

Evaluation of Glycated Albumin and Glycated Hemoglobin as Glycemic Indices in Diabetic Patients with Chronic Kidney Disease

Hoda Abdelbadie Hussein¹, Mohamed S Gomaa¹, Maher A Alamir¹, Mustafa M Hasan² and Mohamed S Abdallah³

Abstract

In diabetic patients with chronic kidney disease (CKD) or on regular hemodialysis, glycated hemoglobin (HbA1c) can be affected by multiple factors such as reduced red-blood cell lifespan, recent transfusions, iron deficiency, metabolic acidosis or frequent erythropoietin injection, while glycated albumin (GA) is not affected by these factors. *Aim of the study* is to measure glycated albumin and glycated hemoglobin in these patients to evaluate the significance of each of them as a glycemic index. *Subjects and methods:* Glycated hemoglobin and glycated albumin levels were measured in 75 patients: 25 with DM were on regular hemodialysis (group I), 25 with DM had chronic kidney disease at different stages (group II), and 25 regular hemodialysis patients without diabetes mellitus (control).

Results: GA/HbA1c ratio was significantly increased in dialysis patients (3.8 ± 0.38 %) and CKD patients (3.6 ± 0.2 %), compared with the control (2.7 ± 0.5 %). correlation of GA or HbA1c with fasting blood sugar (FBS) in groups I and II showed; poor level of GA (of 24%) and HbA1c (of 8%) resulted in a FBS of 140-160 and 180-200 mg/dl respectively. Therefore, the GA of 24% was reflected by the FBS of 150 mg/dl. However, categorization of the HbA1c of 8% was an underestimation, as it was reflected by FBS as high as 190 mg/dl. *Conclusion:* The study concluded that HbA1c may underestimate glycemic control status in diabetic patients with CKD and hemodialysis patients. In these patients, GA can reflect a reliable glycemic control status.

Keywords: glycated hemoglobin; glycated albumin; hemodialysis; chronic kidney disease

¹Cor. Author, Internal medicine department, faculty of medicine, Fayoum University, Egypt

²Internal Medicine, Faculty of Medicine - Cairo University

³Clinical & Chemical Pathology, Faculty of Medicine - Cairo University

1 Introduction

It is known that glycation among various proteins is increased in diabetic patients compared with non-diabetic subjects. Among these glycated proteins, glycated hemoglobin (HbA1C) is commonly used as the gold standard index of glycemic control in the clinical setting [1].

HbA1c reflects glucose control over the preceding 8–12 weeks, the time period being dictated by the red blood cell lifespan. Although HbA1c correlates well with glucose concentrations in diabetic non-CKD patients the relationship between HbA1c and glucose is complex in patients with CKD [2].

Falsely low HbA1c is seen mainly in conditions with high red cell turn over, such as hemoglobinopathies [3] including variant hemoglobin, sickle cell disease, glucose-6-phosphate dehydrogenase deficiency, treatment of anaemia with iron [4] or erythropoietin [5], and auto immune haemolytic anaemia. Recent blood loss and blood transfusion result in greater proportion of reticulocytes or transfused red cells in blood stream thereby reducing the average age of red cells. Patients of chronic kidney disease on dialysis and chronic liver failure [6] may also have less than expected level of HbA1c. All these conditions result in shortened average age of erythrocytes, resulting in decreased exposure time of hemoglobin to glucose and therefore less percentage of HbA1c [7].

HbA1c may therefore not accurately reflect long-term glucose exposure and might not be a reliable measure of glycemic control in CKD [8].

However, despite these concerns, current guidelines still recommend HbA1c as the preferred biomarker of glycemic control in CKD patients with target values the same as those without diabetic nephropathy.

Glycated albumin (GA) is a keto-amine formed by binding of albumin and glucose by non-enzymatic oxidation reaction. GA is an index of glycemic control not affected by disorders of hemoglobin metabolism. Additionally, it reflects the short-term status of glycemic control compared with HbA1C [9].

It was suggested that GA may provide a significantly better measure to estimate glycemic control in hemodialysis (HD) patients with diabetes and that the assessment of glycemic control by HbA1c in those patients might lead to underestimation [9, 10].

Serum glycated albumin (GA) has been proposed as an alternative marker of glycemic control in CKD patients (especially who are on dialysis), since levels of GA are unaffected by changes in RBC survival time or erythropoietin use. Compared to HbA1c, GA responds much sooner to changes in glycemic levels, reflecting short-term glycemic control over the previous 2 - 3 weeks [11].

2 The Aim of the Work

The aim of this study is to compare between GA and HbA1c in diabetic patients with different stages of CKD and ESRD on HD. This can determine the significance of measurement of GA compared to HbA1c for accurate assessment of glycemic control in these patients.

3 Patients and Methods

The study included 75 Egyptian patients from nephrology unit of Fayoum University hospital. Fifty patients with diabetes mellitus and chronic kidney disease divided into two groups: group I which included 25 patients on regular hemodialysis, group II which included 25 patients with chronic kidney disease in different stages (II,III and IV) based on estimated GFR. Group III which included 25 non diabetic patients with end stage renal disease on regular hemodialysis was a control group. The control group was selected without diabetes to determine that normal levels of HbA1C and GA in these patients are not affected by hemodialysis process itself.

Inclusion criteria:

Patients with type I or II diabetes on either insulin or oral hypoglycemic drugs.

Exclusion criteria:

Patients with liver cirrhosis, hypoalbuminemia, proteinuria more than 1gm/day, variant hemoglobin, hemolytic anemia, or with hemoglobin less than 10gm/dl. These exclusions were selected to rule out the effect of any of these factors on either HbA1C or GA.

Patients were subjected to the following: fasting blood glucose (FBS) levels on three different occasions through successive three months (1st, 2nd, 3rd month), then mean FBS was measured. At the end of these three months: HbA1C was measured, using 5ml of venous blood collected in the EDTA containing tubes and Glycated albumin (GA%) was measured using 5ml of venous blood collected in serum separator tubes.

3.1 Assay of Glycated Hemoglobin HbA1c

HbA1c was measured using Cobas Integra Tina-quant Hemoglobin A1c Gen.2 kit (*Roche Diagnostics, GmbH, Sandhofer Strasse 116, D/68305 Mannheim, Germany*). The HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed blood. The anti-coagulated whole blood specimen is hemolyzed automatically on the COBAS INTEGRA 400/400 plus/800 analyzers with COBAS INTEGRA Hemolyzing Reagent Gen.2. Glycated hemoglobin in the sample reacts with anti-HbA1c antibody to form soluble antigen-antibody complexes (R1). Then addition of polyhapten to react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex (R2), which can be determined turbidimetrically.

3.2 Assay of Glycated Albumin GA

GA was measured using human glycated albumin (GA) ELISA kit (*WKEA med supplies, 450-11th AVE, New York 10123, USA*). The GA determination is based on the enzymatic labeled immuno-sorbent assay ELISA. Using purified human GA to coat micro-titer plate wells, to make solid-phase antibody, then add GA to wells, combined GA with antibody which enzyme labeled, become antibody-antigen-enzyme-antibody complex (double-antibody sandwich), then after complete washing, add substrate which becomes blue color and the color change is measured spectro-photometrically at a wavelength of 450 nm. GA % value was calculated as the percentage of measured GA (g/dl) relative to total albumin (g/dl) measured using the same sample.

4 Main Results

Some demographic and laboratory investigations are shown in Table 1. We observe that RBCs indices (Hb, MCV, MCH) and serum albumin were not different considerably between the three groups. This means that these parameters didn't affect the results of HbA1c. Estimated GFR in group II was (mean \pm SD; 41.32 ± 18.9 ml/min) and CKD staging was; 6 patients stage II, 11 patients stage III and 8 patients stage IV. Ten patients of them were on erythropoietin (EPO) injection therapy. All patients in group I and III were on regular EPO injection therapy during hemodialysis sessions (the doses were not recorded).

Table 1: Demographic and Laboratory data of each group

	Group I DM&ESRD/HD	Group II DM&CKD	Group III ESRD/HD
Age	52.2 \pm 4.9	47.3 \pm 10.7	49.8 \pm 8.2
Sex M/F	18/7	13/12	19/6
Hb mean (g/dl)	11.2 \pm 0.64	11.1 \pm 0.83	11.2 \pm 0.71
MCV mean (fl)	78.6 \pm 2.7	76.2 \pm 4.9	79.5 \pm 2.9
MCH mean(pg)	26.7 \pm 2.2	25.4 \pm 2.9	27.2 \pm 1.9
PLT (x1000/cmm)	236.7 \pm 47.8	261.5 \pm 65.3	206.3 \pm 47.1
WBCs (x1000/cmm)	6.9 \pm 1.6	6.9 \pm 1.4	6.5 \pm 1.5
Creatinine mean(mg/dl)	5.58 \pm 1.2	3.4 \pm 1	5.45 \pm 1.4
s.Albumin mean(g/dl)	3.8 \pm 0.2	3.8 \pm 0.2	3.9 \pm 0.3
e GFR (ml/min)	-	41.32 \pm 18.9	-
ACR (mg/mmol)	-	39.6 \pm 16.4	-

DM: diabetes mellitus, ESRD: end stage renal disease, HD: hemodialysis, CKD: chronic kidney disease, Hb: hemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, PLT: platelets, WBCs: white blood cells, eGFR: estimated glomerular filtration rate, ACR: albumin/creatinine ratio

Table 2 shows glycemic control markers of the three groups. The GA/HbA1c ratio in Group III was (2.7 ± 0.5 %), while it increased significantly in Group I (3.8 ± 0.38 %) and in Group II (3.6 ± 0.2 %).

Table 2: Glycemic control markers data of each group

	Group I	Group II	Group III	p-value
FBS mean (mg/dl)	161.6 ± 33.7	170.3 ± 36.4	95 ± 9.1	<0.001*
GA %	26.4 ± 4.4	27.6 ± 4	12.1 ± 2.4	<0.001*
HbA1c %	7.1 ± 1.2	7.7 ± 1.03	4.5 ± 0.71	<0.001*
GA/HbA1c %	3.8 ± 0.38*	3.6 ± 0.2*	2.7 ± 0.5	<0.001*

FBS: fasting blood sugar, GA%: glycated albumin ratio, HbA1c: glycated hemoglobin ratio.

Groups I and II were classified into four levels (Table 3) according to serum HbA1c values: Excellent (HbA1c ≤ 6%), good (HbA1c between 6-7%), fair (HbA1c between 7-8%), and poor (HbA1c > 8%). In group I; There were (16%), (16%), (52%), and (16%) of patients in each level, respectively. While in group II there were (4%), (28%), (28%), and (40%) of patients in each level, respectively. As reported before, GA values are approximately three times greater than HbA1c values, glycemic control also was assessed according to the GA values: Excellent (GA ≤ 18%), good (GA between 18-21%), fair (GA between 21-24%), and poor (GA > 24%). In group I; there were (0%), (8%), (28%), and (64%) patients in each of the respective groups. While, in group II; There were (0%), (8%), (20%), and (72%) patients in each of the respective groups.

Table 3: Distribution of the degrees of glycemic control on the basis of HbA1c and GA values:

Glycemic control	HbA1C	GA
	patients number (%)	Patient number (%)
Group I		
<i>Excellent</i>	4 (16%)	0 (0%)
<i>Good</i>	4 (16%)	2 (8%)
<i>Fair</i>	13 (52%)	7 (28%)
<i>Poor</i>	4 (16%)	16 (64%)
Group II		
<i>Excellent</i>	1(4%)	0 (0%)
<i>Good</i>	7 (28%)	2(8%)
<i>Fair</i>	7 (28%)	5(20%)
<i>Poor</i>	10 (40%)	18(72%)

In Table 4, there were significant and positive correlations between FBS and GA in group I ($r = 0.97$ and $p\text{-value} < 0.001$) or HbA1c ($r = 0.84$ and $p\text{-value} < 0.001$) and in group II, correlations between FBS and GA ($r = 0.97$ and $p\text{-value} < 0.001$) or HbA1c ($r = 0.9$ and $p\text{-value} < 0.001$). There was non-significant correlations between FBS and GA ($r = 0.38$ and $p\text{-value} 0.06$) or HbA1c ($r = 0.31$ and $p\text{-value} 0.1$) in non-diabetic patients with ESRD/HD.

Table 4: Correlation between FBS and HbA1C and GA in the three groups

	FBS	
	r	p-value
Group I		
HbA1c	0.84	<0.001*
GA	0.97	<0.001*
Group II		
HbA1c	0.9	<0.001*
GA	0.97	<0.001*
Group III		
HbA1c	0.31	0.1
GA	0.38	0.06

Table 5 shows a significant and positive correlation between GA and HbA1c in Group I ($r = 0.9$ and $p\text{-value} < 0.001$), Group II ($r = 0.9$ and $p\text{-value} < 0.001$), and Group III ($r = 0.4$ and $p\text{-value} < 0.03$).

Table 5: Correlation between GA and HbA1c among study groups

HbA1c	GA		
	R	p-value	Sig.
Group I	0.9	<0.001*	HS
Group II	0.9	<0.001*	HS
Group III	0.4	0.03	S

Correlation of GA or HbA1c with fasting blood sugar (FBS) in groups I and II showed that a *poor* category of GA of 24% and HbA1c of 8% resulted in a FBS of 140 - 160 and 180 - 200 mg/dl, respectively (figures 1,2 and 3).

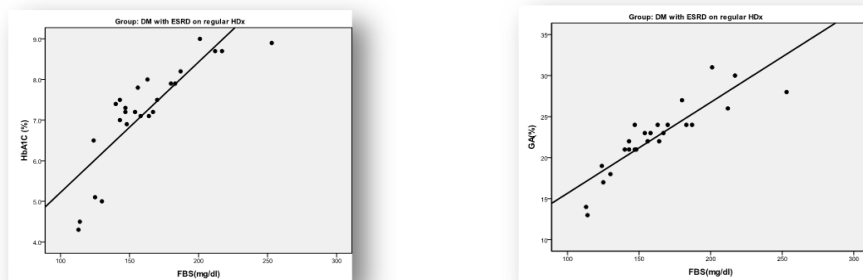


Figure 1 a and b: Correlation between FBS and HbA1C and GA in Group I

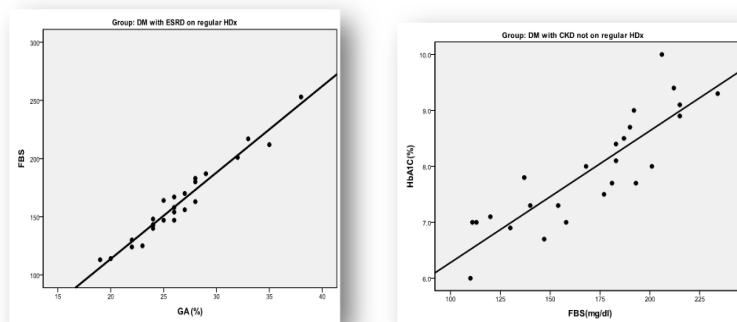


Figure 2 a and b: Correlation between FBS and HbA1C and GA in Group II

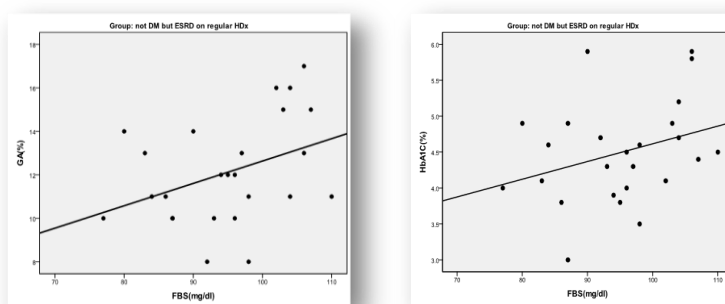


Figure 3 a and b: Correlation between FBS and HbA1C and GA in Group III

Table 6 shows the parameters of glyceimic indices of the patients in group II when correlated with their CKD stage. We observed that FBS/HbA1C and GA/HbA1C ratios had no much difference between stages II, III, or IV.

Table 6: correlation of glyceimic indices to different stages of chronic kidney disease (according to e GFR) in group II:

Stage of CKD	FBS/HbA1C (mean)	GA/HbA1c (mean)
Stage II	23.5	3.9
Stage III	26.2	3.7
Stage IV	24.8	3.7

5 Discussion

Our study showed that the *GA/HbA1c* ratio in Group III was ($2.7 \pm 0.5\%$) which is consistent with the reported ratio of 3% (GA is three times HbA1c values). The GA value relative to HbA1c was increased significantly to ($3.8 \pm 0.38\%$) in Group I and ($3.6 \pm 0.2\%$) in Group II. This means that HbA1c levels in both groups were lower than expected as regard glyceimic control. Also, in Group I and II, the glyceimic control that were based on the HbA1c values differ markedly from those based on the GA values (p -value < 0.001). It was found that a *poor* level of GA of 24% and HbA1c of 8% resulted in a FBS of 140 - 160 and 180 - 200 mg/dl, respectively. Therefore, the GA of 24% was categorized into a

poor level, as reflected by the FBS of ~150 mg/dl. Categorization of the HbA1c of 8% into a poor level was an underestimation, as the FBS was high (~190 mg/dl). From the above data, we found that HbA1c may underestimate glycemic control in diabetic patients with CKD and ESRD on HD. In such patients, GA may reflect the reliable glycemic control status and should be the preferred marker of it.

In group II; when we correlated both FBS/HbA1C and GA/HbA1C ratios to the stage of CKD based on eGFR, there was no much difference in the results. So, it seems that HbA1C underestimated the glycemic control in all stages of CKD. It means that HbA1C may not reflect the reliable glycemic control even in patients with mild CKD.

The results of our study agree with that found by Jwa-Kyung et al. [11], who assessed the glycemic markers in 185 ESRD patients, including 154 diabetic and 31 non-diabetic participants. In patients with diabetic ESRD, GA reflects the glycemic control more accurately than HbA1c according to the following result; GA/HbA1c ratio was 2.8 ± 0.9 in all patients and 3.1 ± 0.8 in diabetic group. This result is in good agreement with previous studies and could give additional support to the usually recognized simple equation of $GA = HbA1c \times 3$.

Frederiek et al. [12] measured HbA1c, GA and fructosamine concentrations in 25 subjects with diabetic CKD stages 4 and 5 matched with 25 subjects with diabetes and no evidence of nephropathy. The GA/HbA1c ratio was significantly higher in diabetic patients with CKD compared with controls. HbA1c values were significantly lower in CKD patients, relative to non-CKD patients at comparable mean glucose concentrations.

In a previous study [10], HbA1c and GA were measured in 519 diabetic patients; 415 on HD, 55 on PD and 49 non-nephropathy. The relationship between HbA1c and GA differed in diabetic patients with end-stage renal disease who perform either PD or HD compared to those without nephropathy. HbA1c significantly underestimated glycemic control in peritoneal and hemodialysis patients relative to GA.

Also, two studies [9,13] evaluated 796 diabetic HD patients and 877 diabetic subjects without nephropathy, demonstrated that HbA1c underestimated true glucose control relative to GA. The HbA1c assay was impacted by performance of HD, erythropoietin dosage, and hemoglobin concentration, while the GA assay consistently provided more accurate assessment of recent glycemic control in Japanese, African-American, and European-American HD patients.

That HbA1c value is influenced by various clinical factors explains our data. Reduced red-blood cell lifespan, recent transfusions, iron deficiency, metabolic acidosis or frequent erythropoietin injection; all of these factors exist in CKD and ESRD patients [11]. On the other hand, levels of GA are unaffected by changes in RBC survival time or erythropoietin use.

As albumin homeostasis may be abnormal in patients with ESRD, and it is important to consider potential inaccuracies in glycated albumin in addition to the limitations of HbA1c as a potential explanation for the higher HbA1c–glycated albumin ratio in ESRD. So, Mehrotra et al in their study [14] stated that it may be easier to implement different thresholds for HbA1c in ESRD and non-ESRD populations than undertake the studies necessary to replace HbA1c with glycated albumin. In our study, patients with either hypoalbuminemia or proteinuria were excluded to decrease this possibility of test inaccuracy. So, the nutritional states of our patients didn't affect the results.

Other disorders can affect the GA values, causing underestimation of glycemic status. GA shows lower values in relation to blood glucose in patients with nephritic syndrome, hyperthyroidism [15], glucocorticoid administration, smokers, hyper-uricemic [16],

hyper-triglyceridemia [17] and men with nonalcoholic fatty liver disease [18]. Also, in obese subjects GA values were found to set lower in relation to glycemia [19]. These factors were not studied in details in our research. However, our study showed higher glycated albumin values in comparison to glycated hemoglobin as a glycemic index.

6 Conclusion

HbA1c may underestimate glycemic control in diabetic patients either with CKD or with ESRD on hemodialysis. In these patients, GA can reflect the reliable glycemic control status, so to be the preferred marker of it.

ACKNOWLEDGEMENTS: We are grateful to the consideration and cooperation of all the participants of the study. This research did not receive any funding from any organization or company. The study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- [1] Koga M and Kasayama S. Clinical impact of glycated albumin as another glycemic control marker. *Endocr J* (2010)57: 751–762.
- [2] Agarwal R and Light R. Relationship between glycosylated hemoglobin and blood glucose during progression of chronic kidney disease. *Am. J. Nephrol.* (2011) 34: 32 – 41.
- [3] Kosecki SM, Rodgers PT and Adams MB. Glycemic monitoring in diabetics with sickle cell plus beta-thalassemia hemoglobinopathy. *Ann Pharmther.* (2005) 39: 1557–1560
- [4] Koga, M., Murai, J., Saito, H., Matsumoto, S. and Kasayama, S. . Usefulness of glycated albumin as a glycemic control marker after iron treatment for diabetic patients with iron deficiency anemia. *J Jpn Diab Soc* (2009)52: 341- 345.
- [5] Uzu T, Hatta T, Deji N, Izumiya T, Ueda H, Miyazawa I et al. Target for Glycemic Control in Type 2 Diabetic Patients on Hemodialysis: Effects of Anemia and Erythropoietin Injection on Hemoglobin A1c. *Therapeutic Apheresis and Dialysis* (2009) 13(2):89–94
- [6] Koga M, Kasayama S, Kanehara H and Bando Y. CLD-HbA1c as a novel indicator for estimation of mean plasma glucose in the patients with chronic liver disease. *Diab Res Clin Pract* (2008)81: 258-262
- [7] Yavari A. Glycosylated Hb; the importance in management of type 2 DM; *journal of stress physiology and biochemistry* (2011) vol 7 (4); 122-129.
- [8] KDOQI Diabetes Guideline update. KDOQI Clinical Practice Guideline for Diabetes and CKD. *Am J Kidney Dis.* (2012) 60 (5):850-886.
- [9] Inaba M, Okuno S, Kumeda Y, Yamada S, Imanishi Y, Tabata T et al., (Osaka CKD Expert Research Group). Glycated albumin is a better glycemic indicator than glycated hemoglobin values in hemodialysis patients with diabetes: effect of anemia and erythropoietin injection. *J Am Soc Nephrol* (2007)18: 896-903.

- [10] Freedman BI, Shenoy RN, Planer JA, Clay KD, Shihabi ZK, Burkart JM et al. Comparison of glycated albumin and hemoglobin A1c concentrations in diabetic subjects on peritoneal and hemodialysis. *Perit Dial Int* (2010) 30: 72-79.
- [11] Jwa-Kyung Kim, Jung Tak Park, Hyung Jung Oh, Dong Eun Yoo, Seung Jun Kim, Seung Hyeok Han et al. Estimating Average Glucose Levels from Glycated Albumin in Patients with End-Stage Renal Disease. *Yonsei Med J* (2012) 53(3): 578 – 586
- [12] Frederiek E Vos, John B Schollum, and Robert J Walker. Assessment of markers of glycaemic control in diabetic patients with chronic kidney disease using continuous glucose monitoring. *Nephrology* (2011)17; 182–188.
- [13] Peacock TP, Shihabi ZK, Bleyer AJ, Dolbare EL, Byers JR, Knovich MA et al. Comparison of glycated albumin and hemoglobin A1c levels in diabetic subjects on hemodialysis. *Kidney Int* (2008)7: 1062-1068
- [14] Mehrotra R, Kalantar-Zadeh K, and Adler S. Assessment of Glycemic Control in Dialysis Patients with Diabetes: Glycosylated Hemoglobin or Glycated Albumin? *Clin J Am Soc Nephrol* (2011)6: 1520–1522.
- [15] Koga M, Murai J., Saito H, Matsumoto S and Kasayama S. Effects of thyroid hormone on serum glycated albumin levels: study on non-diabetic subjects, *Diabetes Res Clin Pract* (2009) 84; 163-167.
- [16] Koga M, Murai J, Saito H, Mukai M and Kasayama S . Serum glycated albumin, but not glycated hemoglobin, is low in relation to glycemia in hyper-uricemic men. *Acta Diabetol* (2010)47: 173-177.
- [17] Koga M, Murai J, Saito H, Mukai M and Kasayama S . Serum GA, but not HbA1C, is low in relation to glycemia in men with hyper-triglyceridemia. *J Diab Invest* (2010); 1(5): 202–207.
- [18] Koga M, Murai J, Saito H, Mukai M and Kasayama S . Serum glycated albumin levels, but not glycated hemoglobin, are low in relation to glycemia in non-diabetic men with non-alcoholic fatty liver disease with high alanine aminotransferase levels *Clin Biochem* (2010) 43:1023-1025.
- [19] Koga M, Otsuki M, Matsumoto S, Saito H, Mukai M, Kasayama S, et al. Negative association of obesity and its related chronic inflammation with serum GA but not HbA1C levels. *Clin Chim Acta* (2007) 378: 48-52