Original Article

# Association between serum albumin and glycated hemoglobin in Asian Indian subjects

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## ABSTRACT

**Background:** Protein glycation plays a significant role in diabetic complications. Glycated hemoglobin (HbA1c) is a known predictor of diabetes and its complications. Albumin, found to be profoundly glycated in diabetes, and its level could regulate plasma protein as well as hemoglobin glycation. **Aim:** We aimed to evaluate the association between variations in albumin level with HbA1c in the Asian Indian population. **Materials and Methods:** We screened data of 929 subjects who have had a simultaneous measurement of fasting plasma glucose (FPG), HbA1c and albumin levels via the same blood collection. Data were analyzed by SPSS for 610 subjects who met the study criteria. **Results:** There was a significant negative correlation between HbA1c and albumin concentration ($r = -0.284$; $P < 0.001$). Univariate analysis showed the statistically significant decrease of average HbA1c but not for fasting plasma glucose (FPG) across increasing tertiles of albumin. Stepwise multiple regression model showed a significant correlation between HbA1c and serum albumin ($P < 0.05$), FPG ($P < 0.001$), hemoglobin (Hb) ($P < 0.001$) and serum globulin ($P < 0.05$). FPG was the strongest predictor (63.4%) of variation of HbA1c. The albumin concentration ($r = -0.114$) accounted for 0.3% ($P < 0.05$) of the total variance in HbA1c independent of age, body mass index, FPG, Hb, creatinine, total protein and globulin. It was also observed that HbA1c decreases with increasing albumin concentration in those having FPG between 100 to <126 mg/dl. **Conclusion:** Serum albumin negatively correlates with HbA1c in Asian Indians independent of other variables. This study suggests that predicting diabetes and its complication based on the HbA1c needs to be further investigated in Indian subjects.

**Key words:** Albumin, glycated hemoglobin, glycation

## Introduction

Protein glycation is involved in the long-term complications of diabetes.[1,2] Plasma proteins are the primary targets of glycation following elevated levels of glucose in diabetes.[3] Amongst plasma proteins, albumin is one of the heavily glycated proteins because of its abundance, comparatively longer half-life and a higher number of free lysine and arginine residues.[4] Glycation accelerates albumin degradation via increasing catabolic rate and decreasing protein half-life,[5] thus decreasing the albumin levels in diabetes. It has been mechanistically shown that albumin competes with other proteins for glycation[6] and low albumin level was associated with increased plasma protein glycation in diabetes.[7] This study was corroborated in a recent finding where low albumin levels were associated with increased fibrinogen glycation.[8] It has also been suggested that low plasma albumin predicts the glycated hemoglobin (HbA1c) in type 2 diabetes,[9] thus, strongly implicating albumin in regulation of plasma protein glycation and HbA1c.

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Glycated hemoglobin is an important marker of glycemic control as it estimates average blood glucose of the previous 3 months. Recent guidelines by the American Diabetes Association also recommended HbA1c as a diagnostic tool for diabetes, in addition to its well-known use to define control.[10] Studies showed that its level correlates with average plasma glucose and the progression of diabetes complication.[11,12] However, several biological, ethnic and therapeutic factors are known to affect HbA1c values, one of being albumin levels. Despite a significant negative correlation between plasma albumin levels and HbA1c in type 2 diabetes, levels of albumin are not routinely monitored in diabetes. Only in diabetic nephropathy, albumin levels are routinely monitored. Therefore, we hypothesized that low albumin levels may be associated with higher HbA1c levels and vice versa. Thus we pursued this hypothesis by analyzing the association between albumin and HbA1c in clinical setting.

## Materials and Methods

We analyzed clinical, anthropometric and biochemical data of subjects who attended outpatient's clinic of Chellaram Diabetes Institute, Pune during year 2012-2014 and who have had a simultaneous measurement of fasting plasma glucose (FPG), HbA1c and albumin levels via the same blood collection. We screened data of 929 subjects and excluded 319 cases with anemia (Hb <8 g/dl), renal impairment (serum creatinine >2 mg/dl), pregnancy, chronic liver disease (serum total bilirubin >3 mg/dl; serum direct bilirubin >0.6 mg/dl; serum indirect bilirubin >3 mg/dl; serum aspartate aminotransferase >120 IU/L; serum alkaline phosphatase >387 IU/L; serum alanine aminotransferase >150 IU/L), hypertriglyceridemia (triglycerides >500 mg/dl), iron or Vitamin B12 deficiency and also those who were on drugs that can induce variability in HbA1c estimation.[13] All biochemistry was done using commercially available kits. HbA1c estimation was based on principles of ion-exchange high-performance liquid chromatography using fully automated D-10™ hemoglobin A1c analyzer (Bio-Rad Laboratories, Inc., USA). Albumin was estimated by colorimetric assay with endpoint method using Cobas Integra 400 plus (Roche, Switzerland). Albumin binds with bromcresol green, an anionic dye, at pH 4.1 to form a blue-green complex. The intensity of the blue-green color was measured at 583 nnm which is directly proportional to the albumin concentration in the sample.

### Statistical analysis

Data were analyzed for 610 subjects. Pearson's correlation was used to ascertain the association between albumin and HbA1c levels. Subjects were classified according to their albumin concentrations in tertiles - Q1 ≤ 4.44 g/L, Q2 = 4.44-4.73 g/L and Q3 ≥ 4.73 g/L. The mean (HbA1c) and FPG of these "albumin-level groups" were compared using ANOVA with *post-hoc* Tukey's correction. Step-wise multivariate regression analysis was done to find out the independent predictors of HbA1c. Based on FPG, subjects were grouped into group 1 (FPG < 100 mg/dl); group 2 (FPG = 100 to < 126 mg/dl); group 3 (FPG ≥ 126 mg/dl) and the mean (HbA1c) level was compared across the "albumin-level groups" by ANOVA with *post-hoc* Tukey's correction.

## Results

We studied the results of 610 subjects (Male = 545; Female = 65) with simultaneous measurement of serum albumin, FPG and HbA1c. The mean age of the subjects was 38.9 ± 13.2 years. There was a significant negative correlation between HbA1c and albumin concentration ($r = -0.284$; $P < 0.001$). Initially, we applied univariate approach by working out the tertiles of albumin versus HbA1c. The tertiles (three sets of albumin data grouped) showed statistically significant differences of average HbA1c across three groups (tertiles) of albumin [Figure 1]. The average HbA1c was significantly higher in the lower tertile compared to the second and third tertiles of serum albumin concentration ($P < 0.05$ for both). The average HbA1c is significantly higher in the second tertile compared with the third tertile of serum albumin concentration ($P < 0.01$). The average FPG did not differ significantly between first and second tertile ($P = 0.4$) though the difference was significant between second and third tertile ($P < 0.05$) [Table 1].

We tried to confirm the association by stepwise multiple regression model which showed a significant correlation between HbA1c and fasting glucose ($P < 0.001$), hemoglobin (Hb) ($P < 0.001$), serum albumin ($P < 0.05$) and serum globulin ($P < 0.05$). The most influential

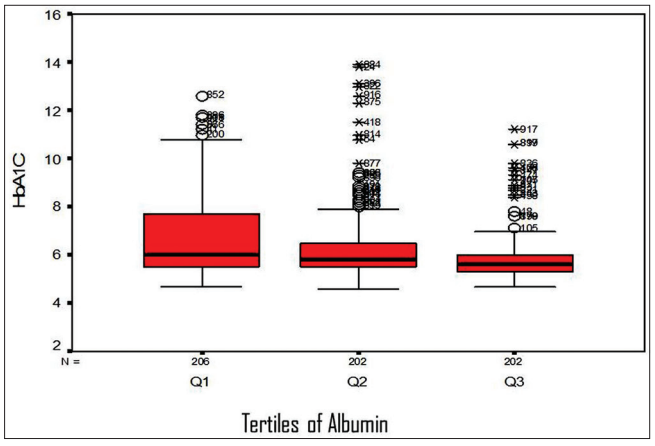


**Figure 1:** The distribution of glycated hemoglobin across tertiles of albumin concentration (box-plot)

**Table 1: The statistical comparison of HbA1c across tertiles of albumin concentration**

| Variable (mean±SD) | Tertiles of serum alubim | | | Inter-tertiles comparison | | |
|---|---|---|---|---|---|---|
| | Q1 (*n*=206) <4.44 g/L | Q2 (*n*=202) 4.44-4.73 g/L | Q3 (*n*=202) >4.73 g/L | Q1 versus Q2 | Q1 versus Q3 | Q2 versus Q3 |
| HbA1c | 6.83±1.88 | 6.39±1.69 | 5.94±1.16 | 0.013 | 0.001 | 0.010 |
| FPG | 122.96±45.85 | 117.86±49.83 | 107.64±28.50 | 0.4 | 0.001 | 0.043 |

*P* values by one-way ANOVA with *post-hoc* Tukey's correction due to multiple group comparisons. $P<0.05$ is considered to be statistically significant. SD: Standard deviation, ANOVA: Analysis of variance, HbA1c: Glycated hemoglobin, FPG: Fasting plasma glucose

predictor was FPG, which accounted for 63.4% of the total variance in HbA1c. Albumin concentration ($r = -0.114$) accounted for 0.3% ($P < 0.05$) of the total variance in HbA1c among the 610 patients independent of age, body mass index (BMI), FPG, Hb, creatinine, total protein and globulin [Table 2].

We also tried to explore if the association of HbA1c with albumin occurred irrespective of or in synchrony with different levels of FPG. Our results showed that the average HbA1c is significantly higher in Q1 of serum albumin compared with Q2 and Q3 of serum albumin in group 2 of FPG (FPG between 100 mg/dl and 126 mg/dl) ($P < 0.01$ for all). However, the average HbA1c did not differ significantly ($P > 0.05$) between Q2 and Q3 of serum albumin in Gp2 of FPG [Table 3].

## Discussion

The results of our study showed a statistically significant negative correlation between HbA1c and serum albumin levels. This persisted despite adjusting for confounding factors like FPG age, BMI, Hb, serum creatinine, serum globulin, total protein. Notably, common clinical conditions like anemia and drugs interfering with HbA1c estimations like aspirin were excluded.

While the magnitude of HbA1c change with serum albumin variations was admittedly small (0.3%) compared to a previous study[9] we believe that beyond altering HbA1c, this phenomenon may have other important physiological effects too. For example, if serum albumin was to compete with hemoglobin for glycation and lower the HbA1c negatively, it is possible that other proteins could be designed that may become progressively glycated, and therefore, prevent tissue glycation, and alter the prevalence of complications. Indeed this has been tested *in vitro* and *in vivo*, with some suggestions of benefit.[7-9]

Interestingly, our results also suggest that the HbA1c-albumin association existed only at non-hyperglycemic FPG ranges, but not at hyperglycemic ranges. This leads us to speculate whether this means that higher glucose levels were able to glycate both hemoglobin and albumin-thus warding off any correlation between the two at higher levels.

**Table 2: Multivariate regression analysis (stepwise method) to obtain the independent predictors of HbA1c**

| Variables | Standard beta | *T* value | $R^2$ change | *P* for $R^2$ change |
|---|---|---|---|---|
| Fasting blood glucose (mg/dl) | 0.739 | 30.451 | 0.634 | 0.001 |
| Hemoglobin (g %) | −0.145 | −5.788 | 0.032 | 0.001 |
| Age (years) | 0.125 | 5.214 | 0.017 | 0.001 |
| Serum albumin (g/dl) | −0.069 | −2.731 | 0.003 | 0.016 |
| Serum globulin (g/dl) | 0.051 | 2.113 | 0.002 | 0.035 |

Variables included in the model (age, BMI, hemoglobin, fasting glucose, serum creatinine, serum globulin, total protein, serum albumin). Variables such as BMI, serum creatinine and total protein were excluded from the final model due to statistical insignificance. $P<0.05$ indicates the change in $R^2$ is statistically significant. SD: Standard deviation, BMI: Body mass index, HbA1c: Glycated hemoglobin

It is well known that HbA1c may falsely overestimate pre-diabetes in Indians, and this has been attributed to iron deficiency anemia, among other factors.[14,15] In the study by Hardikar *et al.*, it has been shown that among 116 subjects (HbA1c and OGTT measured on the same day), HbA1c overestimated prediabetes (23.3%) compared with OGTT (7.8%), but did not overestimated diabetes (2.3% by both HbA1c and OGTT) and this has been attributable to iron deficiency.[14] It is possible that additional factors like low albumin level could also, play a role in overestimating HbA1c in Indians with pre-diabetes. The results of our study are consistent with this finding as the increased HbA1c correlated with low albumin in those with FPG between 100 to <126 mg/dl, but not with FPG ≥ 126 mg/dl. Whether this increase in HbA1c attributable to a lower albumin might still link to diabetic complications is an issue that deserves further study.

Our study in Indian subjects suggests that higher serum albumin levels may decrease HbA1c levels and that lower serum albumin levels may raise HbA1c levels as reported previously from western studies.[9] As we did not measure glycated albumin levels, we can only cautiously speculate that this could be due to higher albumin levels competing with Hb to get excessively glycated.

Further, we caution that our study may be interpreted as hypothesis-generating, rather than hypothesis proving results, as this study has several limitations-importantly, it was a retrospective study. Also, we classified subjects into hyperglycemia and non hypergylcemia and did not

**Table 3: The distribution of average HbA1c according to the tertiles of albumin concentration and three levels of FPG**

| FPG groups | Tertiles of serum albumin | | | Inter-tertiles comparison (*P* values) | | |
|---|---|---|---|---|---|---|
| | Q1<4.44 g/L | Q2 4.44-4.73 g/L | Q3>4.73 g/L | Q1 versus Q2 | Q1 versus Q3 | Q2 versus Q3 |
| Group 1 | 5.7±1.00 | 5.6±0.5 | 5.4±0.4 | 0.889 | 0.115 | 0.281 |
| Group 2 | 6.3±1.2 | 5.9±0.6 | 5.8±0.7 | 0.008 | 0.001 | 0.905 |
| Group 3 | 8.7±1.7 | 8.8±2.3 | 8.3±1.7 | 0.996 | 0.722 | 0.702 |

Values are mean±SD of HbA1c. *P* values by one-way ANOVA with *post-hoc* Tukey's correction due to multiple group comparisons. *P*<0.05 is considered to be statistically significant. Group 1 (FPG<100 mg/dl), Group 2 (FPG = 100 to <126mg/dl), Group 3 (FPG ≥126mg/dl). SD: Standard deviation, FPG: Fasting plasma glucose, HbA1c: Glycated hemoglobin, ANOVA: Analysis of variance

group them into diabetes and non-diabetes. Hence, those without hyperglycemia could have been nondiabetic or could have been well-controlled diabetes. Nevertheless, in this limitation lies an opportunity of two different interpretations. First, the group of both prediabetes and well-controlled diabetes under a single group of FPG between 100 to <126 mg/dl mean that our data could be generalizable to both diagnostic and therapeutic settings. Second, the finding of an association of statistically increasing HbA1c with low albumin tertiles (in the subgroup of FPG 100 to <126 mg/dl) suggest that albumin could be one more factor that alters HbA1c in prediabetes subjects. This further strengthens the current understanding that HbA1c may not be as reliable in diagnosing prediabetes among Indian subjects.

We believe that the results of this study, which showed statistically significant negative correlations between HbA1c and albumin in the Indian population, could lead to new approaches in studying the ways in which glucose and proteins (albumin and Hb are examples of such proteins) might interact with one another and such studies could have an impact on understanding hyperglycemia and it's estimation.

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