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STUDY OF MAGNESIUM, GLYCOSYLATED HEMOGLOBIN AND LIPID PROFILE IN DIABETIC RETINOPATHY

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ABSTRACT

The present study was undertaken in 100 subjects, 30 diabetics without complication (group I), 40 diabetics with retinopathy (group II) and 30 non diabetic as normal control group (group III). Blood sugar levels, magnesium, cholesterol and triglyceride were analyzed from plasma and serum. The results were correlated with degree of diabetic control from the levels of glycosylated hemoglobin. Serum magnesium levels in group II were found to be significantly lowered than in group I. There was also significant difference in magnesium levels of group I and group III. We found a significant correlation between the glycosylated hemoglobin and magnesium levels in our study.

The results also indicate that the patients with diabetic retinopathy showed significant rise in serum cholesterol and triglyceride. Probably hypomagnesemia and increased serum cholesterol and triglyceride levels are responsible for microvascular changes in diabetes leading to retinopathy. The purpose of this study was thus to gather information about the degree of control of diabetes and magnesium status.

INTRODUCTION

Hypomagnesemia has long been known to be associated with diabetes mellitus, Mather *et al.* confirmed the presence of hypomagnesemia in nearly 25% of their diabetic out patient (1). Low serum magnesium level has been reported in children with insulin dependent diabetes mellitus (2, 3). The association between diabetes mellitus and hypomagnesemia is compelling for its wide ranging impact on diabetic control and complications. Magnesium depletion has been linked to the development of retinopathy (4, 5).

Although it is generally believed that strict metabolic control delays the development of late complications in diabetes mellitus(6), it has not been demonstrated conclusively that such control holds back the development of diabetic retinopathy (7). Glycosylated hemoglobin have been postulated as a biochemical model for the pathogenesis of diabetic sequelae through the glycosylation reactions (8).

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Hence this work was carried out to study the correlation between glycosylated hemoglobin, magnesium status, serum lipids in diabetic patients with retinopathy.

MATERIAL AND METHODS

The study was carried out in Govt. Medical college and Hospital Aurangabad. Total 100 cases were selected and divided into 3 different groups. The 70 diabetics (with diabetic history of 10 to 20 years) subjects were included in study who were attending diabetic clinic. The subjects were in the age group of 40 to 65 years. They were screened for the presence of retinopathy. Retinopathy was assessed by direct and indirect ophthalmoscopy, fundus photography. On this basis they were divided in to two groups. Group I included 30 diabetics without retinopathy or with very minor changes, group II included 40 diabetics with retinopathy i.e. having microaneurysm with exudates, hemorrhages and proliferative changes. Group III was the control group which included 30 non-diabetic age and sex matched individuals. Hypertension, chronic diarrohea, alcoholism, use of diuretics, reduced renal function were exclusion criteria.

Glycosylated Haemoglobin (HbA_1c) estimation was carried out by a modified calorimetric method of Fluckiger and Winterhalter (9). 5ml heparinised venous blood was centrifuged to collect RBCs. The packed cells were washed 3-4 times in normal

saline. After final wash 0.5ml of distilled water and 0.5 ml of CCl4 were added and mixed vigorously then centrifuged. The supernatant haemolysate were separated and its Hb concentration was adjusted to 10 gms% with distill water.

To 2 ml of haemolysate, 1ml of 0.3 N oxalic acid was added and heated in incubator at 100°C for 60 min. After cooling 1 ml of 40% T.C.A. was added, shaken vigorously and centrifuged. To 2 ml of this supernatant 0.5 ml of 0.05 M thiobarbituric acid was added and incubated at 37°C for 30 min. The resultant yellow color was read on colorimeter at 443 nm, HbA_1c was calculated on assumption that 1% HbA_1c corresponds to an absorbance of 0.029 at 443 nm.

Fasting samples were used for analysis, magnesium was estimated by Arsenazo method, cholesterol by COD-POD method and triglyceride by Trinder's GPO-POD method. Statistical analysis was done by using students 't' test.

RESULTS

The average concentration of magnesium in groups I (diabetic without retinopathy), group II (diabetic with retinopathy), and group III (control) were measured as 2.13 ± 0.32, 1.2 ± 0.38 and 2.60 ± 0.37 meq/l respectively. The patients in group II has hypomagnesemia ($p < 0.01$) when compared to group I. Also group I showed hypomagnesemia ($p < 0.01$) when compared to group III. Group I, group II and III were also compared with respect to glycosylated hemoglobin, blood glucose, triglyceride and cholesterol levels.

The HbA_1c (%) values in group I, group II and group III were measured as 7.62 ± 0.69, 10.48 ± 1.22 and 4.99 ± 0.98 respectively. These values

Table I Showing Mean ± S.D. of HbA_1C and blood Glucose in different groups

Group	Blood Glucose (mg%)		HbA_1C %
	Fasting	Postmeal	
I (Diabetic Without Retinopathy)	163±20.31	208±18.5	7.62±0.69
II (Diabetic Retinopathy)	235±20.03	267±25.6	10.48±1.22
III (Normal Control)	95.12±9.31	140±9.4	4.99±0.98
Group II versus group I		$p < 0.001$	
Group I versus group III		$p < 0.01$	

Table 2 Showing mean ± S.D. of Magnesium, Cholesterol and Triglyceride in different groups

Group	Magnesium (mg%)	Cholesterol (mg%)	Triglyceride (mg%)
I (Diabetic Without Retinopathy)	2.13±0.32	213±11.51	151±10.86
II (Diabetic Retinopathy)	1.2±0.38	262±11.30	174±7.87
III (Normal Control)	2.60±0.37	164±13.39	120±9.21
Group II versus group I		$p < 0.001$	
Group I versus group III		$p < 0.01$	

were found to be significantly higher (group II $p < 0.001$ and group I $p < 0.01$) and correlated positively with blood glucose levels.

The serum cholesterol levels were estimated as 213 ± 11.51, 262 ±11.30, 164 ± 13.39 mgs/dl for group I, group II and group III respectively.

The serum triglycerides levels were 151 ± 10.86, 174 ± 7.87, 120 ±9.21 for group I, group II and group III respectively.

The serum cholesterol and triglyceride values were significantly higher in group II ($p < 0.001$) and group I ($p < 0.01$) as compared to group III.

DISCUSSION

The possible atherogenic potency of hypomagnesemia prompted this study (10). Magnesium is involved on multiple levels in insulin secretion, binding and activity. Magnesium activates more than 300 enzymes in body and is a critical cofactor of many enzymes in carbohydrate metabolism. Cellular magnesium deficiency can alter the activity of membrane bound sodium-potassium ATPase (11) which is involved in maintenance of gradients of sodium, potassium and in glucose transport. Low levels of magnesium can reduce secretion of insulin by the pancreas (12). In diabetes there is a direct relationship between serum magnesium level and cellular glucose disposal that is independent of insulin secretion. This change in glucose disposal has been shown to be related to increased sensitivity of the tissues to insulin in presence of adequate magnesium levels (13).

Our observations revealed a definite lowering of serum magnesium in diabetic patients without retinopathy and more over patients who had

retinopathy were found to have lowest concentration of serum magnesium. These observation are similar to other workers (4, 5, 14). Consequently we suggest hypomagne-semia as a possible risk factor in the development and progress of diabetic retinopathy. The exact cause of diabetic hypomagnesemia is still unknown but an increased urinary loss of magnesium may contribute to it. Two factors may work together in this respect namely, the osmotic action of glucosuria and the hyperglycemia per se, the latter being known to depress the net tubular reabsorption of magnesium in normal man (15-17).

Glycosylated Haemoglobin (HbA_1c) results from post translational changes in the haemoglobin molecule, and their levels correlate well with glycemic levels over the previous six to ten weeks. Glycosylation of haemoglobin takes place under physiological condition by a reaction between glucose and N-terminal valine of Beta chain of Hb molecules.

We measured HbA_1c levels in a series of 70 diabetics (with and without retinopathy) and in confirmation to previous reports (18, 19) found a significantly higher HbA_1c levels compared to healthy control. The mean HbA_1c levels in diabetic with retinopathy were higher than in diabetes without retinopathy and it was stadstically significant ($p < 0.001$). From our results we are able to predict that higher levels of HbA_1c indicate risk for development of microangiopathy in diabetic. HbA_1c has special affinity for oxygen there by causes tissue anoxia and plays a role in causation of micro and macroangiopathy (20). Our study has also been confirmed by Boucher *et al.* (21) who documented that levels of HbA_1c above 12.6% indicate a risk for development of micro-angiopathy. We also obtained a significant positive correlation of HbA_1c with fasting blood glucose levels as reported earlier in diabetics with microangiopathy (22).

Thus the measurement of glycosylated haemoglobin not only shows promise of being a successful approach to the monitoring of diabetic patient but also provides a conceptual frame work for the pathogenesis of secondary sequelae of diabetes. Statistically significant correlation was found between HbA_1c and serum cholesterol and triglycerides. Our results in this regard are similar to the findings of other authors (23-25).

Other workers have documented that the oral magnesium supplementation improves insulin sensitivity and metabolic control in type II diabetics with decreased serum magnesium levels (26) and have also shown that it has beneficial effect on lipid profile of these subjects. Hence further studies on oral magnesium supplementation to prevent late complication of diabetics will be interesting and helpful (27).

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