

STUDIES ON GLYCOSYLATED PLASMA PROTEINS IN DIABETIC PATIENTS

Pages with reference to book, From 16 To 18

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ABSTRACT

The level of glycosylated haemoglobin in the blood is considered as an index of long term blood glucose levels in diabetic patients. Glycosylation of structural proteins also occur. The present study describes the levels of glycosylated blood proteins (nonenzymatic) and blood glycoproteins (enzymatic glycosylation) in four groups of diabetic patients (n = 80) as well as non-diabetic control subjects (n = 20). The patients were divided according to the treatment they received. Glycosylated haemoglobin, glycosylated plasma proteins, fasting plasma glucose, hexosamine, sialic acid and mucoproteins were measured in each subject. All of these measurements were significantly increased in diabetic patients however, glycoproteins were not significantly correlated with glycosylated haemoglobin or fasting glucose (JPMA 41: 17, 1991).

INTRODUCTION

Total concentration of protein-bound carbohydrate is approximately three times that of free glucose in blood. Protein-bound hexosamine is present in about the same concentration as glucose in blood and is associated with rise in blood glucose level¹. Siddiqui and Rehman² observed that bound- hexosamine decreased after insulin injection in normal and diabetic rats. The association between free and bound carbohydrate indicates the changes in glycol proteins. Jons and Wales³ suggested that rising levels of certain glycoproteins in the blood of diabetic patients may indicate the development of diabetic vascular complications. A positive correlation between glycosylated haemoglobin and other indices of blood glucose control has been established^{4,5}. The half life of glycosylated haemoglobin is 60 to 90 days and hence its reduction may not be apparent for weeks after establishment of glycaemic control. Its rate of formation is much faster than disappearance, thus levels may be disproportionately representative of high rather than average glucose concentration⁶. Several workers have reported a significant correlation between the degree of glycosylation of proteins and haemoglobin and have concluded that measurement of glycosylation of plasma proteins can serve as a sensitive, short term integrator of glucose homeostasis in diabetes⁷⁻¹⁰. The purpose of the present study was to determine the changes in the amount of bound-carbohydrate in comparison with other established indices of glycaemic control in normal and diabetic patients.

PATIENTS AND METHODS

A total of 80 patients suffering from diabetes mellitus of varying severity attending out-patients departments of the Diabetic Association of Pakistan and Jinnah Postgraduate Medical Centre, Karachi, were selected. Only male patients of type I and type II diabetes were included in the study. Type II diabetic patients were maturity onset but were not insulin deficient. They were divided into four groups depending on the treatment they were receiving i.e. those on diet control, those receiving a single oral dose of antidiabetic drug, and those receiving more than one oral drug and more than once daily (combination of sulphonylurea and biguanides). Type I patients were receiving insulin treatment. Twenty age and sex matched healthy subjects belonging to the same socioeconomic class with no

family history of diabetes were selected as controls from general population. About 10 ml blood was drawn from the antecubital vein after an overnight (10 to 12 hours) fast. EDTA was used as an anticoagulant. The treatment was not withheld before withdrawing blood. Glycosylated haemoglobin was determined by the Helena Quick Column method with a kit supplied by Helena Laboratories, Beaumont, Texas 77704, USA, plasma glucose by hexokinase and glucose-6-phosphate dehydrogenase method with a kit obtained from the General Diagnostics, Warner Lambert Co., New Jersey, USA; Sialic acid by the method described by Natelson¹¹; mucoproteins by Winzler et al. method¹², plasma protein by Reinhold method¹³ and hexosamine (after hydrolysis with 3N hydrochloric acid at 106 C in a sealed tube) by the method of Cessi and Piliago¹⁴. The glycosylation of plasma protein was determined by treating the trichloroacetic acid precipitate of protein by thiobarbituric acid and the absorbance measured at 443 nm was expressed as glycosylation per gm protein⁸. The analytical precision of each variable was checked against the standards or procedures stated in the methods used.

RESULTS AND DISCUSSION

Various workers have studied the non-enzymic glycosylated protein and its correlation with glycaemia and other indices of metabolic disorders in diabetes mellitus^{6,15}. In this study the age and weight of the patients were not different from the control subjects except the patients treated with insulin were younger and lighter in weight (Table).

TABLE I. Age, weight, duration of disease and blood constituents of Control and Diabetic patients.

Parameters	Control (20)	On Diet Control (20)	Drugs		
			Oral Single Drugs (19)	Combination of Drugs (20)	On Insulin therapy (20)
Age (Years)	51.4 ± 6.4 (38-60)	56.6 ± 3.9 (48-61)	54.6 ± 9.0 (46-60)	56.5 ± 4.1 (49-60)	44.2 ± 5.2 (35-52)
Weight (Kg)	60.3 ± 4.7 (52-69)	59.5 ± 2.4 (55-65)	60.7 ± 3.0 (55-65)	63.1 ± 4.5 (58-72)	52.9 ± 2.4 (50-57)
Duration of Disease (years)	-	6.0 ± 3.2 (1-11)	4.7 ± 2.2 (1-10)	6.7 ± 2.4 (2-10)	4.2 ± 2.6 (1-11)
Glucose (m moles/L)	4.0 ± 0.3 (3.3-4.7)	5.3* ± 1.0 (4.0-7.8)	6.6* ± 0.8 (5.0-7.7)	9.8* ± 2.2 (7.8-16.4)	10.9* ± 3.5 (5-15)
Glycosylated hemoglobin(%)	6.8 ± 0.5 (5.9-7.4)	8.3* ± 1.0 (7.2-10.5)	9.5* ± 1.0 (8.4-12.2)	10.9* ± 1.6 (9.2-13.2)	11.7* ± 2.2 (8.0-15.5)
Glycosylated protein (per gm protein)	6.1 ± 0.5 (5.4-6.9)	7.2* ± 0.5 (6.5-8.3)	7.7* ± 0.5 (6.7-8.5)	8.9* ± 0.7 (8.2-10.8)	9.0* ± 1.0 (7.2-10.0)
Mucoprotein (mg/dl)	74.4 ± 17.6 (35-99)	118.8* ± 29.4 (78-194)	131.7* ± 35.1 (86-204)	114.9* ± 30.5 (72-194)	124.6* ± 39.0 (57-229)
Hexosamine (mg/dl)	74.7 ± 12.8 (50-98)	97.5** ± 26.1 (56-137)	105.1* ± 21.0 (68-154)	114.1* ± 21.7 (72-164)	125.5* ± 29.2 (78-187)
Sialic acid (mg/dl)	38.5 ± 3.0 (31-45)	53.5* ± 9.9 (39-78)	54.0* ± 6.9 (40-64)	58.3* ± 10.9 (39-78)	62.9* ± 14.8 (45-102)

The difference is statistically significant as compared with control group, *P < 0.001; **P < 0.01

The values are expressed as mean ± S.D. Ranges are given in parenthesis.

All other parameters were increased in all patients irrespective of the treatment they were receiving as compared to the control subjects. The values of glycosylated haemoglobin were similar to those found by Aleyassine et al¹⁶ and that of glycosylated plasma proteins by Ma et al⁸ and the increase in the

values followed the pattern of hyperglycemia. A significant correlation was found between fasting plasma glucose and glycosylated haemoglobin ($r = 0.91$, $P > 0.001$) as well as with glycosylated plasma proteins ($r = 0.87$, $P < 0.001$). Day et al. also reported a similar correlation. When the correlation was considered in different groups, it was found that glycosylated haemoglobin was more significant in patients receiving insulin as compared to those on diet control. In case of glycosylated plasma proteins, the correlation was significant in patients on oral hypoglycemic drugs where glucose level was comparatively more stable and was least correlated with insulin treated patients where glucose levels were more labile. It has been suggested earlier⁶ that glycosylated plasma proteins reflect the more recent changes in blood sugar level and therefore, is a better index of previous glycemia as compared with glycosylated haemoglobin. Moreover, in certain conditions like haemolytic anaemias, recent blood transfusions and presence of some abnormal haemoglobin values do not reflect the exact state of previous glycemia. Glycosylated plasma protein level remains unaffected by these conditions. There was a significant increase in mucoprotein, hexosamine and sialic acid concentration in diabetic patients which followed the pattern of hyperglycemia but this increase was not correlated with fasting plasma glucose or glycosylated haemoglobin levels. Siddiqui and Rehman² have also found increased level of hexosamine in diabetic rats while Khan and Rehman¹ did not find any change in hexosamine in diabetic patients from control subjects during glucose tolerance test indicating that hexosamine in serum reflects the glycemic state rather than the immediate change in blood glucose level as in glucose tolerance test.

REFERENCES

1. Khan, I.A. and Rehman, M.A. Variation of succharoid fraction in diabetes mellitus. *Nature*, 1967; 215: 979.
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3. Jons, A. and Wales, J.K. Blood glycoprotein levels in diabetes mellitus. *Diabetologia*, 1976; 12: 245.
4. Schultz, T.A., Lewis, S.B., Davis, J.L., Rost, C.R. and Bliziots, M.M. Effect of sulfonylurea therapy and plasma glucose levels on hemoglobin A in type II diabetes mellitus. *Am. J. Med.*, 1981; 70:373.
5. Fraser, D.M., Smith, A.F., Gray, R.S., Borseley, D.Q., Sinclair, M.E., Clarke, B.F. and Duncan, L.J.P. Glycosylated hemoglobin concentrations in newly diagnosed diabetics before and during treatment. *Br. Med. J.*, 1979; 1:979.
6. Kennedy, A.L. and Merimee, T.J. Glycosylated serum protein and hemoglobin A level, to measure control of glycemia. *Ann. Intern. Med.*, 1981; 94 : 56.
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11. Natelson, S. *Microtechniques of clinical chemistry*. 2nd ed. Springfield. Ill. Thomas, 1961, p.378.
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14. Cessi, C. and Piliego, F. The determination of amino-sugars in the presence of amino-acids and glucose. *Biochem. J.*, 1960; 77: 508.

15. Koenig, R.J., Peterson, C.M., Kilo, C., Cerami, A. and Williamson, J.R. Hemoglobin A as an indicator of the degree of glucose intolerance in diabetes. *Diabetes*, 1976; 25 : 230.

16. Aleyassine, H., Gardiner, R.J., Tonks, D.B. and Koch, P. Glycosylated hemoglobin in diabetes mellitus; correlations with fasting plasma glucose, serum lipids, and glycosuria. *Diabetes Care*, 1980; 3:508.

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7. Day, J.F., Ingebretsen, C.G., Ingebretsen, W.R. Jr., Baynes, J.W. and Thorpe, S.R. Non-enzymatic glycosylation of serum proteins and hemoglobin; response to changes in blood glucose levels in diabetic rats. *Diabetes*, 1980; 29 : 524.
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