SERUM MAGNESIUM, LIPID PROFILE AND GLYCATED HAEMOGLOBIN IN DIABETIC RETINOPATHY

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ABSTRACT

BACKGROUND
Diabetic retinopathy is one of the important microvascular complications of diabetes mellitus of long duration. Alterations in trace metals like magnesium and lipid profile was observed in diabetic retinopathy with hyperglycaemic status.

AIM
The study was taken up to assess the role of magnesium, lipid profile and glycated haemoglobin in diabetic retinopathy.

MATERIALS AND METHODS
A total of 80 subjects between 40-65 years were included in the study. Group 1 includes 20 age and sex matched healthy controls. Group 2 includes 30 cases of Diabetes mellitus without retinopathy. Group 3 includes 30 cases of Diabetes mellitus with retinopathy.

RESULTS
Magnesium was found to be significantly low in the diabetic group with retinopathy. Serum cholesterol and triglycerides were significantly elevated in the diabetic group with retinopathy. Fasting and Postprandial plasma glucose and glycated haemoglobin (HbA1c) levels confirmed the glycaemic status of each of the groups.

CONCLUSIONS
Hypomagnesemia, hypercholesterolaemia, hypertriglyceridemia was observed in diabetic retinopathy along with increased levels of glycated haemoglobin in our study.

KEYWORDS
Magnesium, Glycated Haemoglobin, Diabetic Retinopathy, Glycaemic Status, Insulin Resistance, Insulin Sensitivity, Glucose Disposal.

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INTRODUCTION: As per the statistics, globally 300 million are suffering from Type 2 diabetes mellitus (T2DM). India has maximum number of diabetic cases with 31.7 million in the year 2000 and expected to rise further to 69.9 million by 2025 and the prevalence is estimated to double in the coming decades.[1] Type 2 diabetes mellitus is characterised by insulin deficiency and insulin resistance. Hyperglycemia occurs when endogenously produced insulin can no longer match the increased demand as a result of insulin resistance.[2] Hyperglycemia can lead to acute and chronic complications. Chronic microvascular changes in the eye, kidney and nerves lead to retinopathy, nephropathy and neuropathy respectively.

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Chronic macrovascular changes lead to coronary artery disease, cerebrovascular disease and peripheral vascular disease.[3]

Diabetic Retinopathy Can be classified into the Following Sub-Divisions by Funduscopy:

1. Back ground or Non-Proliferative retinopathy and is characterised by micro-aneurysms, haemorrhages, hard exudates, cotton-wool spots and venous dilatation.
2. Background retinopathy with macular oedema (Diabetic maculopathy) characterised by visual impairment which is due to fluid accumulation in the macula.
3. Pre-proliferative and proliferative retinopathy characterised by venous changes, clusters of larger blot haemorrhages, multiple cotton wool spots and occluded arteries.
4. Advanced diabetic eye disease: The characteristics features are pre-retinal or vitreous haemorrhages, fibrous tissue in front of the macula, retinal traction and detachment and neovascular glaucoma.[4]

Magnesium our molecule of interest has insulin dependent and insulin independent actions on glucose metabolism and it is one of the important cofactor to many enzymes in carbohydrate metabolism.[5] Hypomagnesemia has been incriminated in the development of microvascular complications of diabetes mellitus.[6] The cause of Hypomagnesemia in diabetes mellitus is still unclear. One possible hypothesis is that hyperglycemia is accompanied by excretion of glucose and magnesium. In addition, the hyperglycemia per se reduces the net tubular reabsorption of magnesium.[7] Elevated serum cholesterol and triglyceride levels have long been associated with diabetes and its complications (Micro and macrovascular complications). Serum triglycerides correlate more closely with glycated haemoglobin (HbA1c) which confirms the undeniable association between hyperglycemia and elevated triglycerides.[8]

HbA1c reflects the mean plasma glucose levels over the previous 8-12 weeks.[9] The greatest advantage of glycated haemoglobin over fasting plasma glucose and postprandial plasma glucose is that it can be performed at any time of the day without any prior preparation. In recent times, there is a growing interest in using it as a diagnostic marker for diabetes after the WHO in 2009 recommended it as a diagnostic test for diabetes mellitus, provided the assays are standardised.[10] The present study was aimed to estimate the levels of serum magnesium, lipid profile and glycated haemoglobin in diabetic retinopathy.

MATERIALS AND METHODS: The present study was conducted in the Department of Biochemistry in collaboration with the Department of Ophthalmology at Prathima Institute of Medical Sciences, Nagunur, Karimnagar, Telangana state. This study was approved from the Institutional Ethics Committee. Informed consent was obtained from all the subjects prior to sample collection.

80 subjects were included in this study between the ages of 40-65 years and divided into 3 groups.

- Group I- 20 normal healthy subjects as controls.
- Group II- 30 diabetic patients without retinopathy.
- Group III- 30 diabetic patients with retinopathy.

Patients suffering from Hypertension and Renal failure were excluded from the study. Retinopathy was assessed by direct and indirect ophthalmoscopy and fundus photography.[11,12,13,14] 3 mL of venous blood sample was collected after overnight fasting in plain tube and allowed to clot. The samples were then centrifuged at 3000 rpm for 10 minutes to get clear serum and the following parameters were analysed:

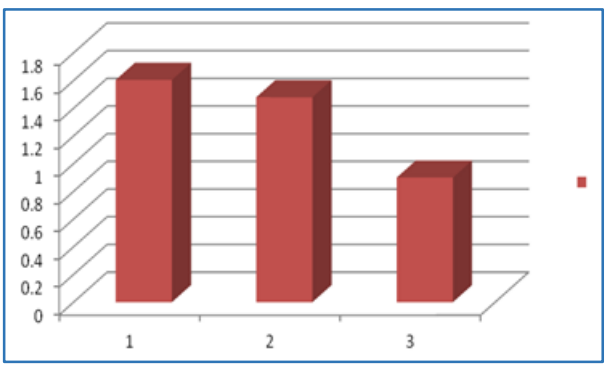
- Magnesium was assayed by Xylidyl blue method (Karen diagnostics).
- Cholesterol was estimated by cholesterol oxidase method (Nicholas Piramal kit).
- Triglycerides was estimated by glycerol-3-phosphate oxidase method (Nicholas Piramal kit).

2 mL of fasting venous blood was collected in fluoride tube and allowed to clot. Then, the vacutainers were centrifuged at 3000 rpm for 10 minutes to get clear plasma for estimation of fasting plasma glucose. Glucose was assayed by glucose oxidase-peroxidase method (Nicholas Piramal kit). HbA1c sample was collected in an EDTA vacutainer and whole blood was used to assay glycated haemoglobin by ion exchange method from Biosystems. 2 mL of sample was collected exactly after 2 hrs. of having a meal for estimation of post prandial plasma glucose. The sample was allowed to clot and then it was centrifuged at 3000 rpm for 10 minutes after which postprandial plasma glucose was assayed. Statistical analysis of the data obtained was done by ANOVA using SPSS version 17. 'p'-value of <0.05 was considered as significant.

RESULTS:

Analyse	Group-I	Group-II	Group-III	Group I vs. Group II	Group I vs. Group III	Group II vs. Group III
Magnesium	1.605±0.11	1.48±0.17	0.9±0.18	<0.05	<0.001	<0.001
Cholesterol	160.85±22.8	215.3±20.32	260±22.2	<0.001	<0.001	<0.001
Triglycerides	132.6±14.48	152.1±20.2	171±20.57	<0.01	<0.01	<0.001

Table 1: Serum Magnesium, Cholesterol and Triglycerides in controls (Group I), Diabetes without Retinopathy (Group II) and Diabetes with Retinopathy (Group III):

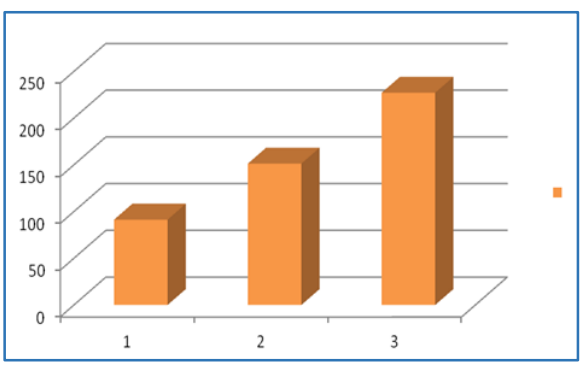


Bar Diagram 1: Serum magnesium Levels in the Control Group, (Group I), Diabetes without Retinopathy (Group II) and Diabetes with Retinopathy (Group III).

The mean serum magnesium of group I, group II and group III are 1.605+/-0.11, 1.48±0.17, 0.9±/-0.18 respectively. The lowest serum magnesium is seen in diabetes group with retinopathy which was statistically significant ('p'-value <0.0001). The mean serum cholesterol of Group I, Group II and Group III are 160.85±22.8, 215±20.32, 260±22.2 respectively. From the above data, it is clear that, Group III i.e. diabetes group with clinical signs of retinopathy recorded the highest mean serum cholesterol. The mean serum triglyceride concentration in Group I, Group II and group III are 132.6±14.48, 152.1±20.2 and 171.2±20.57 respectively. Significant increase in triglyceride concentration of was found in diabetes with retinopathy.

Analyse	Group-I	Group-II	Group-III	Group I Vs. Group II	Group I Vs. Group III	Group II Vs. Group III
FPG	91.3±6.3	151.3±20.11	227.1±21.4	<0.001	<0.001	<0.001
PPPG	131.15±6.36	202.31±8.21	272.5±22.09	<0.001	<0.001	<0.001
HbA1c	4.95±0.74	7.450±.69	9.95±1.39	<0.001	<0.001	<0.001

Table 2: Fasting Plasma Glucose, Post Prandial Plasma Glucose and Glycated Haemoglobin (HbA1c) of Controls, Diabetes Mellitus without Retinopathy and Diabetes Mellitus with Retinopathy



Bar Diagram 2: Fasting Plasma Glucose in Controls (I), Diabetes Mellitus without Retinopathy (II) and Diabetes Mellitus with Retinopathy (III)

Fasting plasma glucose, post prandial plasma glucose and glycated haemoglobin (HbA1c) confirmed the glycemic status of each of the groups.

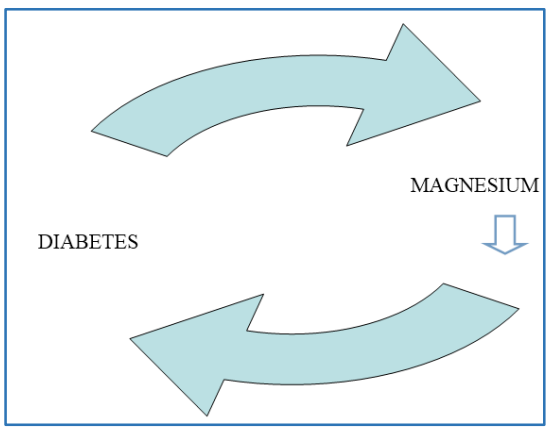
DISCUSSION: Hypomagnesemia is defined as a serum Mg^{++} concentration ≤ 1.6 mg/dL.[15,16] According to this cut off point all the three groups in our study have Hypomagnesemia, with a mean of 1.605±0.11,1.48±0.17 and 0.9±0.18 mg/dL. Unquestionably, diabetic retinopathy group recorded the lowest mean magnesium concentration with a mean of 0.9 mg/dL followed by the diabetic group. Does this mean our control group are prone to type 2 diabetes mellitus? Only prospective studies can throw light on this aspect. Another thought provoking question that plagued most investigators including us is that Mg++, being predominantly an intracellular cation and whose effects on glucose metabolism are mediated via intracellular magnesium, is it worthwhile to measure serum magnesium concentrations? According to a few authors, intracellular magnesium assays are more important than serum magnesium concentrations. Some authors still support that, serum Mg++ concentration is sufficient to confirm the diagnosis. Amidst all these controversies, most authors have relied predominantly on serum Mg++ concentrations.[17] probably in the firm belief that serum magnesium concentration is a reflection of intracellular magnesium.

A strong association between Hypomagnesemia and diabetes mellitus was debated since quite a few decades. Many studies showed Hypomagnesemia further promoted progression of the disease and an increased risk for diabetic complications. In our study too, we found a significant Hypomagnesemia in diabetic retinopathy cases. The exact cause for this decreased magnesium levels is not yet known clearly, but an increased urinary loss of magnesium may contribute to it.[18]

Secondly, hyperglycemia itself reduces the net reabsorption of magnesium from the tubules. As per the recent review article by Lisanne M.M. Gommers, Joost G.J, insulin regulates renal Mg^{++} channel transient receptor, melastatin type 6, which determines the urinary Mg^{++} excretion. Hypomagnesemia in a setting of diabetes mellitus will have a major impact on glucose metabolism. Decreased serum magnesium levels can reduce further insulin secretion by pancreas.[19] Investigators after considerable research opined that intracellular Mg^{++} regulates glucokinase, K^{ATP} channels and L-type Ca++ channels in pancreatic β cells. Thereby, promoting insulin secretion from them. Hypomagnesemia inhibits insulin secretion. According to Yajnicks CS and Smith et al, magnesium in addition potentiates the effect of insulin and also plays a major role in glucose disposal through its action on 300 enzymes.[20] Intracellular Mg^{++} stimulates insulin action by regulating insulin receptor's intrinsic tyrosine kinase activity/auto-phosphorylation. 2 Mg^{++} ions bind to the tyrosine kinase domain.[21]

Magnesium increases the affinity of the receptor to bind with ATP.[22,23] and stimulates downstream signalling of insulin. Magnesium is quintessential for auto phosphorylation of the β subunits of the insulin receptor. Magnesium also promotes glucose uptake in the skeletal muscle by increasing GLUT 4 expression.[24] In short, Mg++ regulates insulin secretion and insulin action- the two physiological factors that regulate glucose homeostasis. A Hypomagnesemia can decrease insulin secretion and also inhibit insulin action, thereby resulting in a hyperglycemia. Magnesium is an anti-inflammatory molecule. So, it can be

rationalised that Hypomagnesemia propels an inflammatory environment.[25] Diabetic patients enter a vicious circle in which diabetes leads to Hypomagnesemia which propels hyperglycemia further.



The severity of diabetic retinopathy is directly proportional to the increase in the cholesterol and triglycerides levels. In the present study it was observed that serum cholesterol and triglyceride levels were significantly higher in the group with diabetic retinopathy. Increased triglycerides in diabetes mellitus is due to increased synthesis of triglycerides as well as a decreased clearance of triglycerides. Increased synthesis of triglycerides is due to an increased FFA influx to the liver and the decreased clearance of triglycerides is due to a decreased lipoprotein lipase activity, a sequel of the insulin resistant state. The raised triglyceride levels leads to increased blood viscosity and altered fibrinolytic activity which leads to formation of hard exudates. Thus increased triglyceride level alters the fluidity of cell membrane leading to haemorrhage and oedema. This in turn leads to endothelial cell dysfunction and local inflammatory response releasing cytokines and growth factors which are responsible for neovascularisation in retina.[26]

Glycated haemoglobin results from post translational changes in haemoglobin molecules and their levels correlate well with glycemic status over the previous 6-10 weeks. Higher levels of glycated haemoglobin favours the risk for the development of microangiopathic complications in diabetes mellitus like diabetic retinopathy. The attributed mechanism is that glycated haemoglobin has special affinity for oxygen which leads to tissue anoxia and plays a role in causation of microangiopathic changes.[27] our study also showed higher levels of glycated haemoglobin in the group with diabetic retinopathy.

CONCLUSION: Hypomagnesemia, elevated serum cholesterol and elevated triglycerides are associated with diabetes and its microvascular complications like diabetic retinopathy. Annual eye check-ups along with serum magnesium, glycated haemoglobin and lipid profile assays go a long way in preventing the micro-vascular and macro-vascular complications of diabetes mellitus.

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