

STUDY OF MICRO ELEMENTS IN DIABETIC RETINOPATHY

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ABSTRACT: Diabetic retinopathy is one of the most devastating complications of diabetes. The prevalence of Diabetic retinopathy in India is relatively on the lower side as compared to the western countries. Indian studies have recorded prevalence rates of 34.1% and 37% in two studies from south India. Our aim of the study is to measure and compare the serum levels of zinc and magnesium in normal individuals and in diabetic retinopathy patients. We found that serum magnesium levels in diabetic retinopathy mean is $11.98 \pm 2.20 \mu\text{ gm/ml}$ levels were significantly lower ($P < 0.001$) than the controls, the value being $18.89 \pm 4.26 \mu\text{ gm/ml}$. Serum zinc levels in cases were being 0.422 ± 0.182 significantly lower than the control group $1.10 \pm 0.20 \mu\text{ gm/ml}$. ($p < 0.001$). Hypomagnesemia may be implicated in the etiology of diabetic retinopathy by causing reduction in the rate of inositol transport, causing subsequent intracellular inositol depletion and inhibition of $\text{Na}^+ \text{K}^+ \text{ATPase}$ activity thus leading to the development of this complication. Zinc has been shown to stimulate insulin action and insulin receptor tyrosine kinase activity. Its deficiency interferes with its normal physiological enzyme and hormone action causing impairment of insulin action and development of insulin resistance leading to diabetes mellitus and its complications. The decrease in these micro elements could be due to poor glycemic control, osmotic diuresis and altered metabolism.

KEY WORDS: Diabetic retinopathy, zinc, magnesium, metallo-enzyme

INTRODUCTION: Diabetes mellitus is a multi-factorial disease characterized by deficiency of insulin or resistance to insulin. Diabetic Retinopathy is one of the most common microvascular complications of diabetes, affecting 80% of patients over 20 years duration of diabetes. It is the major cause of blindness among working age individuals in developed countries and in developing countries like India also, it may become one of the major cause of blindness in view of prevailing diabetes epidemic which can eventually lead to blindness¹. Biochemical and physiological changes that occur very early in the retina of diabetic patients are the major signaling determinants of future damage to the retina. Research during the past few decades has provided ample evidence that hyperglycemia is one of the main factors driving the onset and progression of diabetic retinopathy². Several factors have been implicated in the pathogenesis of diabetic retinopathy. These include non enzymatic glycation, glycooxidation, accumulation of advanced glycation end products, Free radical mediated protein damage, up regulation of metalloproteinases. Direct association of trace elements with diabetes mellitus has been observed in many research studies³.

Zinc is metalloenzyme which forms Zn-enzyme complex. In presence of zinc insulin molecules assemble to a dimeric form in vivo and hexameric form in vitro. This hexameric form is clinically used insulin⁴. In diabetic retinopathy there is a disturbance of this vital trace element⁵. Zinc plays an important role in insulin synthesis and storage of insulin which is secreted as zinc crystal. It helps in maintaining structural integrity of insulin⁶. Zinc also plays a key role in the cellular antioxidative defense mechanisms. Zinc deficiency causes oxidative stress, which damages the cell irreversibly, producing or exacerbating some of the classic complications of diabetes⁷.

Magnesium is a cofactor in the phosphorylation of glucose and in many other enzymatic reactions⁸. Its deficiency drives in insulin resistance, carbohydrate intolerance, dislipidemia and complications of diabetic retinopathy. Studies reported that the diabetes induced damage to the eyes is more likely to occur in magnesium deficient patients with insulin dependent diabetes mellitus and suggested hypomagnesemia as a possible risk factor in the development and progress of diabetic retinopathy.^{9,10} It is suggested that the hypomagnesemia leads to reduction in rate of inositol transport and subsequent inositol depletion that might enhance the development of diabetic complications. This inositol depletion allows hypomagnesemia and the polyol pathway to be unified into one mechanistic model for the development of the diabetic retinopathy. The polyol theory states that hyperglycemia drives the intracellular accumulation of sorbitol with subsequent inositol depletion and inhibition of Na⁺-K⁺ ATPase activity causing alteration in the maintenance of Na⁺-K⁺ gradient and alteration in glucose transport. Most Na⁺ extrusion and K⁺ influx are dependent upon the Na⁺-K⁺ pump which is ouabain sensitive Na⁺-K⁺ ATPase. It is necessary for maintaining intracellular K⁺ concentration and it is a magnesium dependent enzyme. It has been reported that this impaired enzyme activity plays a role in the pathogenesis of diabetic retinopathy¹¹.

It is to be known whether decreases in trace elements levels are a consequence of diabetic retinopathy and hyperglycemia or their deficiencies contribute to the expression of the diabetes¹². It is generally believed that strict metabolic control delays the development of late complications in DM, it has not been demonstrated conclusively that such control holds back the development of diabetic retinopathy.

We conducted this study to evaluate levels of zinc and magnesium in diabetic retinopathy patients to further clarify their role in this disease.

MATERIAL AND METHODS: The blood samples in the present study were obtained from medical department and ophthalmology department of the Gandhi hospital and from Sarojini Devi Eye hospital for diabetic retinopathy patients. Fasting blood sugar, HbA1C, zinc and magnesium levels were estimated in the blood samples out of which 30 healthy patients taken as controls and 30 diabetic patient with non proliferative retinopathy. Prominent clinical features included are micro aneurysms, venous abnormalities, hard yellow exudates, intra retinal microvascular abnormalities and cotton wool spots.

Controls selected in our study ranged from 35 years to 60 years of both sexes subjects were divided into two groups controls as group 1 and patients with diabetic retinopathy taken as group 2 subjects who are not taking any kind of trace element supplements.

Samples were collected to estimate fasting glucose by GOD-POD method. This method was used because of its specificity, reliability and simplicity. Glycated hemoglobins estimated by total glycohemoglobin Ion exchange resin method.

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Analysis of minerals was done in plasma by using Atomic Absorption Spectroscopy, Varian Spectra AA 220 model atomic absorption spectrophotometer, Varian Australia Pvt Ltd, (Mulgrave Victoria). For the analysis of Mg, and Zn, flame ionization method was used.

REFERENCE VALUES: (Teitz text book of clinical chemistry 3rd edition13)

Normal serum level of zinc is 0.8-1.2 μ gms/ml.

Normal magnesium levels are 16-26 μ gms /ml.

Study was in accordance with the ethical standards.

RESULTS: The data was analysed by using SPSS 15.0 version and Microsoft Excel software. The results were expressed as Mean and Standard deviation (S.D). Independent sample 't' test was used to assess the significance of difference of means between the cases and controls. $P < 0.05$ was considered as significant. The results were represented in the form of tables and bar diagrams.

TABLE- I: The Mean \pm S.D. of fasting Blood glucose level (mg %) in the various study groups.

TABLE: II : The Mean \pm S.D of HbA1C(%) in study groups.

TABLE-III :The Mean \pm S.D of Serum Magnesium levels (μ gms/ml).

TABLE- IV :The Mean \pm S.D of Serum Zinc levels (μ gms/ml) in various study groups

Were taken for analysis.

DISCUSSION: Diabetic retinopathy is common micro vascular complications of both type I and type II diabetic retinopathy that can severely impair visual acuity, eventually leading to blindness. The prevalence of retinopathy increases with the duration of diabetic retinopathy¹⁴.

In the present study, blood glucose and HbA1c levels were significantly raised in the diabetic group, being 159.22 ± 45.23 mg/dl and $7.896 \pm 1.170\%$ in group II patients respectively. (Table- I and Table- II)

Our observations showed a significant lowering ($p < 0.001$) of serum magnesium in diabetic retinopathy from Table- III and figure I, which correlates with study of Ceriello et al¹⁵ Elamin A et al and 16Harold W et al¹⁷. Altered metabolism, poor glycemic control and osmotic diuresis may be factors¹⁸ in causing hypomagnesemia and hyperglycemia^{19,20,21}.

Magnesium is an essential ion involved in glucose metabolism. It plays an important role in the activities of various enzymes involved in glucose oxidation, and may play role in the release of insulin^{4,21,22}. It is mainly intracellular and its intracellular uptake is stimulated by insulin²². Cellular magnesium deficiency causes impaired inositol transport and inositol depletion which alter the activity of membrane bound Na-K ATPase^{10,19}. Low concentrations of magnesium can decrease secretion of insulin by the pancreas. In diabetic retinopathy there is a direct relationship between serum magnesium level and cellular glucose disposal that is independent of insulin secretion¹⁹.

In the present study, serum zinc levels were significantly lowered in diabetic group with retinopathy ($p < 0.001$) from Table- IV and figure II, correlation with Garg V²⁴. The cause of decreased serum zinc levels in diabetic retinopathy is may be due to increase in urinary loss. Zinc is found to amplify the effect of insulin in vitro and zinc deficiency may intensify the insulin resistance in type II diabetic retinopathy²³.

Zinc plays a important role in glucose metabolism relationship between diabetic retinopathy, insulin and zinc is complex with no clear cause and effect relationships. Zinc is

involved in the synthesis, storage and secretion of insulin as well as conformational integrity of insulin form. It has a protective role against β cell destruction. The complications of diabetic retinopathy may be mediated, at least in part, through oxidative stress, and zinc plays a key role in the cellular anti oxidative defense. It has been suggested that the zinc metabolism certainly play a important role in the pathogenesis of diabetic retinopathy 24.

Increased fasting blood glucose is the hallmark of diabetic retinopathy. In the present study fasting blood glucose is significantly raised in diabetic groups ($p < 0.001$) when compared to normal controls. It has been suggested that damaging effect on endothelial cells in diabetic retinopathy are due to high glucose. Although endothelial cells have insulin receptors, most of glucose enters the cells through facilitated transport of D-Glucose²⁵ and this transport mechanism is significantly increased in diabetic retinopathy.

CONCLUSION AND SUMMARY: Retinopathy is a common microvascular complication of diabetic retinopathy leading to blindness. It proves to be zinc and magnesium depletion reduces insulin sensitivity and may increase risk of secondary complications. Hence it may be prudent in clinical practice to periodically monitor plasma magnesium and zinc concentrations in diabetic patients. So therapeutic intervention to increase dietary intake and oral supplementation of zinc and magnesium may be advantageous.

Conflict of interest: Declared none.

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TABLE- I: The Mean \pm SD of fasting Blood glucose level (mg %) in the various study groups.

Group	Mean \pm SD	S.E
Group-I (Normal controls)	84.36 \pm 15.3	2.978
Group-II Diabetic patients with Retinopathy	159.22 \pm 45.23	8.76

TABLE: II: The Mean \pm S.D of HbA1C (%) in study

Group	Mean \pm SD	S.E
Group-I (Normal controls)	5.06 \pm 0.587	0.117

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Group-II Diabetic patients with Retinopathy	7.89±1.17	0.234
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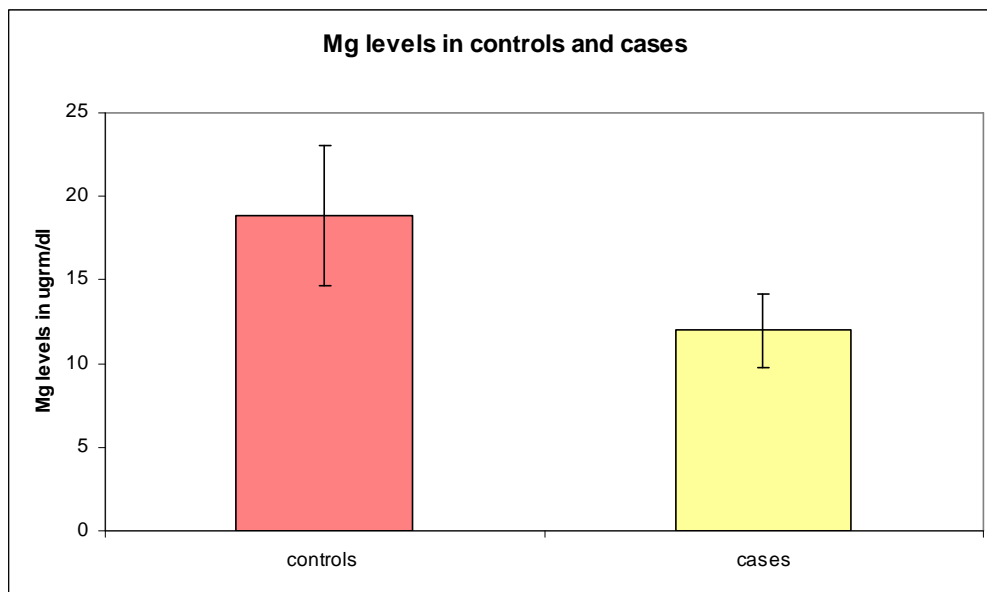
TABLE-III: The Mean ± S.D of Serum Magnesium levels (µgms/ml)

Group	Mean ± SD	S.E
Group-I (Normal controls)	18.89±4.26	0.852
Group-II Diabetic patients with Retinopathy	11.98±2.2	0.441

TABLE- IV: The Mean ± S.D of Serum Zinc levels (µgms/ml) in various study Groups

Group	Mean ± SD	S.E
Group-I (Normal controls)	1.1±0.202	0.0404
Group-II Diabetic patients with Retinopathy	0.422±0.182	0.0364

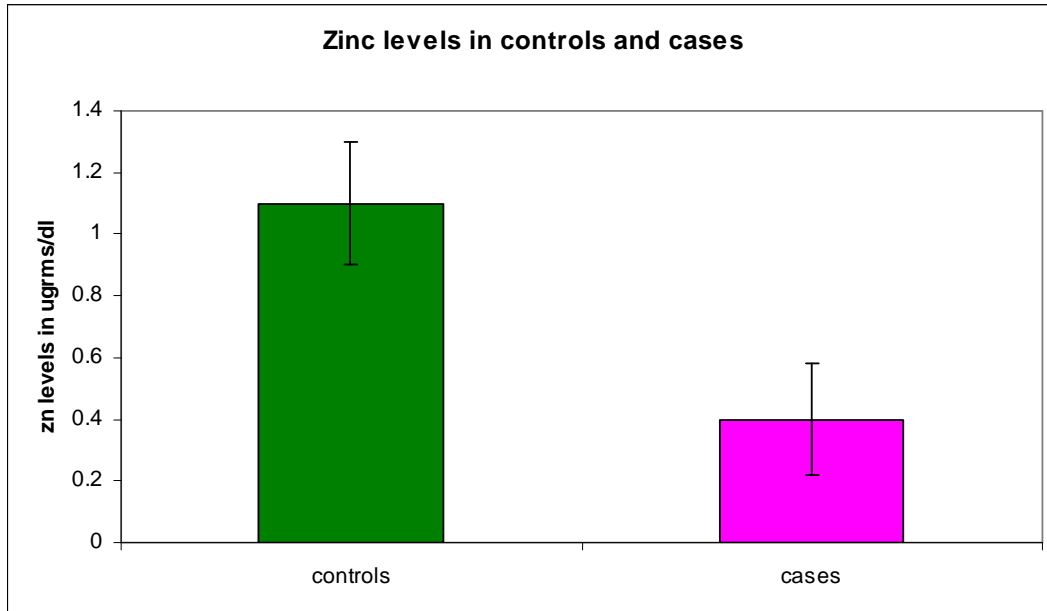
Figure 1: Comparison of Mean values of Serum Magnesium levels (µgms/ml) in the study groups



* The mean difference is significant at the .05 level.

Serum magnesium levels in diabetic patients with retinopathy were $11.98 \pm 2.20 \mu$ were significantly lower ($P < 0.001$) than the controls, the value being $18.89 \pm 4.26 \mu \text{ gm/ml}$.

Figure 2: Comparison of Mean values of Serum Zinc ($\mu\text{gms/ml}$) in the study Groups



Serum zinc levels in group II were $0.422 \pm 0.182 \mu \text{ gm/ml}$ significantly lower than the control group $1.10 \pm 0.202 \mu \text{ gm/ml}$. ($p < 0.001$)