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Original Article

Evaluation of Biochemical parameters evincing atherogenic potency in Type 2 Diabetic Retinopathy.

Dayanand CD [a]* , KrishnaMurthy N [a] , Asha kiran S [a], Naveen [a]

a Department of Biochemistry , Sri Devaraj Urs Academy of Higher Education and Research, Tamaka Kolar Karnataka India PIN-563101*

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ABSTRACT

Objectives- Poor glycemic control leads to hypomagnesemia, uncontrolled hyperglycemia associated with high incidence of micro vascular complications such as retinopathy, nephropathy and cardiovascular problems in type 2 Diabetes mellitus. The chief aim of the study is to obtain possible biochemical indices level in all the groups. Materials and methods- The current study was under taken in total 90 subjects divided in to three groups, includes thirty control (Group-1), thirty diabetics with out complications of retinopathy (Group-2) and thirty diabetics with retinopathy (Group-3). Fasting Blood sugar level, fructosamine, magnesium, microalbuminuria, cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol were analyzed in all the groups. Results-Decreased serum magnesium observed in Group-3 compared to Group-1 was a significant observation and also significant correlation present between fructosamine, magnesium and microalbuminuria level. Our study results also indicate that the diabetic retinopathy patients have hypercholesterolemia, hypertriglyceridemia, elevated LDL-cholesterol lipoprotein fraction and significantly decreased HDL-cholesterol lipoprotein fraction which are responsible for micro vascular changes. Conclusion- Hypomagnesemia, hyperglycemia, increased fructosamine and increased lipid profile with decreased HDL-cholesterol level indicates the probability and possibility of atherogenicity.

1. Introduction

Diabetes is a disease of impaired carbohydrate metabolism characterized by hyperglycemia and glycosuria. The onset of uncontrolled hyperglycemia causes various micro vascular complications such as damage of retina, nephron, neuron, and cardiovascular tissues due to complex and multifunctional metabolic changes [1-2].

Diabetic retinopathy caused by cataract formation, ocular nerve palsies and retinal artery occlusion (optic neuropathy). Good glycemic control can reduce the progression of retinopathy. Therefore, early detection for treatment can save the vision in affected subjects and also from the cardiovascular diseases.

Therefore, in the present study, an attempt is made to find out the biochemical parameters and their correlations. The biochemical analytes are serum magnesium, lipid profile, extent of control of glycemic status in type 2 diabetes mellitus by fructosamine and microalbiminuria in clinically proven diabetics with and without complications of retinopathy.

The study on magnesium, glycemic status and proteinuria and retinopathy in type-1 diabetes mellitus available [3] and also the study on magnesium, glycosylated hemoglobin [HbA1c], lipid profile in diabetic retinopathy available [4] The concept of hypomagnesaemia in Diabetes Mellitus is established[5] Similarly, hypomagnesaemia is also linked to development of retinopathy[6-7] Microalbuminuria is associated with diabetic retinopathy in type 2 Diabetes mellitus therefore, it is a reliable marker of retinopathy[8]

* Corresponding Author : Dr.Dayanand C.D
Department of Biochemistry
Sri Devaraj Urs Academy of Higher Education and Research.
Tamaka Kolar Karnataka India PIN-563101.Tel: +91-08152-243003
Email: cd8905@yahoo.co.in

Poor diabetic control is associated with hypercholesterolemia and hypertryglyceridemia [9] However, there is need to study the correlation between the fructosamine, Magnesium level, microalbuminuria, and serum lipids in diabetic retinopathy patients of Southern Karnataka in India.

2. Material and Methods

The study was undertaken in tertiary care hospital in rural area of Kolar. The study group comprises 90 subjects and these were divided in to 3 groups, thirty control Group-1, thirty patients of type 2 Diabetes without retinopathy is Group -2, and thirty patients of type 2 diabetes with retinopathy Group-3. The subjects were in the age group of 46-79 years. Inform consent was obtained in written proforma and ethical clearance is obtained to carry out the study. They were screened for retinopathy by opthalmoscopic examination and fundus photography. In the control group the exclusion criteria like alcoholism, smoking, hypertension, diarrhea, use of diuretics, reduced renal function were excluded. Serum fructosamine (glycated albumin) estimation was carried out by a method described by Johnson [10]. This is used to identify the degree of glycemic control over an intermediate short period of time [11] where HbA1c used for assessing glucose level preceding six weeks.

2.1. Procedure

Five ml of fasting venous blood allowed for spontaneous retraction of clot, the separated unhemolysed serum (0.05ml) containing fructosamine allowed reacting with Nitro blue tetrazolium (NBT) during incubation of 10 minutes at 37^0 C. Absorbance measured at 550nm and also after intervals of 5 minutes again absorbance measured.

The difference in absorbance used in calculation.[12] The samples were used for analysis, Magnesium was estimated by spectrophotometric Xylidyl blue method[13], glucose determination by Glucose oxidase –Peroxidase method [14], cholesterol by Cholesterol Oxidase –Peroxidase method[15] and triglycerides by Trinders Glycerol Phosphate Oxidase-Peroxidase method [16] HDL by Poly ethylene glycol [PEG] precipitation method[17] LDL cholesterol by calculation method according to Freidewald Formulae [18] and microalbimunuria by micral test (Roche diagnostics Ltd Canada) which is immunological rapid semi quantitative dipstix method. Statistical analysis of the results was done using student 't' test.

3. Results

The mean values of serum magnesium (mg/dl) and fructosamine (mMol/L) in Group-1 (control normal healthy individuals), Group-2 (diabetics without retinopathy) and Group-3 (diabetics with retinopathy) were measured.

The serum magnesium and fructosamine in Group 1, Group 2, and Group 3 were 1.9 ±0.25, 2.16 ±0.26, 1,43 ±0.17 and 1.8 ±0.41, 2.06 ±0.47, 2.37 ±0.18 respectively. The patients of Group 1 and Group 2 indicated normal magnesium in comparison to Group 3 where hypomagnesemia observed (P= 0.22). Similarly, although the serum fructosamine indicated normal range in Group 1, but significantly the values were raised in Group 2 and Group 3. Fructosamine and hypomagnesemia observed in Group 2 and 3 significantly higher. The increased fructosamine positively correlated with the fasting blood glucose 143 mg/dl as tabulated in table 1 and figure 1& 2 .

Table 1 indicating the mean and ±SD of FBS, Fructosamine, and magnesium in different groups

Groups	FBS (mg/dl)	Fructosamine (mMol/L)	Magnesium (mg/dl)
Normal Control(G1)	80.0 ±8.92	80.0 ±8.92	1.9 ±0.25
Type2 DM without Retinopathy (G2)	104.43 ±11.68	104.43 ±11.68	2.16 ±0.28
Type2 DM with Retinopathy(G3)	143.13 ±19.27	143.13 ±19.27	1.43 ±0.17

Fig 1 Comparison of serum magnesium (mg/dl) levels between control and diabetic with and without retinopathy

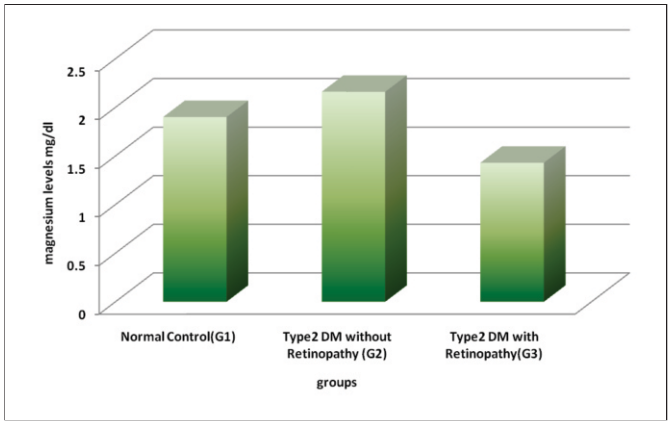
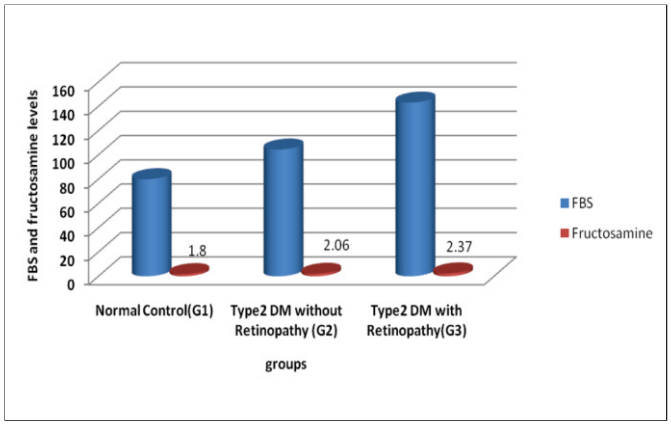


Fig 2 Comparison of Serum fructosamine with FBS levels between control, diabetic with and without retinopathy



The serum cholesterol levels were estimated as 150 ±13.67, 171.66 ±16.70, and 202.93 ±51.00 mg/dl for Group 1, Group 2 and Group 3 respectively. The High Density Lipoprotein –cholesterol (HDL) calculated by Friedewald's formulae and was estimated as 35 ±6.60, 44.13 ±8.16, and 34.56 ±5.98 mg/dl for Group 1, Group 2 and Group3 respectively. The significant observation is that increased cholesterol and decreased HDL-cholesterol observed with increased cholesterol/HDL-cholesterol ratio (5.9).

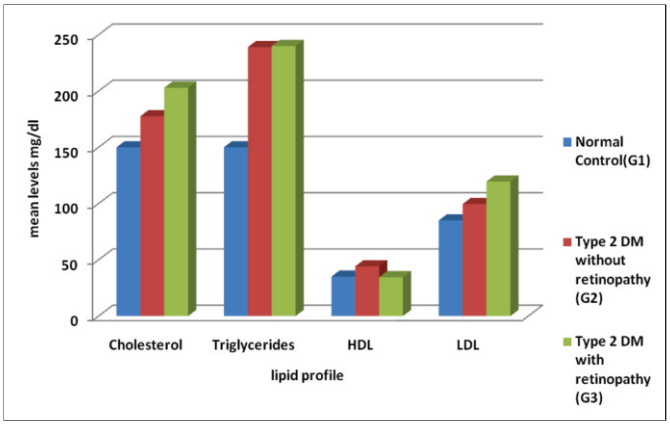
The serum triglycerides level were estimated as 150 ±21.20, 239 ±31.67, 240 ±101.70, mg/dl for Group 1, Group 2, Group 3 respectively. Hypertryglyceridemia observed in Group 2 and Group 3 ($p < 0.001$). The serum cholesterol and triglyceride value significantly higher in Group 2 and Group 3 however, HDL cholesterol is significantly lowered in these groups as tabulated in table 2 & figure 3.

Table 2 Indicating the mean and ±SD of fructosamine, Magnesium, cholesterol: HDL ratio, Triglycerides, LDL-cholesterol in different groups.

Groups	Fructosamine (mMol/L)	Magnesium (Mg/dl)	Cholesterol (mg/dl)	HDL cholesterol (mg/dl)	Cholesterol/ HDL- ratio	Triglycerides (mg/dl)	LDL-cholesterol (mg/dl)
Normal Control G1	1.8±0.41	1.9±0.23	150±13.67	35±6.40	4.28	150±21.20	85±15.86
Type 2 DM without Retinopathy G2	2.06±0.47	2.16±0.26	177.6±16.20	44.13±8.16	4.01	239±33.67	99.53±18.58
Type2 DM with Retinopathy	2.37±0.18	1.43±0.17	202.96±51.0	34.46±5.98	5.9	240±101.70	119.50±43.66
P value	<0.001	P=0.22	<0.001	P=0.020	-	<0.001	<0.001

P >0.05 non significance. P< 0.05 significant. P < 0.001 highly significant.

Fig.3 Comparison of Mean serum lipids (mg/dl) between control (Group 1), Diabetics without retinopathy (Group 2) and diabetic retinopathy Group 3.



The microalbuminuria in 24 hours urine sample measured between the Group 2 and Group 3 using a semi quantitative immunological method. Accordingly, in our study microalbuminuria observed in 19 patients from the total of thirty patients of Diabetic retinopathy. This accounts about 63.33 percent prevalence rate in diabetic retinopathy population. This result is slightly higher than the observation made by other studies that represents between 25 to 35 percent occurrence rates. The higher prevalence could be due to the poor glycemic state as implied from the fructosamine level 2.37 ±0.18 in comparison to normal healthy control subjects 1.8 ±0.41. This could be also due to the smaller group of population selected for the study might be the contributing factor.

The rate of percent of microalbuminuria in diabetics with retinopathy is higher than the diabetics without retinopathy showing no case for presence of microalbuminuria, which is similar to that of other studies observation. These findings in this study suggest the presence of microalbuminuria is a predisposing factor in diabetic retinopathy.

4. Discussion

In the study, there is a correlation between normal magnesium level and insulin secretion and also its requirement for enzyme activity in the body. Therefore, altered magnesium level can affect the cellular function particularly membrane bound sodium-potassium ATPase [19] hypomagnesemia and reduced insulin secretion is reported [20].

There is direct relationship between serum magnesium and glucose utilization in the cell. [21] Fructosamine (a glycosylated albumin protein) served as a simple screening procedure for diabetes mellitus. It was an index of intermediate term (1 to 3 weeks) blood glucose control [22].

Fructosamine used as an index to monitor short term diabetic control, and its measurement is sensitive to changes in diabetic control due to shorter life span of albumin. Thus, it alerts the physician to understand the glycemic status much earlier than Glycosylated hemoglobin (HbA1c) therefore; a higher fructosamine value indicates the poor glycemic control [23-24].

We measured fructosamine level in a series of 60 diabetics with and without retinopathy and found a significant higher level in Group 2 (2.06 ±0.47) with mean Fasting blood glucose 104.43 mg/dl and 2.37 ±0.18 for Group 3 with mean fasting blood glucose 143.13mg/dl compared to Group 1 normal healthy control (1.8 ±0.41) with fasting blood glucose 80.0mg/dl. The mean fructosamine level in diabetics without retinopathy and it was found stastically significant ($p < 0.001$).

The measurement of fructosamine not only gives idea of successful glycemic control but also provides the information on secondaries of diabetes, statistically significant correlation was found between fructosamine and serum lipids such as cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol, the ratio of HDL/Cholesterol found that statistically significant on our results. And also supports the findings of the authors [25-26]. These, observation indicate the evidence for biochemical parameters causing atherogenic potency in diabetic retinopathy that results alarming indication of cardiovascular complications.

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