GLYCOSYLATED LIPOPROTEINS, HAEMOGLOBIN AND PROTEINS IN DIABETES MELLITUS WITH SPECIAL REFERENCE TO ITS COMPLICATIONS

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Summary

In 76 patients of diabetes mellitus, and 26 age and sex matched healthy controls, glycosylated haemoglobin (GHB) glycosylated plasma proteins (GPP) and glycosylated low density lipoproteins and very low density lipoproteins (GSLDL + VLDL) were measured. Diabetics were found to have significantly higher values of GHB, GPP, GSHDL and GSLDL + VLDL. With the fair and good control of diabetes mellitus the value of these parameters showed a significant fall. It was found that the GPP levels decreased within 15 days of fair to good control and did not show any further decline with continued metabolic control of diabetes, while GHB, GSHDL & GSLDL+VLDL levels took approximately 2 months to decline significantly, however the values were still higher compared to normal. The mean levels of GHB, GPP, GSHDL did not differ significantly ($p>0.1$) in poorly controlled patients with or without diabetic complications. However, GSLDL + VLDL were significantly higher ($p<0.001$) in those who had long term complications of diabetes mellitus. It seems that higher GSLDL + VLDL may to some extent either independently or in association with other alterations found in hyperglycemia, contribute to the occurrence of these complications. GSLDL + VLDL values do not have any correlation with individual complications, thereby suggesting that it is a function of hyperglycemia *per se* than being a specific marker for complications of diabetes mellitus.

Introduction

Glycosylated haemoglobin and glycosylated plasma protein represent the summated glycemic control of preceding few months and few weeks respectively. The glycosylation of other proteins, apart from reflecting the glycemic control, may indicate the change in organ structure. Glycosylation of lipoproteins is one important group which has so far not been studied in detail. The present study was done in order to elucidate the change in serum glycosylated lipoproteins, glycosylated haemoglobin and glycosylated plasma proteins in patients of Diabetes Mellitus with and without its long term complications and

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whether the abnormalities of lipoproteins glycosylation relate to these long term complications.

Material & Methods

One hundred and two subjects were included in the study. Seventy six were diabetics from OPD Diabetic Clinic or those who were admitted in medical wards of Gandhi Memorial & Associated Hospital, Lucknow.

Twenty six non diabetic age and sex matched healthy persons from amongst the relatives of patients constituted the control group. In control group, diabetes was excluded by standard OGTT.

Modified criteria as laid down by National Diabetes Data Group (USA) 1979[1] for true blood sugars were used.

The patients were divided on the basis of past records of out patient diabetic clinic and follow up for atleast previous two months, in following groups :-

Group I - Healthy subjects

Group II- Diabetic patients without complications

(A) Patients with poor control & good control (with treatment). (At least 70% values were in conformity with the standards of poor, fair & good control before inclusion in a given group).

(B) Patients under fair control for 15 days but previously they were uncontrolled.

Group III - Diabetics with complications.

Each person included in the study was subjected to thorough history taking particularly regarding duration of diabetes, treatment taken, previous glycemic control and any other factor and symptoms suggesting involvement of cardiovascular, cerebrovascular, peripheral vascular insult, nephropathy, retinopathy or peripheral neuropathy with its duration followed by complete physical examination.

All subjects were put to the following investigations.

(a) Complete haemogram

(b) Urine examination --- albumin/sugar/microscopy

(c) True blood sugar --- Fasting
--- Two hour post prandial
OGTT whenever indicated

(d) Blood urea

(e) Serum creatinine

(f) Standard 12 lead E.K.G.

(g) Nerve conduction velocity where peripheral neuropathy was suspected.

(h) Glycosylated haemoglobin

(i) Glycosylated plasma protein

(j) Glycosylated serum HDL

(k) Glycosylated serum LDL and VLDL

Those who could not be controlled on dietary regime were put on insulin or oral hypoglycemic agents. They were followed up and degree of diabetic control was judged by the criteria of Marble and Camerini-Davalos (1957)[2] as given below :

Relation to food	Degree of control according to blood sugars (mg/dl)		
	Good	Fair	Poor
Fasting	100	130	All other values
1 hour P.C.	150	l80	more than
2 hour P.C.	130	150	"Fair control"
3 hour P.C.	110	140	

All patients were reassessed weekly or biweekly by blood sugar measurements regarding diabetic control for at least two months. Measurements of glycosylated haemoglobin, glycosylated plasma proteins, glycosylated HDL and glycosylation of LD and VLD lipoprotein mixture were again done at the end of two months. Glycosylated haemoglobin was measured by method of Fluckiger and Winterhalter[3] with minor modifications of Goldstein[4,5] and expressed as nannomole of 5-HMF/mg of Hb (NMF/mg of Hb).

Measurement of Glycosylated Plasma Proteins : Plasma was dialysed against 25 nm hepes buffer solution and 0.15 M sodium chloride solution for 24 hours to remove free sugar. Total protein measurement was done by the method of Lowry *et al.,* (1951).

Glycosylation was estimated as stated above. Results were expressed as n mole of 5 HMF per mg of protein

Measurement of Glycosylation of Lipoproteins : One ml serum was separated and 5 ml of 10% Dextran sulphate and 50 µl of 1 M manganese chloride (MnCl2) were added to it. LDL and VLDL were precipitated by it and solution of these were made according to the method of Brunstein *et al* (1970)[6]. HDL was separated from the supernatant of above mentioned reaction according to the method of Burstein et al. (1970)[6]. Glycosylation of HDL and LDL-VLDL mixture were estimated as above. Results were expressed as nannomole of 5 HMF per mg of protein. (NMF/mP)

Results

The patients with other complications like cerebrovascular disease, retinopathy, peripheral vascular disease and other complications were few, so that no comparisons could be made. Similarly from subsequent data it was observed that among the fairly controlled and well controlled diabetic groups having these individual complications, microvascular and macrovascular as compared to those not having these complication, there was no statistically significant difference between the two.

Discussion

Vascular complications are attributable to long term hyperglycemia, but the way these complications are produced is still not clear. One of the important factors held responsible is disturbed lipoprotein metabolism. It is well known that various body proteins are glycosylated and these glycosylated proteins behave in a different manner than normal body proteins. Simitarly altered level of glycosylated lipoproteins may contribute to the vascular complications of diabetes mellitus.

In healthy non diabetic normal subjects GHB, GPP, GSHDL & GSLDL + VLDL values were found to be 3.86±0.47 NMF/mHB; 5.47±0.27 NMF/mP; 4.57±0.44 NMF/mP and 5.16±0.61 NMF/mP. Absolute values for GSHDL and GSLD + VLDL are not reported in the literature. If GHB values were interpreted in terms of percentage of haemoglobin, they would come out to be somewhat similar to those observed by other workers in healthy persons[7,8,9].

However, it is acceptable that each laboratory should establish its own values as difference in methodology, average haemoglobin levels apart from blood sugars may have a bearing upon GHB values[4,5]

The degree of glycosylation of HDL also does not directly correlate with the degree and duration of hyperglycemia in a manner similar to that of GHB and GPP. After control of glycemia, percent glycosylation of HDL goes down significantly.

It seems that more than 15 days are required before GSHDL level changes materially after control of diabetes, suggesting that GSHDL level reflects glycemic status of previous 15 days to 2 months. Unfortunately, there is no cornparable observation in the available literature *in vivo* studies [14,15].

It has been suggested that glycosylation of HDL causes a proportionate increase in clearance of HDL. This in turn may account for lower levels of glycosylated HDL cholesterol found in diabetics and with a good control of diabetes degree of glycosylation reduces significantly which in turn leads to accumulation of HDLC. This may be a mechanism by which maintenance of euglycemia retards the progression of diabetic vascular complications.

As opposed to HDL, high level of LDL and VLDL are found in diabetics. Their glycosylation also runs parallel to the degree of hyperglycemia, being higher in diabetic than in normal subjects (Table 1). With better control of hyperglycemia, their levels progressively come down (Table 1) irrespective of the mode of therapy.

The GSLDL + VLDL values were significantly higher in poorly controlled patients with complications as compared to those who were not having complications (Table 2). This would suggest that patients having a propensity to develop complications have an inherent tendency to produce higher glycosylated LDL + VLDL which gets metabolized very slowly, thus increasing the total levels of LDL + VLDL which in turn increases the tendency to develop premature atherosclerosis[14,15].

Table 1

GHB, GPP, GSHDL and GSLDL+VLDL in patients with poor, fair and good control of diabetes compared to healthy subjects.

Types of cases	No. of cases	GHP NMF/mHb Mean±SD	GPP NMF/mP Mean±SD	GSHDL NMF/mP Mean±SD	GSLDL+VLDL NMF/mP Mean±SD
A. Poorly controlled	15	12.07±1.32	11.6 ±1.32	6.36±0.65	9.41±0.65
B. Fairly controlled	13	7.63±0.65	6.72±0.53	5.33±0.51	7.46±0.60
C. Well controlled	10	5.50±0.43	6.18±0.27	5.30±0.42	6.35±0.54
D. Healthy subjects	26	3.86±0.47	5.47±0.27	4.57±0.44	5.16±0.61

Table 2

GHB, GPP, GSHDL & GSLDL+VLDL in poorly fair & well controlled diabetics with complications

Types of cases		GHP NMF/mHb Mean±SD	GPP NMF/mP Mean±SD	GSHDL NMF/mP Mean±SD	GSLDL+VLDL NMF/mP Mean±SD
A. Poorly controlled					
l. With complications	10	12.04±1.03	11.21±0.95	6.55±0.78	10.51±0.52
2.Without complications	15	12.07±1.32	11.06±0.01	6.36±0.65	9.41±0.65
p value between A_l&A_2		p> 4.1	p> 0.1	p > 0.1	p < 0.001
B. Fairly controlled					
l. With complications	10	7.08±0.03	6.37±0.69	6.03±0.52	7.89±0.64
2.Withoutcomplications	13	7.63±0.65	76.72±0.53	5.33±0.51	7.46±0.60
p value between B_l&B_2		p > 0.05	p > 0.1	p > 0.01	p > 0.1
C: Well controlled					
1. With complications	8	5.42±0.55	6.27±0.44	5.83±0.29	6.83±0.49
2. Without complications	10	5.50±0.43	6.18±0.27	5.30±0.42	6.35±0.54
p value between C_1&C_2		p>0.1	p>0.1	p>0.05	p>0.05
D. Healtby subjects	26	3.86±0.47	5.47±0.27	4.57±0.44	5.16±0.61

Patients who are brought under a fair control for 2 weeks or so show significant lowering of GSLDL+VLDL, thus suggesting that high glycosylation of LDL+VLDL can be reserved quickly.

Could high levels of GSLDL+VLDL relate to specific complications of diabetes mellitus? With this possibility in mind patients with and without individual complications were compared. Their GSLDL and VLDL values suggest that there are no significant differences in patients having different complications (Table 3).

Thus, it seems that GSLDL and VLDL is a function of hyperglycemia rather than being a specific marker for complications of diabetes mellitus.

Table 3

GHB, GPP, GSHDL & GSLDL+VLDL in diabetics with poor control having individual complications.

Types of cases	No. of cases	GHP NMF/mHb Mean±SD	GPP NMF/mP Mean±SD	GSHDL NMF/mP Mean±SD	GSLDL+VLDL NMF/mP Mean±SD
A_1 Patients with angina pectoris	5	12.19 ±1.05	11.28 ±0.85	6.67±0.86	9.90±0.52
A_2 Patients without angina pectoris	5	11.89±1.10	11.13±1.14	6.43±0.77	10.60±0.47
p values between A_l&A_2		p>0.1	p>0.1	p>0.1	p>0.05
B_1 Patients with nephropathy	4	12.68 ± 1.06	11.00±0.71	6.25±0.53	10.52±0.77
B_2 Patients without Nephropathy	6	11.62±0.83	11.35±1.13	6.74±0.90	10.07±0.41
p value between B_1&B_2		p>0.1	p>0.1	p>0.1	p>0.1
C_1 Patients with peripheral neuro-pathy	3	12.03±0.64	11.52±0.71	6.21±1.04	10.46±0.64
C_2 Patients without peripheral neuropathy	7	12.05±1.20	11.08±1.06	6.69±0.69	10.16±0.60
p value between C_l&C_2		p>0.1	p>0.1	p>0.1	p>0.1

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