Original article

Trace Minerals And Oxidative Stress In Diabetic Retinopathy

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Abstract:
There is accumulating evidence showing relationship between trace elements and Diabetes mellitus (DM). This study evaluated the role of magnesium, Zinc and other indices of peroxidative status ie MDA, Vitamin C and Vitamin E in pathogenesis of Retinopathy in type 2 diabetes. Seventy two type 2 diabetes cases were enrolled in the study, of which 42 were with retinopathy and 30 without. Patients with nephropathy were excluded. Forty age- and sex-matched subjects were served as health controls. The results showed that the mean values of Mg and Zn were significantly lower reduced in diabetes more so in diabetic retinopathy cases as compared to control subjects ($p<0.05$). Lipid peroxidation marker MDA was a significantly higher in both the diabetes groups whereas serum Vitamin C and vitamin E levels were significantly low ($p<0.05$) as compared to controls. Our correlation study revealed that MDA was negatively associated with serum Mg ($r=-0.73$, $p<0.01$) as well as serum Zn ($r= -0.82$, $p<0.01$), pointing towards the role of these trace elements in retarding the oxidative process prevailing in diabetic retinopathy.

Key Words: Trace elements, peroxidative status, diabetic retinopathy.

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Introduction:

Perturbation in mineral metabolism is more pronounced in diabetes population with complications like microangiopathy. Interest in trace elements has been steadily increasing over the last 20 years as alteration in the metabolism in several trace elements including Zinc[1] and magnesium[2,3] have been associated with impaired insulin release, insulin resistance and glucose intolerance in experimental animals and humans. The resulting chronic hyperglycaemia damages blood vessels and nerve cells producing micro vascular complications such as diabetic retinopathy.

Zinc an essential trace element is a component of many enzymes involved in synthesis, storage, release and conformational integrity of insulin monomers[4]. Zn assembles to dimeric forms for storage and secretion as crystalline insulin[5]. Zn has been found to enhance the effectiveness of insulin in vitro and it has been postulated that Zn deficiency may aggravate the insulin resistance in diabetic retinopathy[6]. Magnesium is an essential nutrient involved in glucose homeostasis. Hypomagnesaemia has been reported in diabetic retinopathy[7] while hypermagnesaemia has been noted in diabetic subjects particularly in a controlled state[8]. Differences in the above results may reflect study populations that varied greatly with respect to diabetic control and presence of micro vascular diseases. Changes in oxidative status may also be anticipated when trace element status is altered. Elevated levels of lipoperoxides have been reported and peroxidative damage has been proposed as a contributory factor in Diabetic retinopathy[9,10].

In this study we evaluated the Zn, Mg status in diabetic retinopathy subjects. Lipid peroxidation marker Malondialdehyde (MDA) and antioxidant vitamins i.e. vitamin C and vitamin E were also evaluat-

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ed to analyze the oxidative status in diabetic retinopathy. Correlation study was done to find out the relationship between the Lipid Peroxidation marker(MDA) and trace elements i.e. Zn and Mg. towards the progression of the disease.

Material and Methods:

The present study was conducted in the department of Biochemistry, Hi Tech Medical College, Bhubaneswar, from March 2011 to July 2012. Forty two diagnosed diabetic retinopathy cases were randomly selected from the OPD and indoor of the Department of Ophthalmology, Hi tech medical College, BBSR. Thirty type 2 diabetes subjects without retinopathy were also enrolled in the study. Type 2 diabetes subjects with nephropathy, cardiovascular involvement, neuropathy, history of smoking, alcoholism and hypertension were excluded from the study. Forty age- and sex matched apparently healthy individuals with normal plasma glucose, normal vision and no symptoms suggestive of diabetes were recruited from the staff of the department as controls. All diabetic cases were on oral hypoglycaemic drugs. None of the subjects was on oral Zn supplementation, antioxidant supplementation or on lipid lowering drugs.

Table-1: Age, Weight and BMI of Control and Cases

Parameters	Control (n=40)	Diabetes Without Retinopathy (n=30)	Diabetic Retinopathy (n=42)
Age (yrs)	58.26±7.41	56.30±6.29	64.07±6.23
Weight (Kg)	58.26±4.42	61.80±6.29	60.64±4.21
Body Mass Index (Kg/m^2)	23.21±3.21	24.27±2.71	24.20±2.51

Venous blood was collected in the morning after an overnight fast. Both cases and controls were subjected to estimation of glycaemic indices like FPG[11] and 2hr PPPG[11]. Estimation of serum Zn was done colorimetrically by Nitro PAPS method[12]. Zinc reacted with nitrophenol Adenosine Phosphosulfate (PAPS) producing a purple coloured complex which was measured at 578 nm[12]. Serum Mg was measured by Tabata et al (1985) reaction rate method[13] based on activation of Hexokinase by Magnesium ions. Rate of production of NADPH was measured at 340nm which is directly proportional to the concentration of Mg in the sample. Malondialdehyde (MDA), specific marker of lipid peroxidation product, forms a chromogenic adduct with TBA which was measured spectrophotometrically after butanol extraction[14]. Plasma vitamin C was measured by conversion to dehydroascorbic acid which reacted with acidic 2,4 dinitrophenyl hydrazine in presence of thiourea as a mild reducing agent , to form a red coloured compound bis-ydrazone, measured at 520 nm in spectrophotometer[15]. Serum Vitamin E was estimated by method of Baker and Frank[16].

Table-2: Biochemical Parameters in The Study Groups (Mean± Sd)

Parameters	Control (n=40)	Diabetic Without Retinopathy (n=30)	Diabetic Retinopathy (n=42)
FPG (mg/dl)	86.15 ± 6.50	186.38 ± 34.12 †	218.70 ± 37.64 †*
2hr PPPG (mg/dl)	104.42 ± 5.62	242.52 ± 31.06 †	288.28 ± 14.66 †*
Mg (mg/dl)	2.44 ± 0.56	1.48 ± 0.37 †	1.05 ± 0.35 † *
Zn (µgm/dl)	106.0 ± 12.03	77.60 ± 12.21 †	59.90 ± 5.97 †*
Vitamin C (mg/dl)	1.16 ± 0.19	0.70 ± 0.26 †	0.61 ± 0.17 †*
Vitamine E (mg/dl)	1.14 ± 0.14	o.68 ± 0.10 †	0.51 ± 0.12 †*
MDA (nmol /ml)	0.93 ±0.0.13	1.69 ± 0.15 †	2.02 ± 0.14 †*

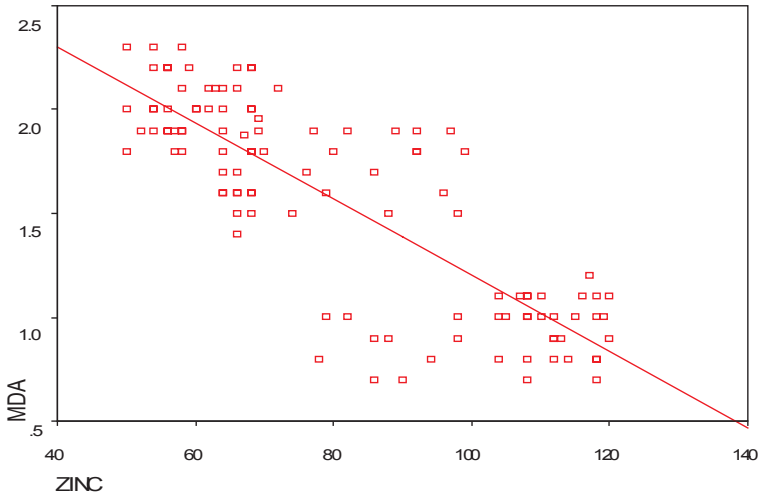
Results were expressed in mean±SD. One way Analysis of Variance (ANOVA), Post Hoc Turkey test and regression analysis were used for statistical analysis by SPSS software. The significance of observed differences among the groups were evaluated with Scheffe's Post hoc test, $P<0.05$ was significant. Correlation study was done using Pearson correlation coefficient (r value) and $P< 0.01$ was considered significant.

Results:

Age, weight and BMI were not significant predictors in the study groups (Table-1). Biochemical parameters of the study groups (Table-2) revealed high FPG and 2 hr PPPG in diabetes as compared to healthy control. Plasma level of trace elements (Mg and Zn), antioxidant Vitamins (Vitamin C and Vitamin E) were significantly low in diabetic groups than control whereas MDA registered a high value in diabetic retinopathy than control group. Multiple comparison demonstrated a significant low Mg and Zn level in diabetes without retinopathy and diabetic retinopathy when compared to control . Serum Mg and Zn levels were significantly low in diabetic retinopathy as compared to diabetes ($p<0.05$). Lipid

peroxidation marker MDA was observed to be significantly high ($p<0.05$) in diabetic retinopathy and diabetes without retinopathy as compared to control. Moreover diabetic retinopathy group registered significantly higher MDA values than diabetes without retinopathy, indicating peroxidative stress prevailing in diabetic retinopathy.

Correlation study evaluated by Pearson's correlation Coefficient and regression graph revealed a significant negative association ($r = - 0.73$, $p< 0.01$)between plasma MDA and plasma magnesium (graph-1) and with plasma Zn (graph-2)($r = - 0.82$, $p < 0.01$) pointing towards the protective consumption of Mg and Zn in the oxidative process.



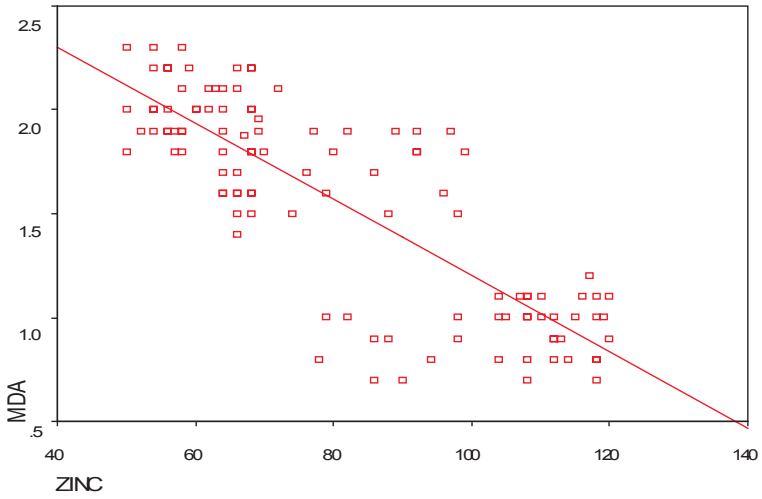
GRAPH-1: Correlation between serum Zn and plasma MDA

Discussion:

Trace elements have diverse metabolic characteristics and functions like catalytic, structural, regulatory functions by interacting with macromolecules such as enzymes, pro hormones, pre-secretary granules and biological membrane[17]. Mg plays a major role in phosphorylation of glucose. A significant relationship between Mg, glucose homeostasis and insulin sensitivity has been demonstrated[18]. Although there have been numerous studies evaluating the mineral status of Diabetic subjects, these studies yielded inconsistent results i.e. low and high(8) concentration of plasma Zinc have been reported in NIDDM as compared to control subjects . Blood Magnesium levels also revealed inconclusive findings in different studies. In present study hypomagnesaemia observed in diabetic retinopathy may be due to its enhanced utilization in enzyme activities involved in glucose oxidation. Mg may play a role in the release of insulin[19]. Mg mainly being intracellular, its intracellular uptake is stimulated by insulin[20] resulting in hypomagnesaemia. Consistent with the finding of some investigators[21] we observed lowered plasma Zn concentration in diabetic retinopathy group than control subjects. Zn plays an integral role in insulin production in the pancreas as well as glucose utilization and insulin secretion. Lowered intestinal absorption of Zn was also documented in chronic diabetes. Zn is highly concentrated in the eyes, mostly in the choroid and retina. Transport of vitamin A from the liver to the retina for synthesis of melanin is also promoted by Zn. Non insulin dependent diabetes mellitus (NIDDM) subjects with retinopathy reported decreased serum Zn concentration as a result of excessive urinary loss of Zn especially when patients had proteinuria[22]. Altered mineral status of diabetes may be due to well recognized cytokine response to vascular damage especially in retinopathy. During the acute phase response, liver metallothionein is induced with a resulting decrease in plasma Zn. Such alteration may reflect a response to retinal or vascular injury via an acute phase response in diabetic retinopathy. Fasting blood glucose and 2hr PPP glucose concentration were significantly more in diabetic subjects as compared to control. Hyperglycaemia generates oxidative stress which is amplified by metabolic stress. The increased production of Reactive oxygen species (ROS) is due to glucose auto oxidation, glyco oxidative activation of protein kinase-C (PKC) and increased Polyol pathway. Excessive lipid peroxidation has been suggested to be a potential biochemical lesion associated with the development of diabetic angiopathy including retinopathy[9].The high concentration of glucose may contribute to the higher plasma MDA levels seen in diabetes. Glucose can enolize and reduce molecular oxygen generating hydrogen peroxide and free radical intermediates[23]. Because glycosylation reactions are more notable in diabetes, particularly when poorly controlled, the increased oxidative activity, free radical production and glycosylation of proteins may play a role in the initiation of retinopathy. Significant fall in both vitamin C and vitamin E in diabetes mellitus more so in diabetic retinopathy

explains their protective consumption in the scavenging process. The uptake of vitamin into the cell is mediated by the process related to glucose transport and the high extracellular glucose in diabetes mellitus may further affect the cellular uptake and decrease its serum level. Therapeutic doses of vitamin C have demonstrated the reversal of early signs of retinopathy in diabetes confirming its role in preventing damage of blood vessels, as well as its therapeutic potential in diabetic retinopathy cases[24]. Further significant negative association between MDA and Zn (r = -0.82) may be attributed to the antioxidant property of Zn . In long term exposure, Zn induces antioxidant metallothionine whereas the acute effects of Zn include protection of protein sulfhydryl or reduction in the formation of OH^- from H_2O_2 through the antagonism of redox active transition metals such as iron and copper .Significant negative association between MDA and Mg (r = -0.73) reveals their protective consumption in the oxidative process prevailing in diabetic retinopathy.

In summary, in this study diabetes was associated with altered Mg and Zn metabolism. The perturbation in mineral status was particularly pronounced in diabetic subjects with retinopathy. Increased lipid peroxidation product MDA, along with weakness of the antioxidant defence system in diabetes, probably serve as a background for the genesis of endothelial dysfunction associated with diabetes ie retinopathy . Significant negative correlation between lipid peroxidation marker MDA and trace elements(Mg and Zn) points towards their protective role in the oxidative process. Further it remains to be determined whether the decreased serum Mg and Zn levels are a simple consequence of diabetes or if they in turn contribute to the clinical expression of the disease.



Graph 2: Correlation between serum Mg and plasma MDA

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