

**Original Research Article****Evaluation of Levels of Glycosylated Hemoglobin (HbA_{1c}) in Patients with Type – 2 Diabetes Mellitus and in Healthy People, Attending P.M.C.H, Patna**

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Abstract

Objective: *The aim of present study was observing the incidence and magnitude of abnormal concentrations of glycosylated hemoglobin (HbA_{1c}) in Type – 2 Diabetes Mellitus (NIDDM) and assessing the adequacy of control of the diabetic patients.*

Materials and Methods: *A total of 106 diabetic patients were selected in both sexes in different age groups, 20 healthy people were selected for control group, Glycosylated hemoglobin was measured by D-10, Column Chromatography methods(HPLC) supplied by Biorad. Blood sugar was measured by Hexokinase methods (Fully automated Roche Integra machine). Urine Sugar is measured by automated urine analyzer and also by rapid strip, supplied by Dirui Industries.*

Results: *Maximum incidence of DM occurred in the 5th decade, there was male preponderance with male and female ratio of 4:1. The level of HbA_{1c} in untreated and treated diabetics was 8.30% + 0.37 (range 6.83% - 10.40%) and 7.72% + 0.44 range (5.52% -12.41%). The corresponding FBS levels were 181 + 17 mg/dl (range 130 – 282 mg/dl) and 151 + 17 mg/dl (range 78 – 310 mg/dl) respectively. The mean of glycosylated Hemoglobin in normal individuals was found to be 5.30% + .24 (range 4.50% - 6.5%) and mean FBS level was 79 +2mg/dl (range 68 – 88 mg/dl).The value of HbA_{1c} was approximately 1.5 times higher in untreated diabetics then in the control group. This was statistically significant. The HbA_{1c} level correlated closely with the FBS level in diabetics.*

Conclusion: *It is concluded that in assessing glycemic control in diabetics with high glycosylated hemoglobin levels, concurrent FBS levels estimations are essential. However, estimation of HbA_{1c} should be viewed as an adjunct to and not a replacement for FBS estimation in the assessment of glycemic control in treated diabetics.*

Keywords: *Diabetes mellitus, Glycosylated Hemoglobin, fasting blood glucose level.*

Introduction

Diabetes mellitus is the most common of the serious metabolic disease. It is a burning medical problem affecting most of the civilized world.

Workers all over the world have been actively working on various aspects of the management of this condition. The characteristic progressive damages to the eyes, kidneys, nerves and the

heightened susceptibility to heart disease, gangrene and stroke are believed to be related to the duration of diabetes and are most probably a direct consequence of inadequate control of the metabolic disorder.

It is also believed that strict glycemic control in diabetics prevents or retards the progress of late vascular or neurological complications. Therefore, proper assessment of glycemic control becomes an important part of the management (Raheja 1981). At present urinary glucose and blood glucose estimations are commonly done for the assessment of glycemic control in these cases.

Vascular complications of diabetes probably occur even in a mildly uncontrolled metabolic state. Since urine analysis does not reflect the state of control at blood glucose levels between 80-180 mg/dl, proper assessment of glycemic control by urinary glucose analysis is not always practicable. In any case urine has to be examined a number of times every day.

Again a fasting or post-prandial blood glucose level reflects only an instant state of glycemia and although good for assessing the control, blood glucose level has to be determined a number of times every day, hardly a practicable proposition.

A single estimation of glycosylated hemoglobin (HbA_{1c}) level on the other hand gives an idea of time averaged blood glucose over the preceding 2-3 months. Ceramic et al (1978) have suggested that the level of HbA_{1c} in blood could be utilized in investigating the effect of long term control of diabetes on the development of complications such as retinopathy, nephropathy and neuropathy.

The major form of glycosylated hemoglobin is termed HbA_{1c}, which normally comprises only 4 – 6% of the total haemoglobin. However, in patients with diabetes its concentration may be increased to as much as 6 – 15% of the total haemoglobin (Harper 2008). HbA_{1c} is altered in many physiological and pathological states apart from diabetes mellitus, viz, pregnancy, chronic renal failure, Iron deficiency anemia, hemolytic anemia and hemochromatosis.

Although, HbA_{1c} is generally considered to be an accurate index of long term blood glucose regulation, several recent studies suggest that it may be responsive even to acute changes in blood glucose levels. In this series scobie et al (1981) observed that even a minor degree of hyperglycemia led to a significant increase in HbA_{1c} levels. The increased appeared 10 days after the test and values remained raised for 30 days, returning to normal only after 60 days. Svendsan et al (1979) have also reported similar acute changes in HbA_{1c} levels.

This study should be of help in improving therapeutic monitoring in treated diabetics.

Materials and Methods

The present study was conducted in the Department of pathology, Patna Medical College, Patna, with the help of Department of Microbiology and Department of Medicine, during the period of September 2016 to August 2018. A total of 106 diabetic patients (71 were obese, 30 of normal body weight and 5 were asthenic). All had first developed symptoms between the 5th and 7th decades of life. 20 healthy individuals were selected as a control group. All the cases in this study were clinically of Type – 2 Diabetes Mellitus (NIDDM).

The Diagnosis of DM was made on the basis of history, Physical examination and the laboratory investigations of urine and blood. The criteria for diagnosis of DM were kept the FBS level more than 126 mg/dl and post prandial blood sugar level after 2 hour of 75 gms of oral glucose intake more than 200 mg/dl.

The glycosylated hemoglobin level (HbA_{1c}) was measured D-10, Column Chromatography methods (HPLC) supplied by Biorad, in the diabetic patients as well as in normal control group. Blood sugar is measured by Hexokinase methods (Fully automated Roche Integra machine). Urine Sugar is measured by automated urine analyzer and also by rapid strip, supplied by Dirui Industries.

Result

The maximum incidence (50%) of type -2 Diabetes mellitus occurred in the 5th decade (41 – 50 years). There was male preponderance with male: female ratio of 4 : 1.

The mean of glycosylated hemoglobin level in normal individuals was found to be $5.30 + 0.24\%$ with a range of 4.50 – 6.5% and the mean fasting blood glucose level was 79 mg/dl with a range of 68 – 88 mg/dl. Fasting blood glucose and glycosylated hemoglobin correlated with age ($41 + 3.2$ years).

The level of glycosylated hemoglobin in untreated and treated diabetics was $8.30 + 0.37\%$ and $7.72 + 0.44\%$ (range 5.52 – 12.41%) of total haemoglobin respectively. The corresponding fasting blood glucose levels were $181 + 17$ mg/dl (range 130 – 282 mg/dl) and $151 + 16$ mg/dl (range 78 – 310 mg/dl) respectively. There was statistically significant differences in glycosylated hemoglobin levels between untreated and treated diabetics. The value of glycosylated hemoglobin was approximately 1.5 times higher in untreated diabetics than in the control group. This was statistically significant.

The glycosylated hemoglobin level correlated closely with the fasting blood glucose level in diabetics.

To assess the glycemic control, the treated diabetics were divided into three groups, depending upon FBS levels. Good control was assumed when it was less than 110 mg/dl, poor control when it was less than 130 mg/dl but more than 110 mg/dl and bad control when it was more than 130 mg/dl. In the good control group (n=12), FBS ranged between 78 – 108 mg/dl and glycosylated hemoglobin ranged between 5.52 – 5.8% with a mean of $5.72 + 0.063\%$ of total haemoglobin. In the poor control group (n=4) fasting blood glucose level was 130 mg/dl and glycosylated hemoglobin level was 6.21% of total haemoglobin. In the bad control group (n=13) fasting blood glucose ranged between 132 – 310 mg/dl and glycosylated hemoglobin ranged

between (6.40 – 12.41%) with a mean of $8.76 + 0.45\%$ of total hemoglobin.

If however, the level of glycosylated hemoglobin was taken to be the index of diabetic control, the results obtained were somewhat different. The patients were divided into three groups on the basis of glycosylated hemoglobin. Good control was assumed when it was less than 7.4%, poor control when it was less than 10.5% but more than 7.4% and bad control when it was more than 10.5% of total haemoglobin. In the good control group (n = 10), glycosylated hemoglobin level ranged between 5.52– 7.23% of total haemoglobin and fasting blood glucose varied from 91 – 163 mg/dl with a mean of $117 + 8$ mg/dl. In the poor control group (n = 9), glycosylated hemoglobin level varied from 7.68 – 10.25% with a mean + SEM = $8.97 + 0.33\%$ and the fasting blood glucose level ranged between 165 – 310 mg/dl with a mean + S.E.M. of $222 + 21$ mg/dl. In the bad control group (n = 1) glycosylated hemoglobin was 12.41%, total haemoglobin and the fasting blood glucose level was 260 mg/dl.

Discussion

The present study is based on observations of glycosylated hemoglobin (HbA_{1c}) in 20 normal healthy subjects (serving as control group) and 106 Type – 2 diabetic patients. The levels of glycosylated haemoglobin appears to be index of the levels of blood sugar for a period of several weeks (2- 3 month or 8 – 10 weeks) prior to the time of sampling, thereby providing an improved method of assessing diabetic control.

The glycosylated hemoglobin level in control group varied from 4.50 – 6.50% with a mean of $5.30 + 0.24\%$ of the total haemoglobin. The fasting blood glucose level in control group varied from 68 to 88 mg/dl with a mean of $79 + 2$ mg/dl. Values of glycosylated hemoglobin, obtained in this study are comparable to those reported by Raheja et al (1981) $5.9 + 0.6\%$ (range 4.4 – 6.8%) and Srivastava et al (1984) $5.38 + 0.60\%$ (Range 4.4 – 6.5%). They have used the modified method of fluckiger and Winter halter for the estimation

of HbA_{1c} level. The values of glycosylated hemoglobin are lower than 8.2 + 1.2% reported by Graf et al 1978 and 7.45 + 0.11% reported by Gonen et al (1977). They have used the method of Trivelli et al (1971) as modified by Gabbay et al 1977. The difference in methodology may account for these differences.

The fasting blood glucose level of 79 mg/dl is well within normal limits of blood glucose i.e. less than 110 mg/dl (W.H.O. Expert Committee Report on Diabetes Mellitus 1980).

A positive correlation of fasting blood glucose with age and glycosylated hemoglobin with age (41 + 3.2 years) was observed in normal persons.

Graf et al (1978) have observed similar positive correlation of fasting plasma glucose and glycosylated hemoglobin with age (51 + 14 years) in 29 normal subjects. Hence the values obtained in control group are close proximity with those of Graph et al (1978).

The level of glycosylated hemoglobin in untreated diabetics varied from 6.83 – 10.40% with a mean of 8.30 + 0.37% of total haemoglobin and fasting blood glucose levels ranged between 130 to 282 mg/dl with a mean or 181 + 17 mg/dl.

The level of glycosylated hemoglobin when compared with that in the control group, showed a statistically significant higher value in untreated diabetic. The glycosylated hemoglobin in untreated diabetics was approximately 1.5 times higher than that in the control group.

Graf et al (1978) observed a 1.5 fold increase (12.7 + 3.4%) compared to 8.2 + 1.2% and Gonen et al (1977) a 1.6 fold increase (12.5 + 0.77%) compared to 7.45 + .11% in glycosylated hemoglobin in diabetics as compared to the normal. Similar results have been reported by Trivelli et al (1971), Gabbay et al (1977) and Paulson and Koury (1976). Hence the results in this series of study are in conformity with this.

The level of glycosylated hemoglobin in treated diabetics varied from 5.52 – 12.41% with a mean of 7.72 + 7.44% of total haemoglobin and that of fasting blood glucose varied from 78 – 310 mg/dl with a mean of 171 + 16 mg/dl.

This value when compared with that found in control group showed a statistically significant higher value in treated diabetics.

Values of glycosylated hemoglobin in treated diabetics in this study are comparable to those obtained by Raheja et al (1981). They studied 56 treated diabetics and found glycosylated hemoglobin levels ranging from 4.9 – 13.1% of total haemoglobin. Similar results have been reported by Chandalia et al (1980).

The values of glycosylated hemoglobin obtained in untreated diabetics (8.30 + 0.37%) of total haemoglobin, when compared with those in treated diabetics (7.72 + 0.44%), showed statistically insignificant differences. However both in untreated and treated diabetics, glycosylated hemoglobin levels were significantly higher when compared with the control.

It has been proposed that the glycosylated hemoglobin level reflects the ambient glycaemia to which the erythrocytes is exposed during its life cycle (Bunn et al 1976). In this study, levels of glycosylated hemoglobin in untreated diabetics were not significantly higher than those in treated diabetics. This may be due to short duration and/or early detection of diabetes, the untreated group which has thus not caused significant rise of glycosylated hemoglobin in comparison to the treated group. On the other hand glycosylated hemoglobin levels of treated diabetics was significantly raised in comparison to control, due to the prolonged hyperglycemia and inadequate control.

Conclusion

Therefore, in assessing glycemic control in diabetics with high glycosylated hemoglobin levels, fasting blood sugar level estimations are essential to know the current blood glucose level. So estimation of glycosylated hemoglobin should be viewed as an adjunct to fasting blood glucose estimation and not as a replacement.

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