Estimation Of Glycosylated Hemoglobin Among Diabetic Patients A Case Control Study

Arshad Ikram*, Rajesh Gautam, Syed Esam Mahmood*****

* Associate Professor, Department Of Pathology, ** Assistant Professor, Department Of Pathology, *** Associate Professor, Department Of Community Medicine, MRA Medical College, Ambedkarnagar, Up, India

Abstract: Introduction: The main role of Glycosylated haemoglobin is in getting information about degree of control in a diabetic patient. The present study is being undertaken for estimating the glycosylated haemoglobin in various forms of diabetes mellitus and to assess its efficacy further management of patients. Methods: The present study has been conducted on forty patients of diabetes mellitus selected from outpatients department and compared to ten controls. All the cases i.e. patients as well as controls were investigated for post prandial 2 hours blood glucose (Glucose oxidase method), urine sugar (Benedict's test) and glycosylated haemoglobin percentage (Quick column/ion exchange resin method). Results: Among 40 diabetic patients, 8 (20%) wee of IDDM group and 32 (80%) were of NIDDM group. Basal level of glycosylated haemoglobin in control group is 6.30%. Glycosylated haemoglobin value can discriminate cases of diabetes mellitus from non diabetic cases. There is no correlation between age and sex of the patient with glycosylated haemoglobin percentage. Glycosylated haemoglobin decreases with improving quality of control of diabetes mellitus as indicated by decreasing amount of sugar in urine. Diabetes mellitus patients can be categorised into good, fair or poor control on the basis of glycosylated haemoglobin percentage. Glycosylated haemoglobin percentage is very well correlated with average blood glucose value (Post prandial 2 hours) over previous 2 months. Conclusion: The control of diabetes mellitus was shown by the level of blood glucose (post prandial 2 hours) and urine sugar and was also confirmed by percentage of glycosylated haemoglobin. [Arshad I et al NJIRM 2016; 7(6):34-39]

Key words: Diabetes mellitus, glycosylated haemoglobin.

Author for correspondence: Dr Syed Esam Mahmood, C-5 Ahliyabainagar, Kalyanpur-226022, Lucknow, Up , India. M: 8127537806 E-Mail: semahmood@gmail.com

Introduction: Broadly diabetes mellitus may be classified as insulin dependent diabetes mellitus (IDDM) which is due to absolute lack of insulin and non-insulin dependent diabetes mellitus (NIDDM), because of insulin resistance and relative insulin deficiency. The monitoring of patients with diabetes mellitus over long periods of time is usually achieved by regular urine testing and occasional plasma glucose estimation but it is well appreciated that single determination of the blood glucose at periodic visits of the patients furnish an incomplete idea of the long term control of diabetes. However, even testing for urine and blood sugar before each meal and at bed time does not fill the need completely.

Consequently the discovery in 1968, that diabetic patients have elevated levels of haemoglobin A_1 (glycosylated haemoglobin) was welcomed with satisfaction, as means of evaluating the degree of control over an extended period of 2-3 months. HbA_1 is formed by post synthetic nonenzymatic glycosylation of red cells. If blood glucose levels are on average higher, as in diabetes mellitus, than a greater proportion of red cell haemoglobin will become glycosylated. The concentration of HbA_1 (normal range 6.5% - 7.5%) of total Hb can thus be used as an index of adequancy of long term control of blood glucose concentration in diabetes mellitus.

A good deal of work has been done on glycosylated haemoglobin in diabetic patients by different workers [1-8]. Besides its main role in getting information about degree of control in diabetic patients, It may also be studied in patients of chronic renal failure [9] in cases of anaemia [10] in pregnant diabetics as an indicator of glucose control and foetal size [11].

Literature testified the rarity of such kind of study ever conducted in this part of the state, hence present study is being undertaken for estimating the glycosylated haemoglobin in various forms of diabetic mellitus and to assess its efficacy further management of patients.

Method: The present cross-sectional one year study was conducted in Department of Pathology, M.L.B. Medical College, Jhansi on the patients attending out patients department of "Diabetes" as speciality after taking permission from institutional ethical committee. The subjects for the present study were those who were clinically diagnosed as cases of diabetes mellitus and Normal healthy individuals as

controls. Patient's general information and clinical examinations and previous investigations (if any) were recorded on a proforma. History and examination included mainly the duration of disease, presenting complaints such as polyuria, polydipsia, polyphagia, change in weight. Symptoms and signs regarding various complications related to Eye, nervous system involving somatic sensory, motor and autonomic nerves Kidney, circulating system were recorded. Patients were then investigated for blood glucose level and along with them normal healthy individuals who were considered as controls aware also investigated for the same. Patients and controls were then called after 2 months interval and were again submitted to the same investigations for determining the level of blood glucose and presence of urine sugar. In addition they were also investigated for Glycosylated haemoglobin concentration.

Estimation Of Blood Glucose Was Done By Glucose Oxidase Method (Bauer, 1990) [12], Test For Urine Sugar -Benedict's Test, Estimation Of Glycosylated Haemoglobin, Glycosylated (Fast Fraction) Haemoglobin And Quick Column/Ion Exchange Resin Method (Trivelli Et Al, 1971 & Helena Methodology)[13,14]

Grading Of Diabetes on The Basis Of G Hb% Was Done As Follows: Non – Diabetic : 4.5% To 8.0%, Good Control: 8.0% To 9.0% And Fair Control: 9.0% To 10.0%.

Results: Out of 40 diabetic cases in this study, 27 (67.5%) were males and 13 (32.5%) were females. The ratio between male and female diabetics is therefore 2:1. There were ten control cases distributed as 6 (60%) male subjects and 4 (40%) female subjects. 8 (20%) cases belonged to IDDM group (Type I diabetes) and 32 (80%) cases belonged to NIDDM group (Type II diabetes). Age was ranging from 11 to 80 years in diabetic cases. In NIDDM group the maximum number of 10 cases (25%) was seen between 41 to 50 years, followed by 9 cases (22.5%) in the range of 51-60 years, 7 cases (17.5%) in the range of 61-70 years, 4 cases (10%) in 31-40 years and 2 cases (5 %) in 71-80 years age group. In IDDM group there were 8 cases (20%), four (10%) each in the age group of 11-20 years and 21-30 years. Among ten control cases maximum numbers of 3 cases (30%) were in the age group of 11 to 20 years, followed by 2 cases each (20%) in 21-30, 31-40, 41-50 age groups and 1 case in 51-60 years age group. The youngest age group of 11-20 years had mean G-HB as 9.02% and oldest age group of 71-80 years had mean G-Hb of 8.30%. Irregular rise and fall of its percentage was observed between the two extremes of age. There were 10 control cases whose mean G-Hb was 6.3%. Mean G-Hb% was only slightly higher in female cases (9.72%) in comparison to males (9.63%). Mean G-Hb% was 6.30% and 6.32% in male and female control cases respectively.

Table – 1: Correlation of Urine Sugar with Glycosylated Haemoglobin Percentage (Based On Benedict's Qualitative Method)

Urine Sugar	No. of cases	Percentage	Mean G-HB%
NIL	12	(30.0%)	8.25%
+	5	(12.5%)	9.34%
++	13	(33.5%)	9.87%
+++	6	(15.0%)	10.4%
++++	4	(10.0%)	12.4%

Maximum number of 13 cases (33.5%) had urine sugar ++ and minimum number of 4 cases (10%) had urine sugar ++++. The table also shows that as the amount of sugar in urine increases, sugar was ++++ (2 or more than 2 gm) and was minimum (8.25%) when no sugar was present in urine. (Table 1)

Table – 2: Blood Glucose Level (Post Prandial 2 Hr) During 2 Months

Group	**No. of cases**	**Previous blood glucose level**	**Present blood glucose level**	**Average blood glucose level during last 2 months**
Diabetic Cases				
Groups 'A' : Mild (up to 200 mg/dl)	9	146 mg/dl	186 mg/dl	166 (mg/dl)
Groups 'B' : Moderate (200-350 mg/dl)	20	224 mg/dl	260mg/dl	242(mg/dl)
Groups 'C' : Severe (more than 350 mg/dl)	11	440 mg/dl	384 mg/dl	412(mg/dl)
Control Cases	10	128 mg/dl	125 mg/dl	126(mg/dl)

Average (post parandial 2hrs) glucose level determined during these two months period was minimum (166 mg/dl) in group A patients. Value was maximum (412 mg/ dl) in group C patients while Group B patients had moderate rise of blood glucose level (242 mg/dl). All the values were still higher than the control cases where glucose level was 126 mg/dl.

Table – 3 Correlation Of Average Blood Glucose Level With Mean Glycosylated Haemoglobin Percentage

Group	Average blood Glucose (Post parandial 2 hours) mg/dl	Urine Sugar	Mean G-Hb%
Diabetic Cases			
Group 'A' (Good control)	166	NIL	8.76%
Group 'B' (Fair control)	242	++	9.20%
Group 'C' (Poor control)	412	+++	11.20%
Control Cases	125	NIL	6.30%

Average blood glucose level determined during these two months was 166 mg/dl and under good control in Group 'A' patients which is correlated well with mean G-Hb% of 8.76% . Group 'B' patients had glucose level of 242 mg/dl which was under fair control correlated will it mean G-HB% of 9.20% Group 'C' patients had glucose level of 412 mg/dl indicating poor control correlating with mean G-Hb% of 11.20% Urine sugar in Group A, B & C was Nil , ++ and +++ respectively. In contrast, control cases had glucose level of 125 mg/dl without sugar in urine and 6.3% G-Hb.

Discussion: The ratio between IDDM and NIDDM subjects was 1: 4 in our study. All the cases belonging to IDDM group were in the range of 11 years to 27 years and in the group of NIDDM, maximum number of cases (25%) presented in fourth decade of life followed by 22.5% cases in fifth decade. Among total cases 67.5% were male and 32.5% were female. The cases in respect to age, sex and type of diabetes are well correlated with studies given by various workers such as – Genuth [15] and Ganong [16] who have reported that NIDDM usually develops after age of 40 years and IDDM usually appears in the patients under 30 years of age. In our study we matched age of the test subjects with glycosylated haemoglobin percentage. All the values were compared with control cases whose mean G-Hb% was 6.3%. Among the test subjects, youngest age group of 11-20 years hade mean GHb as 9.02% and oldest age group of 71-80 years had mean GHb of 8.30%. Irregular rise and fall of its percentage was observed between the two extremes of age. All the values were in the diabetic range in comparison to control cases but there was no correlation between age of the patient and GHb%. The reason behind variability of the GHb values was based on degree of control of disease. Age group showing maximum GHb% included Patients of poor control and age group showing minimum GHb% had good controlled patients. The similar conclusion was made by Trivelli et al [13]and Jun et al [3].

Glycosylated haemoglobin was also matched with sex of the individual. There were 27 (67.5%) males whose mean GHb was 9.63% and 13 (32.5%) females had mean GHb of 9.72%. The results were comparable to control cases of which males had mean GHb of 6.30% and females had 6.32%. It was concluded, that although the test subjects had GHb in diabetic range but the values were almost equal in both sexes because all the males and females were in same degree of control. The reports of Malati et al [8] also prove the same fact who estimated mean GHb% in males as 9.37% and in females as 9.61%.

Urine sugars also give information about quality of control. It was assessed semi quantitatively and was matched with mean GHb%. There was no sugar in urine in 13 (30%) cases and their GHb was 8.25% When the urine sugar was (+) in 5 (12.5%) cases the value of GHb was 9.34%, for (++) sugar in 13 (33.5%) cases it was 9.87% , for (+++) sugar in 6 (15%) cases it was 10.4% and lastly when the urine sugar was (++++) in 4 (10%) cases, mean GHb% was 12.4%. Thus the above values give the clue that when the sugar in urine increases, GHb% also increases.

These findings are well correlated with other workers such as Lanoe et al[17] who told Hb A_1 varies inversely with quality of control as judged by urinary glucose indices. G-Hb is as effective as and more precise than three times day semi quantitative urinary sugar estimations. If the daily urine analysis is the best way for the patient to estimate the quality of his own control, GHb estimation is better for physicians since it is a single measurement and does not depend on patient's measurement.

Bunn et al [4] found that total glycosylated haemoglobin levels were significantly correlated with amount of glucose excreted in 24 hours period of urine collection. A good correlation was also shown by Jun et al [3].

All the diabetic patients were classified into three groups based on degree of rise of blood glucose level (post prandial 2 hrs). Group A included 9 patients having blood sugar level (post prandial 2 hrs.) below 200 mg/dl, Group B included 20 patients having blood sugar level in the range of 200-350 mg/dl and Group C showed server elevation of blood sugar level above 350 mg/di in 11 patients. Average (post prandial 2 hrs.) blood glucose level during two months was 166 mg/dl in Group A. It was 242 mg/dl in Group B and 412 mg/dl in Group C while 10 control cases had level of 125 mg/dl which was in normal range.

Group A patients were considered to be in good control as their sugar level was 166 mg/dl and urine sugar was nil, Group B patients were under fair control as their blood sugar level was 242 mg/dl and (+++) sugar present in urine. Control cases had no sugar in urine.

These results were matched with mean GHb% in these groups. Group A patients had mean GHb% of 8.76% which is in the range of good control (Range for good control is 8%-9%), Group B patients showed mean GHb% as 9.20% which is indicator of fair control (range for fair control is 9% -10%), Group C patients had mean GHb% as 11.2% which is considered to be value of poor control (value for poor control is more than 10%). All the values of GHb% were in the diabetic range in comparison to control cases whose mean GHb% was 6.30% (range for non diabetics 4.5 -8%).

Our findings are in correlation with various workers Ran[18] estimated post prandial 2 hours blood glucose level in diabetic patients. Average blood glucose level in the cases, under good control, was 169 mg/di, glucose level in cases of fair control was 244 mg/ dl. and glucose level in cases of poor control was 383 mg/ dl GHb% in each of these groups was 8.5%, 8.6% and 11.4% respectively thus proving the fact that GHb% are in correlation with post prandial 2 hours glucose,. Among control cases average blood glucose level was 92 mg/dl and the value of GHb was 8.1% which was at the upper limit of normal range (normal range is 4.5% - 8%).

Malati et al [8] used the same method for estimating GHb% that is cation exchange resin binding method. Percentage of GHb in controls was 7.27% $\pm$ 0.45 (range 6.0% 8.04% and 9.47% $\pm$ 0.98 (range 8.2% - 13.63%) in diabetic group.

Chandalia et al[17] classified the control of diabetes over proceeding 2 months on the basis of daily urine sugar data and periodic glood glucose estimation and found that estimated glycosylated haemoglobin levels correspond well with the degree of metabolic control.
Verma et al [19] found that Hb A_1 levels in controls was 6.39 $\pm$ 0.85 SD (range 4.78 – 8.20%) which is in agreement with earlier reported range for normal given by Jain et al [20]. There was no overlap of Hb A_1 level in controls and diabetics even when 99.7% confidence limit is taken for normal range, so it can be concluded that subjects with Hb A_1 levels less than 8.94% are likely to be non diabetic or diabetics with excellent control. This is almost correlated with other studies [19,20] as we have taken 8% GHb as upper limit of the normal range. The difference in values of upper limit is because, Verma et al[19] also included diabetics with excellent control up to value of 8.94% and we have considered only non diabetics up to 8% value of G Hb in our study.

Raman et al [21] estimated glycosylated haemoglobin in 50 diabetics (37 NIDDM and 13 IDDM) and 10 controls. Control of diabetes mellitus in preceding 2 months was assessed in terms of urine sugar and blood glucose.

Patients under good control had 5.7% $\pm$ 3.36 SD GHb, in fair control group was 7.7% $\pm$ 0.31 SD and GHb in poor control group was 9.5% $\pm$ 0.35 SD. They concluded that the levels of GHb are higher in

uncontrolled diabetics as compared to non diabetic control group (4.69% ± 0.662 SD GHb).

The finding is consistent with that of Leo et al [22] and Bunn et al [4].

In comparison to our results, lowered basal control value of GHb as well as test values is mainly used (Thiobarbituric acid method) but even then all the diabetics had GHb% elevated in comparison to control group and were in the diabetic range.

Larsen et al [23] estimated GHb in IDDM patients and gave the view that proportion of patients with poor control had haemoglobin A_1 value above 10.0% and concluded that regular measurements of haemoglobin A_1 lead to changes in diabetic treatment and improvement of metabolic control, indicated by lowering of Hb A_1 values.

Conclusion: The control of diabetes mellitus was shown by the level of blood glucose (post prandial 2 hours) and urine sugar and was also confirmed by percentage of glycosylated haemoglobin. This confirms the role of these tests in assessment of the degree of control and management in diabetic patients.

References:

1. Koenig, R. J. ;l Peterson, C. M. ; Jones, R.L.; Savadek, C.: Lehrman, M.; Cerami, A. : Correlation of glucose regulation and haemoglobin A_{1c} in diabetes mellitus. The New England Journal of Medicine 1976; vol 295 (8): 417-420.
2. Haney, NL. D. Bunn, H. F. ; Kamin, S.; Gabbay, K.H. ; Gallop. P.N. : The biosynthesis of human haemoglobin A_{1c} slow glycosylation of haemoglobin in vivo . The journal of Clinical Investigation 1976; 57: 1652-59.
3. Jun, W.T. ; Thlomposn, K.H.; Leichter, J. : HbA_{1c} – An indictor of Diabetic control. Thle Journal of Pediatrics 1978; Vol (1) : 13 -16.
4. Bunn, H.F. : Gabby, K.H. : Gallay, P.M. : The glycosylation of haemoglobin : Relevance to diabetes mellitus.Scienlce 1978 vol 200:: 21-27.
5. Boden, G. : Master, R.W.: Gordon, S. S. : Charles, R.: Shuman, C.R. : Owen, O.E. : Monitoring metabolic control in diabetic out patients with glycosylated haemoglobin . Annals of Internal Medicine, 1980: Volume 92: Number-3: pg 357 - 360.
6. Nathan, D.M.; Sinlger, D.E.; Hurxthal, K.R.N. ; Goodson, J.D. : The clinlcial information value of the glycosylated haemoglobin assay. The New Enlgland Journal of Medicine. 1984; 310 (6): 341 - 46.
7. Susheela, L.; Ramachandran, A. ; Mohan, V.; Viswanathan , M. : A semi – micro clorimetric method for estimating glycosylated haemoglobin Indian of Medicine Research 1986; 84:93-98.
8. Malati, T. ; Murali, D.M.k. ; Srinlivasan, V.R. and shantharam, V. : Glycosylated haemoglobin A_1 in a Random group of adult onset diabetes of Indian sub population Indian Journal of Clinical Biochemistry 1992; 7 (2): 138-142.
9. Stanton, K.G. l; Davis, R.; Richmond, J. : Glycosylated haemoglobin A in renal failure. Lancet 1978 :pg 100.
10. Rai, K.B. ;Pattabiraman, T.N. : Glycosylated haemoglobin levels in iron deficiency anaemia. Indian Journal Medical Research 1986; 83:234-236.
11. Nangia, M. ; Jain, S. K. ; Verma, N.P. Sing and Eduljee, S.Y. : Glycosylated haemoglobin in pregnant diabetics. Journal of Diabetics Association of India. 1986;26:36-41.
12. Bauer, J.D. : Clinical Laboratory Method1990: 9th. Edition pg 476-478.
13. Trivelli ,L.I.A. ; Ranmey, H.M.; Lai, H. T. : Haemoglobin components in patient with diabetes mellitus. The New England Journal of Medicine. 1971; 284 (7) : 353.
14. Helena : Product Insert Method catalog # 5340, Beaumont, Texas, Helena Laboratories.
15. Genuth, S. : classification and diagnosis of diabetes mellitus. Thy Medical Clinica of North America. 1982;66 (6) : 1191.
16. Ganong, W. F. : Endocrine functions of pancrease and regulation of carbohydrate metabolism.Review of medical physiology, published by Appleton and lange 1991; 15 th edition, pg 331.
17. Chandalia, H.B. ; Sadikot, S. ; Bhargav, D.K.; Krishnaswamy. P.R. : Estimtion of glycosylated haemoglobin by a simple chemical method & its use in monitoring control of diabetes mellitus. Journal of Association of physician of India. 1980; vol 28 : 285-286.
18. Ran, A.L. :Glycohaemoglobin, its use in the follow up of diabetes and diagnosis of glucose intolerance. Arch. Internal Medicine 1981 ; 141:747-9.

19. Vermasingh ,N.P.l; Khullar, S.K. ; Jain, S.K. : Diagnostic value of glycosylated haemoglobin in diabetes mellitus for Indian population. Journal of Diabetes Association of India 1984; 24: 41-46.
20. Jain, S. K. ; Singh, Verma, N.P.; Haemoglobin A_1 levels and their correlation with blood glucose, serum cholesterol triglyceride, serum Free fatty acids and 25 hours urinary glucose in diabetes mellitus. Thesis for Degree of Doctor of Medicine (Medicine), University of Delhi, 1981.
21. Raman, A. G. ;Lonsare, P.K. : Glycosylated Haemoglobin levels in diabetes mellitus. Journal of diabetes Association of India 1985; 25:25-27.
22. Leo, P. Krall : Oral Hypoglycaemic agents. Joslinl's Diabetes mellitus 1976; 11^{th} edition lea and Febiger Philadelphia pg 304.
23. Larsen, M.; Horder, M.; Mogensen, E.F. : Effect of long term monitoring of glycosylated haemoglobin levels in insulin dependent diabetes mellitus. New England Journal of Medicine 1990; 323 (15) : 1021-5

Conflict of interest: None
Funding: None
Cite this Article as: Arshad I, Rajesh G, Syed M Estimation Of Glycosylated Hemoglobin Among Diabetic Patients-A Case Control Study. Natl J Integr Res Med 2016; 7(6): Page no-34-39