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A Comparative Study of Glycated Hemoglobin and Serum Fructosamine, in Maturity Onset Diabetes Mellitus

Dr. Manish Chakrabarti

Department of Biochemistry. Designation: Professor in Biochemistry, Degree MD Biochemistry. PDU Medical College, Rajkot, Gujarat

Abstract: ***Glycated hemoglobin reflects long tern glycemic control and it is more accurate and stable than fasting blood glucose level. It tracts well over time in persons with Diabetes Mellitus and has less measurement error than fasting blood glucose. Serum Fructosamine level estimation become important in dialysis patients with shortened erythrocyte survival, which may lead to under estimation of hyperglycemia based on Glycated hemoglobin ($HbA1_c$) measurement.1 In this study correlations between above two parameters have been established, and results found that, Glycated hemoglobin and Fructosamine levels are increased proportionately with hyperglycemia in most of the uncontrolled Diabetes Mellitus.***

Keywords: Glycated Hemoglobin, Serum Fructosamine, Maturity onset Diabetes Mellitus, Glycemic control

1. Introduction

Diabetes Mellitus may be characterized as an insufficiency of insulin, relative to the requirements of the tissue for the hormone. Diabetes is a generalized metabolic disorder manifesting itself, in fully developed form, by hyperglycemia, glycosuria, increased protein break down, ketosis and acidosis. If the disease is prolonged, it is usually complicated by degenerative changes of the blood vessels, retina, kidney, and nervous system.

In diabetes Mellitus there are wide spread biochemical abnormalities, But the fundamental defects to which most of the abnormalities can be traced are: a) A reduced entry of glucose into various peripheral tissues. b) An increased liberation of glucose into circulation from the liver because of increased hepatic gluconeogenesis. There is therefore, an extracellular glucose excess and an intracellular glucose deficiency, a situation which has been called, "Starvation in the midst of plenty" and a decrease in the entry of amino acids into the peripheral tissues and an increase in lipolysis.

The cause of diabetic complications is not known and may be multifactorial. Major emphasis has been placed on the Polyol pathway, which is associated with decrease in myoinositol content in cell, abnormal phosphoinositide metabolism and a decrease in Na^+, K^+ ATPase activity.

A second mechanism of potential pathognomic importance is glycation of proteins. The effect of such glycation on hemoglobin has been mentioned but multiple proteins in the body are altered in the same way, often with disturbed function. Example includes plasma albumin, lens protein, fibrin, collagen, lipoproteins and the glycoprotein recognition system of the hepatic endothelial cells.

Clinical significance of Glycated protein:
Measurement of Glycated protein is useful in monitoring long term glucose control in individuals of diabetes mellitus. It provides a retrospective index of the integrated plasma glucose values, over a estimated period of time, and is not subjected to the wide fluctuations observed when assaying blood glucose level. Glycated protein levels are therefore, are a valuable adjunct of glycemic control. However these proteins are not reliable for the diagnosis of diabetes mellitus.[3]

Glycated hemoglobin:
Glycation is the non enzymatic addition of sugar residue to amino groups of proteins, Human adult hemoglobin (Hb) usually consist of HbA (97% of the total) HbA_2 (2.5%) and HbF (0.5%). Chromatographic analysis of HbA identifies a number of minor hemoglobin $HbA_{1a,}$ HbA_{1b} and HbA_{1c}; which are collectively known as HbA_1, fast hemoglobins (because they migrate more rapidly than HbA in the electrical field) or glycated hemoglobins or glycohemoglobins. HbA consists of four polypeptide chains two alfa chains and two beta chains. Glycated hemoglobin (HbA_{1c}) is formed by condensation of glucose of N-terminal valine of each beta chain of hemoglobin A to an unstable Schiff base (aldimine, $PreA_{1c}$), which can undergo an amadori rearrangement to form a stable ketoamine HbA_{1c}. HbA_{1c} is the major fraction, consisting approximately 80% of HbA_1.

Fructosamine:
Non enzymatic attachment of glucose to amino group of proteins other than hemoglobin (e. g. serum proteins, membrane proteins, lens crystallins) to form ketoamine also occur. Because serum proteins turn over more rapidly than hemoglobin (the circulatory half life for albumin is about 20 days), the level of Glycated albumin reflects glucose control over a period of 2 to 3 weeks. Therefore, evidence of both deterioration of control is evident earlier than glycated hemoglobin.

2. Methods

a) **Estimation of glycated hemoglobin**[2]: The glucose moiety of glycated hemoglobin in converted to 5-hydroxy methyl furfural by heating with oxalic acid in boiling water both for 1 hour. The supernatant which

contains 5-hydrxymethyl furfural is allowed to react with2-thiobarbituric acid; the colour developed is measured photometrically at 443 nm.

b) **Estimation of serum Fructosamine**[3]: Under alkaline conditions, product of Amadori rearrangements (such as Fructosamine) has reducing activity that can be differentiated from other reducing substances. Nitroblue tetrazolium (NBT) is reduced in the presence of carbonate buffer, by Fructosamine. The absorbance at 530 nm is measured at 10 and 15 mins and the absorbance change is proportional to the Fructosamine concentration. The 10 minutes incubation is necessary to allow fast reacting interfering reducing substances to react. It is unnecessary to remove endogenous glucose from patients' samples because a pH greater than 11 is required for glucose to reduce NBT. In this study we used dihydro acetone (DHA) as calibrator which is less expensive than DMF (1-deoxy-1-morpholinofructose) calibrator.

c) **Estimation of plasma Glucose:** GOD-POD method.

3. Results

Sixty three subjects suffering from maturity onset diabetes mellitus on oral hypoglycemic agent treatment and thirty healthy controls of both sexes were included in the present study.

The values of glycated hemoglobin, and serum Fructosamine along with plasma glucose concentrations (fasting and post Prandial) were estimated in normal controls and subjects with maturity onset diabetes mellitus. Subject studied were divided into following three groups: Group A: Control, Group B: Subjects with fasting plasma glucose concentration greater than 108 mg% but less than 144mg%, Group C: Subject with plasma glucose concentration equal or greater than 144mg%.

In group A, control subjects aged 20-61 years with 1: 2 male female ratio were studied.

Table 1: Age and Sex distribution

Age distribution

Age (Years)	Group A (Control) (n=30)	Group B (n=30)	Group C (n=33)
20-30	05	01	04
31-40	08	nil	06
41-50	09	12	11
51-60	04	11	08
61-70	04	06	04

Sex distribution

Sex	Group A Controls (n=30)	Group B (n=30)	group C (n=33)
Male	10	16	18
Female	20	14	15

Table 2: Plasma sugar, Glycated hemoglobin, and Serum Fructosamine in control subjects

S. no.	Parameters group A	Mean value	Standard deviation
1	Plasma glucose level (Fasting mg%)	88.2	±12.7
2	Plasma glucose level (Post Prandial mg%)	115.3	±7.19
3	Glycated hemoglobin (percent of total Hb)	7.0	±0.34
4	Serum Fructosamine (mmol/L)	0.395	±0.037

Table 3: Correlation between degree of hyperglycemia with glycated hemoglobin, serum Fructosamine

Study group	Mean plasma glucose, Fasting (mg%)	Mean plasma glucose, Post Prandial (mg%)	Mean glycated hemoglobin (% of total hemoglobin)	Mean serum Fructosamine (mmol/L)
Group A	88.2±12.7	115.3±7.19	7.0±0.34	0.395±0.037
Group B	124.6±9.67	166.1±21.5	8.9±0.87 p<0.01 (SS)	0.454±0.054 p<0.01 (SS)
Group C	195.1±39.4	247.5±50.5	11.6±1.5 p<0.01 (SS)	0.562±0.086 p<0.01 (SS)

SS= statistically significant.

4. Discussion

In this study fasting venous plasma glucose level were increased 1.4 and 2.2 folds in the disease group i. e. group B and group C.

It is to be noted that, Glycated hemoglobin concentration in this study was significantly elevated in disease group. Normal value of Glycated hemoglobin was found 4-7 %. In this study the mean value was within this range i. e.4-7% of total hemoglobin. The result obtained in this study is in good agreement with the studies of other authors [5, 6,.] Thus we can conclude that, HbA_{1c} is present in increased amount in patients with maturity onset diabetes mellitus as a consequence of increased plasma blood glucose level. In addition to the usefulness of HbA_{1c} levels in the management and care of diabetic patients, it is suggested that, serial measurement of HbA_{1c} levels (every 2 months) may allow an accurate estimate of the degree of hyperglycemia in diabetic patients and therefore, allow long term studies on effects of the adequacy of control of hyperglycemia in the prevention of various diabetic complications.

Serum Fructosamine level in this study is increased by 1.1 fold and 1.4 folds as compared to controls in group B and group C patients respectively.

The chemical reaction that form Glycated protein is normally occurs in every serum proteins which is capable of binding to glucose. Proteins are Glycated at their amino terminus or on amino acid side chains such as lysine. The term Fructosamine refers to the ketoamine linkages between glucose and protein; it has nothing to do with fructose except the resulting sugar chain resembles the configuration of fructose. Because the serum proteins turnover is more rapid than hemoglobin (circulatory half-life of albumin is 20 days); the level of Glycated albumin reflects the glucose

control over a period of 2-3 weeks. Therefore, evidence of both deterioration of control and improvement with therapy is evident earlier than with Glycated hemoglobin.

Dominiczak MH et al (1988) [4] in their study shown that, in individual patients the relationship between the two variables (Glycated hemoglobin and Fructosamine) may be weak, however, since they reflect different time period of glycemic control. In this study we found that the increase of Glycated hemoglobin and Fructosamine in individual patients are similar except only in few exceptions.

5. Conclusion

Glycated hemoglobin level increases in maturity onset diabetes mellitus patients and the increase is correlated with plasma glucose level.

Serum Fructosamine level increases in maturity onset diabetes mellitus patients and the increase is correlated with plasma glucose level and glycated hemoglobin level.

From the above study it is also concluded that, estimation of glycated hemoglobin, serum Fructosamine can be additional parameters to monitor the patients' suffering from maturity onset diabetes mellitus in adjunct to plasma glucose estimation.

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