ORIGINAL ARTICLE

# Efficacy of Glycated Albumin (GA) in Comparison with Glycated Haemoglobin (HbA1c) in Type 2 Diabetic Subject in India

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**Abstract**

The study was planned to determine the efficacy of GA in comparison with HbA1c among type2 diabetic subjects. A total of 94 type 2 diabetic (group2) subjects were selected for this prospective study and were compared with 50 non diabetic controls (group1). The subjects were reviewed for a period of 3months. Anthropometric, blood pressure, plasma glucose, GA and HbA1c measurements were done at baseline, 1st and final follow up visits for all the subjects.GA levels strongly correlated with HbA1c% both in control and study group. The mean GA and HbA1c values were significantly lower at the baseline and during follow up visits in controls than in study subjects ($p<0.001$). GA, HbA1c and the ratio decreased significantly within 4weeks, but GA showed a significantly larger decrease than HbA1c. There was no significant difference in the GA% after 3months. GA may be a useful marker for assessing short term glycemic changes in type2 diabetes.

**Key Words**

Glycated albumin, HbA1c, Type 2 diabetes, Asian Indians

## Introduction

Glycated haemoglobin (HbA1c) and fructosamine has been commonly used as the primary glycemic control indicators for type 2 diabetes mellitus, but recently glycated albumin (GA) has gained recognition as a new indicator for diabetes. HbA1c is widely used for evaluation of long term glycemic control and it provides an index of average blood glucose levels during the past 2-3 months (1, 2). Many studies have shown that strict glycemic control as indicated by lower HbA1c values reduce the risk of development of complications in diabetic patients (3, 4). Therefore, use of appropriate and accurate marker for achieving better glycemic control is required for diabetic patients to avoid diabetic complications. Due to longer life span of erythrocytes, the HbA1c test may not be suitable for evaluating short term glycemic control. Other plasma proteins like albumin have a shorter half life (15-20 days), it should detect glycemic changes earlier than haemoglobin. Hence, measurement of GA provides an index of short term glycemic control (2-4 weeks) in diabetic patients (5-8).Takahashi *et.al* (9) reported that GA may be a useful marker for monitoring short term glycemic variations during treatment in Japanese diabetic patients. Another study conducted in Japanese population also showed that GA could be a better marker for glycemic control than HbA1c both in type 1 and type 2 patients (10). It was reported that a higher GA/HbA1c ratio may reflect the post prandial hyperglycemic state in diabetic subjects. The authors concluded that GA/HbA1c ratio may be useful for the management of diabetic patients (11).There is as such no data available from Indian population that defines the clinical use of GA; hence this study was conducted prospectively with the aim of determining the efficacy of GA in comparison with HbA1c among type 2 diabetic subjects without any complications.

## Material & Methods

Subjects: A total of 187 (M:F; 105:82) subjects were selected from the out patient department of a tertiary care centre for diabetes in India for this prospective study. One hundred and twenty nine were type 2 diabetic subjects and 58 were non diabetic control subjects. The control subjects were the attenders of the patients who had participated in the study. There were 35 dropouts among type 2 diabetic subjects and 8 dropouts in the control group (*Fig 1*). A total of 94 (M: F; 53:41) type 2 diabetic subjects (group 2) who had completed all follow up visits were compared with 50 (M: F; 14:36) non diabetic control subjects (group 1). Patients having type 1 diabetes, anemia, liver dysfunction, pregnant and lactating women,

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thyroid disease, malignancy or with other diabetic complications were excluded. Hypertensive patients without any treatment and patients on steroid treatment were also excluded. After the full explanation of the study, written informed consent was obtained from each study subject. The study was approved by the Ethics committee of the institution prior the informed consent was obtained from all the subjects.

Demographic and anthropometric details like age, weight, height and duration of diabetes were recorded for all the study subjects at baseline visit. Family history of diabetes and hypertension, smoking and alcohol consumption habits were obtained from the medical records of the study subjects at baseline visit. Body Mass Index (BMI) ($kg/m^2$) was calculated using the standard formula. Blood pressure was measured in all the study subjects using a standard mercury sphygmomanometer. Blood samples were collected for the biochemical measurements. Fasting and post prandial samples were collected from the known cases of diabetes and other subjects underwent a standard oral glucose tolerance test. The diagnosis of diabetes was based on previous history of diabetes or on the criteria of World Health Organization for the classification of glucose tolerance (12).

All the subjects were asked to report for a review after 4 weeks (follow up 1) and after 3 months (follow up 2). Details like weight, body mass index and blood pressure are determined at all the visits. Fasting and post prandial blood glucose, GA, HbA1c measurements were done at baseline visit, 1st follow up which was after 1 month and at the end of the study. GA/HbA1c ratio was calculated and compared among the study groups. The study subjects were divided into four groups based on their current treatment regimen i.e. sulphonylureas, metformin, glitazones and insulin. The data on GA, HbA1c and the ratios were analysed separately in these treatment groups. During the study period the treatment regimen for diabetes remained unchanged for diabetic subjects.

GA and HbA1c measurements:Plasma GA levels were measured by an enzymatic method using albumin specific protease, ketoamine oxidase and albumin assay reagent (Lucica GA-L, Asahi Kasei Pharma Corp, Tokyo, Japan) (13, 14) on the Hitachi autoanalyser 912. GA was hydrolyzed to amino acids by albumin specific proteinase and then oxidized by ketoamine oxidase to produce hydrogen peroxide, which was measured quantitatively. The GA value was calculated as the percentage of GA relative to total albumin, which was measured with bromocresol purple method. The measured values of GA was not influenced by the substances such as Bilirubin F upto 14.6 mg/dl, Bilirubin C upto 15.2 mg/dl, glucose upto 1000 mg/dl, Ascorbic acid upto 100 mg/dl. HbA1c was measured by the turbidimetric inhibition immunoassay (15) using hemolyzed whole blood on the Hitachi autoanalyser 912. This test was designed for accurate and precise measurement of HbA1c%. The mean coefficient variations in the samples were <4.5%. Plasma glucose was estimated by glucose oxidase peroxidase method. Serum albumin was estimated by bromocresol green method.

**Statistical Analysis**

All statistical analyses were performed using SPSS 10.0 Version software (SPSS Inc, Illinois). Mean and standard deviation, median and range for continuous variables and percentages for categorical variables are reported as relevant. Significant differences between groups were evaluated using the t test, Chi-square test, median test and ANOVA where ever appropriate. Comparison of clinical parameters between baseline and follow up visits was done by the paired 't' test and students 't' test. A p value of $<0.05$ was considered statistically significant.

**Results**

GA levels were strongly correlated with HbA1c levels both in the control group ($r=0.457$, $p = 0.001$) and in the study group ($r=0.534$, $p<0.001$). The median (range) of GA value was 12.9 (9.6 - 17) in group 1 versus 18.0 (10.8 - 45) in group 2. The GA/HbA1c ratio was 2.36 (1.8 - 2.98) versus 2.5 (1.5 - 4.6) respectively. The most common range was 2.4 - 2.6 in group 1 and 2.6 - 3.5 in group 2.

***Fig 1. Shows the Flow Chart of the Recruitment of the Study Subjects***

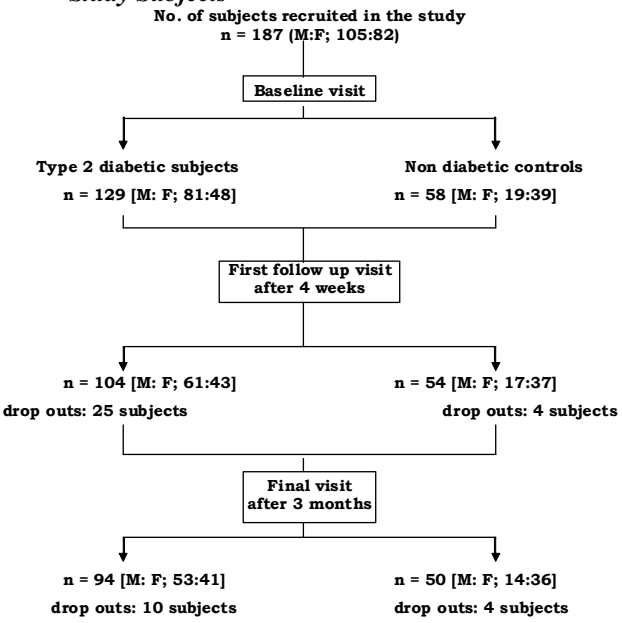

*Table 1. Comparison of Clinical Characteristics at Baseline & at Follow up Visits Between the Control & Study Groups*

| | Group 1 n = 50 (M:F; 14:36) | | | | Group 2 n = 94 (M:F; 53:41) | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | Baseline | Follow up | | p value within group (ANOVA) | Baseline | Follow up | | p value within group (ANOVA) |
| | | First | Final | | | First | Final | |
| Age (years) | 33.5 ± 7.9 | | | | 49.5 ± 7.7* | | | |
| Weight (kgs) | 59.6 ± 8.2 | 59.7 ± 8.0 | 60.0 ± 8.0 | 0.967 | 67.2 ± 13.4* | 66.5 ± 8.7* | 66.9 ± 8.7* | 0.900 |
| BMI (kg/m$^2$) | 24.3 ± 3.3 | 24.3 ± 3.2 | 24.5 ± 3.3 | 0.939 | 26.4 ± 5.1* | 26.1 ± 2.9* | 26.3 ± 3.1* | 0.838 |
| SBP (mmHg) | 119.4 ± 4.7 | 119.6 ± 5.3 | 119.8 ± 4.7 | 0.920 | 120.9 ± 7.04 | 120.7 ± 5.34 | 119.5 ± 4.9 | 0.207 |
| DBP (mmHg) | 80.2 ± 1.4 | 80.6 ± 4.7 | 80.0 ± 4.0 | 0.706 | 80.1 ± 5.6 | 80.4 ± 4.1 | 80.5 ± 4.2 | 0.831 |
| FPG (mg/dl) | 88.9 ± 6.9 | 89.3 ± 7.7 | 87.9 ± 7.1 | 0.610 | 144.7 ± 48.8* | 127.7 ± 31.9* | 117.3 ± 22.6* | <0.0001 |
| PPG (mg/dl) | 98.5 ± 13.4 | 95.2 ± 10.8 | 92.8 ± 10.9 | 0.055 | 222 ± 73.5* | 188.5 ± 50.9* | 165 ± 37.8* | <0.0001 |
| S. albumin (g/dl) | 4.05 ± 0.4 | 3.67 ± 0.42 | 3.6 ± 0.34 | <0.0001 | 3.49 ± 2.04 | 3.45 ± 0.67* | 3.5 ± 0.73 | 0.962 |
| GA (%) | 13.1 ± 1.6 | 14.5 ± 2.5 | 14.3 ± 1.7 | <0.0001 | 24.3 ± 9.5* | 19.2 ± 4.0* | 18.5 ± 6.1* | <0.0001 |
| HbA1c (%) | 5.6 ± 0.29 | 5.6 ± 0.25 | 5.6 ± 0.32 | 1.000 | 8.3 ± 1.5* | 7.7 ± 1.0* | 7.6 ± 0.89* | <0.0001 |
| GA/HbA1c | 2.35 ± 0.25 | 2.58 ± 0.4 | 2.54 ± 0.26 | 0.017 | 2.92 ± 0.89* | 2.4 ± 0.4 | 2.4 ± 0.8 | <0.0001 |

* p<0.05 vs Group 1

*Table 2. Comparison of GA, HbA1c and GA/HbA1c in the Treatment Groups*

| Variables | Sulphonylureas n = 21 | Metformin n = 22 | Glitazones n = 43 | Insulin n = 8 |
|---|---|---|---|---|
| Age (years) | 51.9 ± 9.1 | 48.0 ± 7.2 | 48.0 ± 6.5 | 54.4 ± 8.8 |
| BMI (kg/m$^2$) | 28.8 ± 8.8 | 25.8 ± 2.7 | 25.3 ± 3.0 | 27.7 ± 3.4 |
| GA (%) | | | | |
| Baseline | 25.1 ± 12.5 | 22.0 ± 8.9 | 25.1 ± 8.5 | 24.0 ± 6.8 |
| Follow up:1st | 18.9 ± 3.7* | 17.2 ± 3.4* | 20.0 ± 4.2* | 20.2 ± 4.9 |
| :Final | 19.5 ± 9.8 | 16.8 ± 4.4** | 18.7 ± 4.4** | 19.6 ± 5.7 |
| HbA1c (%) | | | | |
| Baseline | 8.0 ± 1.5 | 7.4 ± 1.1 | 8.8 ± 1.4 | 8.2 ± 1.3 |
| Follow up:1st | 7.4 ± 1.0 | 7.1 ± 0.7 | 8.1 ± 0.9* | 7.6 ± 1.0 |
| :Final | 7.38 ± 0.8 | 7.1 ± 0.8 | 7.9 ± 0.7** | 7.5 ± 0.9 |
| GA/HbA1c | | | | |
| Baseline | 3.0 ± 1.1 | 2.9 ± 0.9 | 2.8 ± 0.7 | 2.9 ± 0.7 |
| Follow up:1st | 2.5 ± 0.4 | 2.4 ± 0.5* | 2.4 ± 0.3* | 2.6 ± 0.6 |
| :Final | 2.6 ± 1.4 | 2.3 ± 0.5** | 2.3 ± 0.5** | 2.5 ± 0.5 |

*P<0.05, * baseline vs 1st follow up P<0.05, ** baseline vs Final follow up*

*Table 1* shows the comparison of clinical characteristics at the baseline and at follow up visits between the groups. The study group subjects were older than control group. The body mass index was higher in the group 2 than in group 1. No significant differences were noted in the systolic and diastolic blood pressure values between the groups. As expected, the fasting and 2 hr plasma glucose values, at baseline and during follow up visits were significantly higher in diabetic subjects than in controls. Both fasting and 2 hr glucose values decreased significantly in the follow up visits compared to baseline values in group 2.

The mean GA % and HbA1c % values were significantly lower at the baseline and during follow up visits in controls than in study subjects (p<0.001). At baseline visit, the mean GA/HbA1c ratio was significantly higher in type 2 diabetic subjects than in the control group (p<0.0001). In group 2, both GA% and HbA1c% decreased significantly in the first follow up visit when compared with baseline values (p<0.0001) The GA/

HbA1c ratio was also significantly lower at first follow up visit than at baseline ($p<0.0001$). Within 4 weeks, GA showed a significantly larger decrease than HbA1c% (mean difference: GA%: $5.15 \pm 8.5$, $p<0.0001$; HbA1c%: $0.56 \pm 0.77$, $p<0.0001$) and there was no significant difference in the GA% after 3 months in comparison with the values at first follow up visit. Similarly, GA/HbA1c ratio also decreased significantly during the initial 4 weeks, which remained similar after 3 months.

*Table 2* shows the GA%, HbA1c% and GA/HbA1c ratio at baseline and follow up visits in the study group according to the treatment regimen. Within 4 weeks, except for insulin treatment, GA levels decreased significantly in other three treatment regimens i.e., Sulphonylureas, Metformin and Glitazones and thereafter, a further reduction was not observed in the GA % at the final follow up visit in all the treatment groups. HbA1c % did not show any significant improvement in the follow up visits in all the treatment groups except glitazones. GA/HbA1c ratio decreased significantly in the metformin and glitazone treated groups within 4 weeks compared to baseline values.

**Discussion**

To our knowledge, this is the first prospective study from India to report the efficacy of GA in evaluation of the short term glycemic control in diabetic patients. The accurate assessment of glycemic control is mandatory in the diabetic patients, as improved glycemic control reduced the development of both micro and macro vascular complications of diabetes (16, 17). In the present study, GA levels were strongly correlated with HbA1c levels both in the control and in patients with type 2 diabetes. Similar observation was noted in the previous reports also (9, 10). As reported earlier (9), GA/HbA1c ratio ranged widely from 1.5 - 4.6 with a median value of 2.5 in diabetic subjects. Our GA/HbA1c ratio values agree with the values reported in Japanese population (9).

The mean GA and HbA1c values were significantly lower in the controls than in type 2 diabetic subjects. The mean GA/HbA1c ratio was significantly higher in type 2 diabetic subjects compared to controls. In a longitudinal study, Takahashi *et.al* (9) also found that the mean GA/HbA1c ratio was significantly higher in patients with poor glycemic control than good glycemic control. Another report showed that the ratio reflects post prandial hyperglycemic state and monitoring both the parameters may be useful in the management of diabetes (11).

In the present study, both GA and HbA1c and the ratio decreased significantly in the first follow up visit, but GA showed a significantly larger decrease than HbA1c. The improvement in the glycemic control as assessed by GA was noted during the initial 4 weeks itself. Further reduction was not seen and the values remained similar at the follow up visit after 3 months. In an earlier report, all the above parameters were assessed at baseline and at 16 weeks after the initiation of intensive insulin therapy. Takahashi et.al reported that both GA and HbA1c and the ratio decreased significantly at 16 weeks than at baseline and GA showed a significantly larger percent decrease than HbA1c (9). The rapid decrease in GA noted in the above study and in the present study reflects the faster turnover of plasma albumin than that of RBC.

To our knowledge, there have been no reports available about the changes of GA, HbA1c and the GA/HbA1c ratio in Indian population during treatment. Our study demonstrated that GA levels decreased significantly within 4 weeks in the oral hypoglycemic agents treated groups. In the insulin treated group also there was a reduction in the GA levels but it was not statistically significant. This may be because of the small number of patients in that group. Except glitazones, HbA1c levels did not show any significant changes in all the treatment groups. GA/HbA1c ratio decreased significantly in the metformin and glitazone treated groups. Overall improvement in the GA and the GA/HbA1c ratio had occurred within 4 weeks. Further reduction was not observed when GA values are compared after 3 months. One of the limitations of the study is that we did not assess glycemic control considering body mass index and other unknown factors which might influence GA. Although subjects with certain disease and drugs which influence albumin turnover and RBC life span were excluded, further studies are needed to evaluate the obesity related mechanism and other possible factors such as insulin resistance, genetic factors which might influence GA or HbA1c formation.

**Conclusion**

The results of the present prospective study suggest that GA may be a useful marker for assessing short term glycemic changes in patients with type 2 diabetes. It may be useful to assess the early improvement in the treatment of diabetes. Further studies are needed to confirm the clinical significance of GA as a marker of diabetic complications.

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