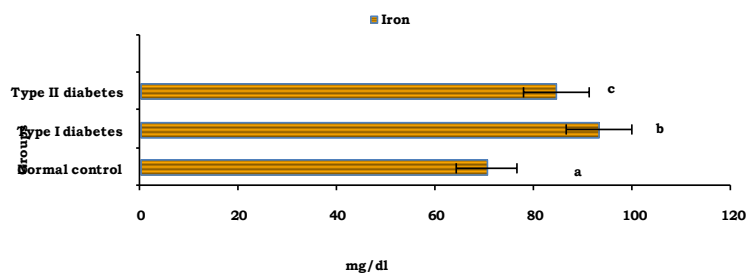


Figure .1 Levels of serum Iron content in normal

control and type I & type II diabetic patients
Results are Mean±SD for 85 samples. Values are
expressed as mg/dl. Values that have a different

superscript (a,b,c) differ significantly with each
other (P<0.05). Duncan's Multiple Range Test

Table .1. Levels of serum Iron in control, Type I DM & in Type II DM

Variables	Controls (n=85)	Type I (n=85)	Type II (n=85)	Significant values		
				Controls- Type I	Controls- Type II	Type 1 vs Type 11
Iron(mg/dl)	70.38±6.25	93.21±6.67	84.45±6.64	<0.001**	<0.001**	<0.001**

Results are presented in Mean ± S.D. P values are obtained by ANOVA with Post-hoc test.

**-Strongly significant

Since iron is a reactive metal ion that is known to catalyze damage to cellular macromolecules caused by oxygen radicals, its reduction from Fe³⁺ to the Fe²⁺ state plays a major role in lipid peroxidation process. As the concentration of iron increases, it finally accumulates in the liver. Ferritin, an iron storage protein may function as a source of iron for promotion of superoxide-dependent lipid peroxidation^[17]. The small size of O²⁻, which is generated by xanthine oxidase in conjunction with its ability to reduce chelated iron, suggests

that it is an excellent candidate for the mobilization of iron from ferritin. All parenterally administered iron in excess of the ferritin storage mechanism accumulates in the liver as hemosiderin. Thus the rise in the amount of iron in the serum of the diabetic patients might be either due to increased release of iron from the body storage depot into the systemic circulation or to attenuation in the process of storage related to oxidative stress. The table .2. shows iron overload states and diabetes.

Table .2 Iron overload states and diabetes

Genetic iron overload

1. HH (C282Y and H63D mutations)
2. Ferroportin disease
3. Hemojuvelin mutation
4. Hereditary aceruloplasminemia

Mitochondrial iron overload

1. Friedreich's ataxia (frataxin mutation)

Transfusional iron overload

Hepatic iron overload

1. Hepatitis C Virus(HCV)
2. Porphyria cutanea tarda

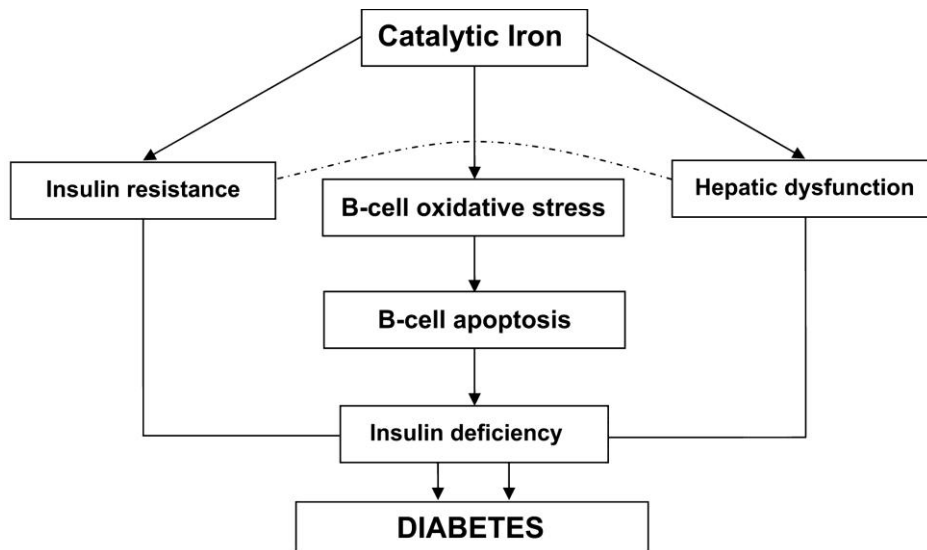


Fig .2 Pathogenic pathways for iron in induction of diabetes

Evidence linking iron to diabetic nephropathy includes 1) animal and epidemiological investigations, 2) researches in which an increased amount of iron has been demonstrated in the kidneys of both animals^[18,19] and humans^[20] with kidney disease, 3) evidence for higher urinary iron in patients with diabetic nephropathy, and 4) the inhibition of progression either by an iron-deficient diet or agents that bind and eliminate iron (chelators)^[21-23]. As shown in the **fig.2**. Earlier experimental investigations offer extensive proof for the role of iron and oxidants in the pathogenesis of diabetic nephropathy^[24-28]. Oxidative stress from factors such as hyperglycemia, advanced glycation end products, and dyslipidemia contribute to the obtainability of intracellular iron that can produce and viciously worsen oxidative deterioration and renal damage. Iron content in the kidney has been demonstrated to be amplified in an animal model of diabetes²⁹, and urinary iron excretion is elevated early in the course of diabetic renal disease in humans^[28,30]. There is substantial proof that, once renal insufficiency progresses, irrespective of

etiology, it inclines to headway over time. This has been interpreted to show certain common pathways for development of kidney disorders. Most notably, the pathogenic part of iron in progression is indicated by the observation that development can be prohibited either by an iron-deficient diet or chelators^[21-23]. A current randomized trial involving 191 patients with diabetes, proteinuria, and a decreased glomerular filtration rate exhibited that a low-iron-available, carbohydrate-restricted, polyphenol-enriched diet compared with a standard protein-restricted diet had a renoprotective activity^[31].

Epidemiologic investigations^[32-34] in explicit iron overload states such as transfusional iron overload and hemochromatosis have indicated that the incidence of coronary heart disease is increased³⁵ and that dietary intake with iron chelation recovers cardiovascular outcome. Likewise, numerous researches have indicated a direct connotation between higher iron intake, body iron reservoirs, and cardiovascular jeopardy in the general population. Elevated intake of heme iron is related with augmented

cardiovascular events^[36-39], and increased body iron stores are linked with myocardial ischemia in a prospective epidemiological investigation⁴⁰. Reliable and sensitive methods need to be developed to precisely measure the free/catalytic iron that participates in oxidative injury. Iron chelation therapy may present a novel way to interrupt the cycle of catalytic iron-induced oxidative stress and tissue injury and consequent release of catalytic iron in diabetes and to prevent diabetes-related complications.

Copper has been known to be essential for health for more than three quarters of a century. Copper functions as a component of a number of metalloenzymes acting as oxidases to achieve the reduction of molecular oxygen (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011)^[41]. The primary criterion used to estimate the Estimated Average Requirement (EAR) for copper is a combination of indicators, including plasma copper and ceruloplasmin concentrations, erythrocyte superoxide dismutase activity, and platelet copper concentration in controlled human depletion/repletion studies.^[42] In the present study, there was a significant elevation observed in serum copper content in Type I and Type II diabetic patients as compared to normal controls. This present finding is in corroboration with earlier reported study^[42], as shown in the Fig.4 and table .3., there is a significant increase in the levels of copper in type 1 DM subjects (Mean \pm SD, 164.02 \pm 13.27) when compared to healthy control subjects (Mean \pm SD, 95.54 \pm 6.78) at $p < 0.001$. There is significant increase in the levels of copper in type 2 DM subjects (Mean \pm SD, 142.86 \pm 10.69) when compared to healthy control subjects (Mean \pm SD, 95.54 \pm 6.78) at $p < 0.001$. Also, there is significant increase in the levels of copper in type 1 DM subjects (Mean \pm SD, 164.02 \pm 13.27) when compared to type 2 DM subjects (Mean \pm SD, 142.86 \pm 10.69) at $p < 0.001$.

Elevated level of copper in type I and type II diabetes mellitus is a major risk factor for the incidence of cardiovascular disease⁴³. Diabetic patients with vascular complications have higher plasma copper levels than diabetic patients without complications or normal controls^[43]. Patients with the "metabolic syndrome" (patients having common risk factors such as obesity, hypertension, glucose intolerance, and dyslipidemia) also have elevated copper levels^[44].

Copper overload in diabetes mellitus differs from that in Wilson's disease through differences in their respective causative molecular mechanisms, and resulting differences in tissue localization and behaviour of the excess copper. Pathogenetic tissue binding of copper is elevated in diabetes. It may well be mediated by advanced-glycation end product (AGE) modification of susceptible amino-acid residues in long-lived fibrous proteins, for example, connective tissue collagens in locations such as blood vessel walls.

Cooper et al. (2004)^[42] have shown that copper metabolism becomes abnormal after induction of diabetes in rats and that the copper chelator trientine, given to these animals, alleviated their heart failure, improved cardiomyocyte structure, and reversed elevations in left ventricular collagen and β_1 integrin without lowering blood glucose. They followed this up with studies in diabetic patients and showed that trientine therapy decreased left ventricular hypertrophy.^[42] Eaton & Qian, (2002)^[45] have shown beneficial effects of trientine in animal studies of diabetic neuropathy. Studies by Jung et al. (2011)^[46] have shown that elevated serum ceruloplasmin levels are associated with albuminuria in Korean men with type 2 diabetes mellitus.

Results are Mean \pm SD for 85 samples. Values are expressed as mg/dl. Values that have a different superscript (a,b,c) differ significantly with each other ($P < 0.05$). Duncan's Multiple Range Test. Reactive oxygen species (ROS) are

induced under diabetic conditions and are likely associated with the development of diabetes. It is also known that ROS production is facilitated in the presence of copper ion through the Fenton reaction^[47]. The results of the present investigation is in line with an earlier reported study^[36], which examined the involvement of

copper ion in the pathogenesis of type 2 diabetes and evaluated the potential usefulness of a copper chelating agent for the treatment of type 2 diabetes. Since copper ion is involved in the development of diabetes, it may be a potential therapeutic target for diabetes.

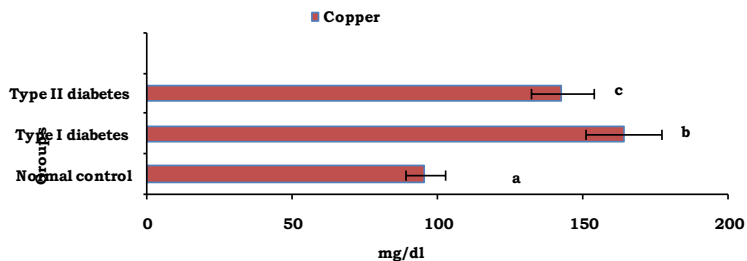


Figure.3 Levels of serum copper content in normal control and type I & type II diabetic patients

The biochemical role for copper is primarily catalytic, with many copper metalloenzymes acting as oxidases to achieve the reduction of molecular oxygen. Many copper metalloenzymes have been identified in humans^[48]. Copper/zinc superoxide dismutase (Cu/Zn SOD) uses two copper atoms for conversion of the superoxide anion (O_2^-) to H_2O_2 and O_2 . Our results also confirmed same pattern and showed significant enhancement in lipid peroxidation level (malondialdehyde release) in diabetic conditions. A concomitant

decline in antioxidant status (FRAP assay) was also observed.

Several other clinical observations deserve further investigation, but there is insufficient evidence to link them to marginal copper status. Glucose tolerance was lower in two of a group of eight men consuming 80 $\mu g/day$ of copper than in men consuming higher levels of copper^[49], but similar observations have not been reported at lower intakes of copper in other studies.

Table .3 Levels of serum Copper in control, Type I DM & in Type II DM

Variables	Controls (n=85)	Type I (n=85)	Type II (n=85)	Significant values		
				Controls- Type I	Controls- Type II	Type 1 vs Type 11
Copper(mg/dl)	95.54±6.78	164.02±13.27	142.86±10.69	<0.001**	<0.001**	<0.001**

Results are presented in Mean ± S.D. P values are obtained by ANOVA with Post-hoc test.

**-Strongly significant.

CONCLUSION

In summary, there is suggestive evidence that iron and copper plays a pathogenic role in diabetes and its complications such as microangiopathy and atherosclerosis. Reliable and sensitive methods need to be developed to precisely measure the free/catalytic iron and copper that participates in oxidative injury. Hence measuring the levels of iron and copper in early age may help to predict the onset of diabetes and its secondary complications which may postpone the diabetes.

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