Altered levels of serum Copper, Magnesium as a marker of oxidative stress in Predicting Systemic Inflammation in Type 2 Diabetes

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ABSTRACT

Introduction: Diabetes Mellitus is a metabolic disease characterized by hyperglycemia due to defective insulin secretion or action. Trace elements like Copper(Cu) and magnesium (Mg) have been found to be altered in diabetes mellitus and induce reactive oxygen species which has got role in progression of disease. Malondialdehyde (MDA) is a surrogate marker of oxidative stress in type 2 diabetes mellitus.

Aims And Objectives: Propose of the study was to estimate serum magnesium (Mg), copper and MDA levels in type 2 diabetes mellitus patients and to compare with that of healthy individuals, also to identify the inter-relationship among these and copper &magnesium can be used as an alternate marker of oxidative stress when MDA not available.

Materials And Methods:The study included 75 control healthy individuals without Type2 DM and 75 Cases with type 2 DM. Serum concentrations of Glucose, Total Cholesterol, Triglycerides, LDL-C, HDL-C, HbA1c, Magnesium, Copper and MDA was measured. Student's t-test, Pearson correlations tests were used for statistical analysis.

Results And Observations: The serum copper level was found to be significantly increased in type 2 DM with HbA1C >7% (157.32±16.89) when compared to control (106.24±25.99). The serum Mg level was found to be significantly decreased (1.62±0.29) (p < 0.0001) in type 2 DM with HbA1c >7 when compared to control (1.82±0.18). We found a significant increased level of serum Total Cholesterol and LDL-c and Triglycerides and decreased HDL-c in type 2 DM subjects with (p < 0.0001) HbA1c >7% when compared to control. MDA (4.62+1.17nmol/L) in type 2 DM patients when compared to controls(1.4+0.8 nmol/L)(p< 0.0001). Overall, mean serum MDA level in the study group was significantly higher than in the controls.

Conclusion: Patients with DM had altered metabolism of Cu (hypercuprimiea) and Mg (hypomagnesaemia). As Cu and Mg has got role in metal induced free radical formation which leads to oxidative stress we compared with novel oxidative marker MDA and concluded that these two trace elements can be used as marker of oxidative stress, which can predict systemic inflammation in type 2 DM to prevent well know complication in advance.

Keywords: Type 2 Diabetes Mellitus, Oxidative Stress, Copper, Magnesium, MDA

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INTRODUCTION

Type 2 diabetes mellitus (DM) is an endocrinological disease associated with hyperglycemia characterized by both insulin resistance and defective insulin secretion [1]. The metabolic derangement is frequently associated with permanent and irreversible functional and structural changes in the cells of the body, those of the vascular system, being particularly most susceptible. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of different organs, and these changes in turn lead to development of well-defined clinical problems, the so called complications, which may affect especially the eyes, kidneys, heart, blood vessels, the skin and the nervous system [2]. It is proved that excessive and/or sustained reactive oxygen species (ROS) production in hyperglycemia can directly or indirectly disturb the integrity and physiological function of cellular macromolecules and also by forming advanced glycation end products (AGE) through non-enzymatic advanced glycation and intracellular glucose auto-oxidation [3]. Oxidative stress is defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). There is considerable current interest in role of transition metal ions in inducing ROS generation. Direct association of minerals, trace elements and vitamins in the pathogenesis and natural course of both type 1 and 2 diabetes mellitus has been observed in many research studies.

Diabetes mellitus is a heterogeneous disease associated with an absolute or relative deficiency of minerals as well as insulin resistance [4]. Some trace elements act as antioxidants; prevent membrane peroxidation while others act directly on glucose metabolism. It is generally agreed that disturbed concentration of Copper (Cu) and Magnesium (Mg) in the body are often found in patients of diabetes mellitus. Copper is the third most abundant essential trace mineral in the body. Copper is toxic in its unbound form, and has a dominant role in diverse proteins such as cytochrome oxidase and cytoplasmic superoxide dismutase causes redox imbalance (oxidation and reduction), which leads to activation of stress sensitive intracellular signaling pathways through Haber-Weiss reaction [5]. Both increased and decreased Cu levels were found in diabetic patients. A deficiency of copper has been shown to result in glucose intolerance, decreased insulin response; increased glucose response, which lead to depression the activity of Cu-Zn SOD. This leads to increase in the amount of free radicals which result in the increase in the oxidative damage. In another study, it was found an increase in the level of copper, in patients with diabetes mellitus, may stimulate glycation and release of copper ions and this accelerates the oxidative stress through Fenton reaction [6]. Cu imbalance is implicated in cholesterol elevation by disrupting normal high density lipoproteins (HDL) and low density lipoproteins (LDL) balance [7].

Magnesium is the fourth most abundant cation in the body and the second most prevalent intracellular cation. Mg is the most abundant macro-nutrient which is essential for the maintenance of proper health. It is required for the activity of more than 300 enzymes, which serve several important physiological functions in the human body [8]. Mg containing enzymes are involved in the glucose homeostasis, nerve transmission, and DNA and RNA production [9]. Intracellular magnesium plays a key role in regulating insulin action, insulin-mediated glucose uptake, and vascular tone. Reduced intracellular Mg concentrations result in a defective tyrosine-kinase activity, postreceptorial impairment in insulin action, and worsening of insulin resistance in diabetic patients [10, 11]. Cellular magnesium is a critical cofactor for the activities of various enzymes involved in glucose transport, glucose oxidation, insulin release, and is a cofactor for ATPase and adenylatecyclase enzymes [8].

Chronic magnesium deficiency has also been associated with elevated concentrations of TNF-alpha, and this may also contribute to post receptor insulin resistance [12].

MDA is a major metabolite of arachidonic acid (20:4) [fatty acid with 20 carbons and four double-bonds]. It is well known that MDA serves as a reliable marker of free radical-mediated lipid peroxidation. It is one of the important indicators of free radical-mediated tissue injury. It participates in a variety of chemical and biological reactions including covalent binding to protein, RNA, and DNA. The endogenous formation of MDA during intracellular oxidative stress and its reaction with biologically important macromolecules makes MDA-DNA adducts a suitable biomarker of endogenous DNA damage [13]. Aims and objectives of the research was to estimate serum magnesium and copper, to compare with MDA levels in type 2 diabetes mellitus patients and to compare with that of healthy individuals. Hence copper and magnesium can be used as alternative marker of oxidative stress in type 2 diabetes mellitus in predicting systemic inflammation.

MATERIALS & METHODS:

A total of 75 patients (aged 30-70 years) with type-2 DM recruited from Institute's Medicine and Endocrinology departments. The study was approved by the Ethics Committee; a written informed consent was obtained from all participants for participation in this study. The diagnosis of type-2 DM was confirmed by biochemical investigations as per WHO criteria. Patients were excluded when diagnosed with type 2 DM, acute complications such as severe infection, major operations, trauma, GI disorders, severe cardiovascular/respiratory diseases, pregnant and breast feeding women. Patients taking supplements such as antioxidants, vitamins, minerals were also excluded. Age and sex matched 75 controls were recruited after clinical and biochemical evaluation.

The baseline demographic data and family history were obtained. 5 mL of venous blood sample was collected. The serum was used for analyzing Fasting Blood Glucose (FBG), Total cholesterol (TC), HDL-cholesterol (HDL-C), Triglycerides (TG). All the above mentioned parameters were measured using the autoanalyzer Beckman Coulterunicel DXC 600. For serum lipid reference level, National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) guideline was referred. According to NCEP-ATP III guideline, hypercholesterolemia is defined as TC>200 mg/dl, high LDL-C when value >100 mg/dl, hyper-triglyceridemia as TG >150 mg/dl and low HDL-C when value 30 mg/dl [14]. Serum copper was measured by modified spectrophotometric micro-method using guanidine hydrochloride and bathocuprinedisulphonate disodium salt [15]. Serum magnesium was measured by using the auto analyzer Beckman coulter DXC600. Serum magnesium was measured by calmagitemethod [16]. HbA1c was estimated by high performance liquid chromatography using D10. HbA1c calculated using NGSP formula. The final result reported as % [17]. Oxidative stress was assessed by quantifying thiobarbituric acid (TBA) reactivity as MDA in a spectrophotometer. To 0.5 ml of the serum, 0.5 ml of 30% Trichloroacetic acid (TCA) (Merck) was added and centrifuged at 3,000 rpm for 5 minutes and supernatant was collected. Thereafter, 0.5 ml of supernatant was added to 0.5 ml of 1% TBA (Merck) in a boiling water bath for 30 minutes following which tubes were kept in an ice-cold water bath for 10 minutes. The resulting chromogen absorbance was determined at the wavelength of 532 nm at room temperature against blank reference. The concentration of MDA was read from standard calibration curve plotted using 1, 1, 3, 3' tetra-ethoxy propane (TEP). The extent of lipid peroxidation was expressed as MDA (nmol/L). After analysis of groups of normal male and female volunteers,

researchers had reported the plasma levels of MDAof 1.076 nmol/L [18].

Statistical analysis: Statistical analysis was done by SPSS version 17.0. Pearson's correlation test was performed to examine correlations between various parameters. Independent samples t-test (2-tailed) was used to compare means of different parameters. All Values are expressed as mean ± standard error of mean. The results were considered statistically significant when P < 0.001.

Table 1: Distribution of age, levels of magnesium, copper, HbA1c, FBS, PPBS and Lipid profile among cases and control

Study Parameters	Controls	Cases	p Values
Age	48.44±11.23	52.12±10.50	
Serum magnesium (mg/dL)	1.82±0.18	1.62±0.29	<0.001**
Serum copper (mg/dL)	106.24±25.99	157.32±16.89	<0.001**
MDA (nmol/L)	1.4 ± 0.8	4.62±1.17	0.0001
HbA1c %	4.71±0.67	9.4±1.8	<0.001**
FBS (mg/dL)	81.96±19.04	185.98±63.11	<0.001**
PPBS (mg/dL)	113.56±42.39	226.84±89.64	<0.001**
Cholesterol (mg/dL)	188.58±39.59	257.30±56.39	<0.001**
Triglycerides (mg/dL)	112.56±55.44	253.18±58.98	<0.001**
HDL (mg/dL)	42.65±6.55	30.98±5.21	<0.001**
LDL (mg/dL)	98.74±28.30	191.17±86.17	<0.001**
VLDL (mg/dL)	31.59±11.56	51.31±11.79	<0.001**
L/H Ratio	3.98±1.84	6.59±1.52	<0.001**

Table 2: Pearson regression analysis between different study parameters

Parameters	Cases r value	Correlations	p value
MDA /FBS	0.72	Positive	<0.001**
MDA/PPBS	0.73	Positive	<0.001**
MDA/Cholesterol mg/dL	0.72	Positive	<0.001**
MDA /Triglycerides mg/dL	0.66	Positive	<0.001**
MDA /HDL	-0.34	Negative	<0.001**
MDA /LDL	0.54	Positive	<0.001**
MDA /VLDL	0.60	positive	<0.01
MDA /L/H Ratio	0.34	positive	<0.001**
Mg/Copper	-0.36	Negative	<0.01
HbA1C%/Mg	-0.35	Negative	<0.01
HbA1C%/Copper	0.28	positive	<0.05
MDA/Copper	0.38	Positive	<0.01
MDA/Mg	-0.42	Negative	<0.001
MDA/HbA1C	0.64	Positive	<0.01

RESULTS & DISCUSSION:

In our study it was observed that serum copper level was elevated in diabetic subject compared to healthy individuals, but serum magnesium level was significantly low. Plasma copper in NIDDM patients is increased when compared to healthy subjects (cases-157.32±16.89 control 106.24±25.99). HBA1C%/Copper, r valve- 0.283 p –value 0.013, which shows positive association which is statistically significant at p<0.05. Similarly FBS/ Copper-r valve 0.245, p valve 0.03 which shows positive association which is statistically significant at p<0.05.

Correlation between the various parameters is shown in Table 2. MDA shows statistically significant positive correlation with HbA1c, cholesterol, triglyceride, LDL-c, copper and negative correlation with HDL-c, magnesium.

Hypercupremia has positive correlation with plasma glucose level, while magnesium has negative correlation; these findings corroborate with the study Kaji et al [19]. Tanaka et al [20] reported that oxidative stress in type 2 DM is facilitated by hypercupremia as excess copper stimulates Fenton reaction. Compelling evidence is now accumulating to support a role of Cu in diabetes-induced oxidative stress. Firstly, ceruloplasmin can be fragmented following non-enzymatic glycosylation and the released Cu2+ can participate in Fenton-type reactions to

produce OH·-, which can further fragment ceruloplasmin. Secondly, glycation of Cu/Zn-SOD in humans with diabetes can lead to a site-specific fragmentation resulting in its inactivation as well as the release of Cu which can further exacebate oxidative stress [21]. Glycation of Cu/Zn-SOD has also been shown to increase the formation of DNA damage in vitro, suggesting that the release of $Cu2+$ from glycated-SOD can participate in cleavage of nuclear DNA. Glycated proteins which are higher in the diabetic patients, have an increased affinity for transition metals such as copper [22, 23, 24]. The redox active metal ions ($Cu2+$ and $Fe3+$) have been implicated in catalyzing the autoxidation of glycoaldehyde and generation of hydroxyl radical, leading to production of glyoxal and associated α - oxoaldehyde derived AGE (Advanced glycosylation end products) formation [25]. A wealth of experimental evidence supports the hypothesis that AGES formed from glyoxal, methylglyoxal and 3-deoxyglucosone have an etiological role in the development of diabetic complications and other diseases. In our study we also found that Hypercuprimia associated with increased HBAIC, cholesterol, LDL, Triglyceride and low HDL possible mechanism as explained above.

The plasma magnesium level is reduced in diabetes mellitus. Our data reveals significant differences in Type2 DM group versus the control group, for plasma magnesium (Table 1). Plasma total magnesium level is reduced in TDM patients compared to healthy controls (1.62±0.29 vs. 1.82±0.18 mg/dl, p< 0.001). There is a negative correlation between HBA1C%/Mg with r -value of -0.35, p-value 0.001 which is statistically significant at <0.01. We found statistically poorer glycemic control in the hypomagnesaemia patients as compared with the normo-magnesemia patients. In accordance with other authors our data reveals the existence of plasma low total magnesium level in patients with Type2 DM when compared to healthy adults. There is a link between hypomagnesaemia, augmentation of free radical production and its contribution to vascular injury and late complications of type-2 diabetes. Hypomagnesaemia may also lead to the induction of proinflammatory and profibrogenic response, cause reduction of protective enzymes against oxidative stress, cause induction or augmentation of vasoconstriction and hypertension, interfere with normal cell growth and apoptosis, and cause stimulation of aldosterone, among others Hypomagnesaemia can increase platelet reactivity, increase vascular and adrenal responses to angiotensin II, enhance thromboxane A2 release and lead to organ damage from free radicals [26].

In Type2 DM an inverse correlation between plasma level of magnesium and insulin resistance has been determined [27]. Intracellular deficiency in $Mg2+$ and Mg ATP2 can be a genetic factor that predisposes to type 2 diabetes mellitus [28]. There are data from previous researches that evinced positive correlations between magnesium deficiency and oxidative stress with reduced plasmatic antioxidant profile and increased lipid oxidation [29]. GSH, a tripeptide containing thiol groups is cofactor of many enzymes as it is glutathione peroxidase , $Mg2+$ being a mandatory cofactor in GSH synthesis as it is in all processes involving ATPase, and deficiency in Mg may induce alteration in GSH synthesis [30]. According to Grafton and Baxter [31], hypomagnesaemia leads to reduction of inositol transport and subsequent inositol depletion that might enhance the development of diabetic complications. Three recently published epidemiological studies from Harvard University and related institutions have reported a significant association between ongoing Mg intake, diabetic risk and fasting insulin levels [32].

In our study we also found that hypomagnesaemia associated with increased cholesterol, LDL, Triglyceride and low HDL possible mechanism being, in lipid metabolism, Mg is necessary for the activity of lecithin cholesterol acyltransferase and lipoprotein lipase, which lowers triglyceride levels and raises HDLcholesterol levels.

Moreover, Mg2+-ATP is also the controlling factor for the rate-limiting enzyme in the cholesterol biosynthesis associated with cholesterol levels [33].

MDA is a highly toxic by-product formed in part by oxidation derived from free lipid radicals, and studies have shown considerably raised concentrations in TYPE 2 DM. MDA reacts both irreversibly and reversibly with proteins and phospholipids with profound effects. In this study highly statistically significant differences in the levels of MDA were demonstrated between cases and the control group (Table 1). MDA showed significant increase ($p<0.01$). This results are in agreement with the findings of ,Mahreen R. et al., [34], and Ozdem et al., [35] MDA showed statistically significant positive correlation with HbA1c ($r=0.640$, $p<0.01$).

MDA also showed statistically significant positive correlation with cholesterol, triglycerides, and LDL-c ($r= 0.72$, $r =0.66$, $r= 0.54$, respectively; $p<0.001$) and showed negative correlation with HDL-c, Magnesium($r= -0.34$, $r= -0.42$ respectively; $p<0.001$.) The most probable causes for the increased MDA levels in serum of diabetic groups may be due to the abnormal lipid metabolism. Hyperinsulinemia and hyperglycemia may enhance the production of free radicals and induce oxidative stress that may also contribute to increased risk for coronary artery disease (CAD) in DM. Free radical interacts in archidonic acid metabolism forming a toxic endoperoxidase. The formed lipid peroxide thus enhances the synthesis of cyclooxygenase, prostaglandin and thromboxane which in turn causes increased platelets aggregation leading to vascular complications [36]. Turk et al., [37], 2002 and Salem et al.,[38] 2010 were observed statistically significant positive correlation in MDA level with HbA1c, our results were also in line with their findings, MDA also showed statistically significant positive correlation with cholesterol, and negative correlation with HDL-C that agree with Salem et al ., [38] and Nacitarhan et al., [39]

revealed significantly higher serum MDA level in patients with hyperlipidemic T2DM than in those with normolipidemic DM .

CONCLUSION:

The results of this study provide evidence that oxidative stress is present in patients with type 2 diabetes mellitus and is influenced by the duration of diabetes.There is growing strong evidence that excess generation of highly reactive free radicals, due to hyperglycaemia, causes oxidative stress, which further exacerbates the development and progression of diabetes resulting in vascular dysfunction, damage to cellular proteins, membrane lipids and nucleic acids.

Abnormalities in the metabolism of several trace elements play a significant role in pathogenesis and progression of the disease. Hypomagnesaemia and Hypercupremia can lead to insulin-resistance, abnormalities in carbohydrate and lipid metabolism. Along with antidiabetic therapy, supplementation of magnesium and chelation of copper can reduce complications of diabetes mellitus. All these observations suggest that serum magnesium and copper estimation should be a part of the screening panel in the risk detection and progression of diabetic complications independent of lipid peroxidation MDA.

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Declaration of Conflicting Interests:

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