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Association of Glycated Albumin with Glycemic Markers, Lipid Profile and Liver Function Tests for the Assessment Control in Saudi Patients with Long-Standing Type-2 *Diabetes Mellitus*

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ABSTRACT

This study is an effort to evaluate the association between glycated-albumin and various biochemical parameters in Saudi type-2 diabetic patients. Ninety long-standing (>10 years) type-2 diabetic subjects (51 males, 39 females) serum was analyzed for glycated albumin, fasting blood glucose, hemoglobin1c, cholesterol, triglyceride, albumin, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, aspartate transaminase, alkaline phosphatase, alanine transaminase and total bilirubin. Correlation, principal components analysis, covariance and statistical differences were conducted using SPSS for both male and female participants. The average age (years) of female and male participants was 51.0±10.2 and 51.6±14.1, respectively. Hemoglobin1C was significantly associated with fasting blood glucose (*r=0.637,P<0.01*). No significant differences between men and women were observed in the glycemic markers, lipid profile and liver function tests. Both men and women showed no significant differences in glycated-albumin irrespective of age and hemoglobin1c covariance. The regression model revealed that low-density lipoprotein, aspartate transaminase and alanine transaminase are significantly associated with glycated-albumin. Men's glycated-albumin was observed to be significantly associated with hemoglobin1c only, while women's glycated-albumin is highly associated with low-density lipoprotein only. Glycated-albumin was also co-varied with low-density lipoprotein. Glycated-albumin could be employed for screening high risk diabetic patients for early diagnosis of dyslipidemia and appropriate intervention with lipid-decreasing drugs. Current findings provide novel insights on the use glycated-albumin as clinical chemistry maker.

INTRODUCTION

Diabetes mellitus (DM) is a state of chronic hyperglycemia due to complete or comparative insulin deficiency (Inzucchi *et al.,* 2012). In diabetes, the body either fails to respond to its own insulin, does not make enough insulin or both. This leads to glucose accumulation in the blood that lead to various complications, and includes diabetic ketoacidosis, relative excess of insulin, non ketotic hyper osmolar coma, retinopathy, nephropathy, neuropathy, atherosclerosis, stroke, cardiovascular disease (Forbes and Cooper, 2013; Pollock and Funk, 2013).

The constant hyperglycemia of diabetes is related with continuing harm, dysfunction, and failure of different body-organs, especially the kidneys, eyes, heart, nerves, and blood vessels (Liu *et al.,* 2013; Qi *et al.,* 2012). In Type 2 diabetes mellitus (T2DM) the severity of hyperglycemia is enough to cause pathological alterations in different tissues but with unseen clinical signs and during this period it is easy to determine the, abnormalities by measuring fasting blood glucose (Ponchiardi *et al.,* 2013; Seshasai *et al.,* 2011). The diagnostic criteria for DM are blood glucose levels, HbA1C and glycated albumin (GA). Glycated hemoglobin is the word utilized to explain the synthesis of hemoglobin compound formed when a glucose reacts with the amino-group of haemoglobin (Dinu and Moţa, 2014; Lee *et al.,* 2013; Saisho *et al.,* 2011). The hemoglobin molecule binds to the glucose molecule to form a ketoamine (Marigliano *et al.,* 2011). The rate of this reaction is directly related to the plasma glucose. Since a typical red blood cell lives for about 120 days, the HbA1C level at any one time reflects the mean blood glucose levels over previous 60-90 days, therefore measurement of glycated haemoglobin provides a guide for the blood glucose concentration over a period of 3 months (Cohen, 2013; Herman and Cohen, 2012; Kuenen *et al.,* 2011; Lo *et al.,* 2014).

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HbA1c is a reliable technique of checking long-term diabetes management rather than random test of blood glucose. The normal values of HbA1C range from 4.5-8.0 mmol/L (Wagstaff and Cheung, 2014). The glycated hemoglobin level is affected by the mean glucose levels and RBCs life span so that if RBCs life span reduced, the hemoglobin will have fewer time to turn into glycated form. In this case the glycated hemoglobin test will be less sensitive (Khera *et al.,* 2015).

Glycation is the mechanism that results from binding of a sugar molecule, such as glucose or fructose, to a protein or lipid biomolecule without the need of a catalytic enzyme (Clark *et al.,* 2013). The augmented glucose levels present in DM produces amplified glycation of all proteins, including, albumin (Barlovic *et al.,* 2011). Quantification of the total glycation reaction consequential from the binding of glucose with free amino groups in proteins available in the blood is utilized to screen the level of blood glucose that has normally been present in body fluids over a previous period. Therefore, serum glycated albumin analysis can be employed to examine the present level of glycation of albumin, the main plasma protein (Barlovic *et al.,* 2011; Joseph *et al.,* 2011).

Some limitations were previously reported on the usefulness of HbA1c due to variable lifespan of RBCs and the presence of some disease that decrease the glycation of hemoglobin (Koga, 2014; Raghav and Ahmad, 2014). Therefore, the current study was designed to assess the association between glycated albumin for the assessment control in Saudi patients with long standing T2DM.

MATERIALS AND METHODS

Quantitative approach of research was adopted, with cross-sectional and analytical design. Samples were collected from different hospitals in Jazan area, Saudi Arabia. Ninety Saudi Arabian long-standing diabetic patients were enrolled in this research. Inclusion criteria were Saudi Arabian patients with diabetes mellitus more than 10 years. Those with diabetes mellitus less than 10 years were excluded. Participants should be with no other chronic diseases.

Permission of this study was obtained from the local authorities in the area of the study. This study was approved by Jazan University Ethical Committee (JUEC-13-15). The aim and benefits of the study were explained to the participant with assurance and confidentiality. An informed consent was collected from all participants. Demographic data such age and gender were collected also. Blood samples were obtained after 12 hrs fasting and kept at -70°C prior to analysis.

Biochemical Analysis

Blood levels of glucose were measured using spectrophotometric technique. Triglyceride (TG), cholesterol, high density lipoprotein-cholesterol (HDL), low density lipoprotein-cholesterol (LDL), alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST) and total bilirubin were analyzed using Hitachi biochemical auto-analyzer (Japan) at Jazan General Hospital, Jazan, Saudi Arabia.

Human glycosylated Albumin

Glycated albumin (GA) in serum samples was measured according to the instructions provided by available commercial kit (Genasia Biotechnology Co., Ltd, Shanghai, China). Diluted serum and standard were added to GA-antigen pre-coated microtiter plates, gently mixed and left to react for 30 minutes at 37° C. After washing with washing buffer, HRP-conjugate was added and left to react for 30 minutes at 37° C and later washed. TMP-substrate was added and incubated for 10 min at 37° C. This blue colored reaction was terminated by the addition of sulphuric acid and the color change (yellow) was detected spectrophotometrically at 450 nm. The concentration of Human GA in the sample was then calculated by comparing the O.D. of the samples to the standard curve.

Glycosylated hemoglobin(HbA1c)

To eliminate the labile Schiff's base, blood was added to a lysing reagent made by mixing borate ions and detergent. The haemolysate was then added for 5 min to a soft binding cation exchange resin to allow HbA0 to be bound with the resin. The resin was then removed using special resin separator to obtain a supernatant fluid with the HbA1. The glycohemoglobin (%) of total hemoglobin is calculated by the determination of the absorbance of the glycohemoglobin and of the total hemoglobin fraction at 415 nm in contrast with a standard glycohemoglobin preparation carried through the test procedure.

Statistical analysis

Collected data were entered, managed and analyzed using SPSS. The mean, SD, frequencies and percentages were obtained. Stepwise linear regression was use for to measure the association of GA and various parameters. *P*-value was obtained to assess the significance of the results. Linear regression was used to analyze the relationship between glycated albumin and various parameters. ANCOVA analysis was used to exclude the main effect of age, as a continuous variable, from the effect of sex on GA levels. Factor analysis using principal components technique was utilized to investigate the covariance of GA and various parameters.

RESULTS AND DISCUSSION

It is well known that glycation among diverse proteins is augmented in diabetic patients compare with healthy subjects. Some of these glycated proteins are proposed to be linked to the occurrence and development of chronic diabetic complication. Among these glycated proteins, glycated hemoglobin (HbA1C) is frequently used as the gold standard index of glycemic control (Liu *et al.,* 2012; Park *et al.,* 2014; Satheesan *et al.,* 2014). However, HbA1C does not precisely mirror the definite condition of glycemic management in some conditions and patients where

plasma glucose modifies during short term, and in anemic patients (Mathur *et al.,* 2014). In contrast, another indicator of diabetic control, GA, more precisely reveals alterations in both short term and postprandial plasma glucose levels. Although GA is not affected by ailments of hemoglobin metabolism, it is influenced by ailments of albumin metabolism (Blaak *et al.*, 2012; Zheng *et al.*, 2012). Therefore, the current study was designed to assess the association between GA, glycemic markers, lipid profile and liver function tests for the assessment control in Saudi patients with long standing T2DM.

Ninety long-standing (>10 years) T2DM subjects were included in the study out of which 51 were males and 39 were females. The average age (years) of female and male participants was 51.0±10.2 and 51.6±14.1, respectively. Table 1 shows the demographic and differences between T2DM male and female in their biochemical parameters, GA and HB1C. The mean value of GA and FBG were higher in males in comparison to female subjects but the differences were non-significant. Kondaveeti *et al.*, (Kondaveeti *et al.,* 2012) was also observed that gender was not a differentiating factor for FBG and GA. ANCOVA analysis was used to exclude the main effect of age, as a continuous variable, from the effect of sex on GA levels. Both men and women showed no significant (*P>0.05*) differences in GA irrespective of age and HB1C. On the other hand, no significant differences between men and women were observed in HB1C, cholesterol, triglyceride, LDL, HDL, ALT, AST, ALP, total bilirubin and albumin. This suggested that gender was not a differentiating factor in the mentioned parameters when excluding the effect of age. Similar results were also observed previously (Barzin *et al.*, 2012; Webber *et al.*, 2010).

Table 1: Demographic and differences between T2DM male and female in their biochemical parameters, GA and HB1C.

Biochemical Parameters	Biochemical Parameters	
	Female (N = 39)	Male (N = 51)
Hemoglobin1C (mmol/L)	9.3 ± 2.5	9.0 ± 2.8
Fasting Blood Glucose (mg/dL)	188.4 ± 65.3	191.1 ± 69.1
Glycated Albumin (mmol/L)	55.6 ± 27.3	59.3 ± 25.0
Total Cholesterol (mg/dL)	206.5 ± 50.5	195.5 ± 40.2
Triglyceride (mg/dL)	160.4 ± 36.9	159.6 ± 35.3
Low-density lipoprotein-cholesterol (mg/dL)	47.9 ± 22.3	50.9 ± 32.0
High-density lipoprotein-cholesterol (mg/dL)	63.6 ± 17.4	62.5 ± 13.2
Alanine transaminase (IU/L)	29.8 ± 14.6	32.4 ± 14.2
Aspartate transaminase (IU/L)	30.1 ± 12.2	31.4 ± 11.5
Alkaline phosphatase (IU/L)	77.3 ± 18.2	74.1 ± 19.2
Total Bilirubin (IU/L)	0.9 ± 0.2	0.8 ± 2
Albumin (g/dl)	3.8 ± 0.4	3.7 ± 0.3

*Data showed no significant differences as analyzed by student t-test.

The present study shows no significant (*P>0.05*) correlation between glycated albumin (GA) and liver function markers, glycemic markers and lipid profile. Hemoglobin1C was significantly associated with FBG (r=0.637, P<0.01), as shown in Table 2. Bivariate Pearson correlation analysis was followed by backward stepwise linear regression modeling with GA as dependent variable. Surprisingly, the regression model after controlling for some insignificant factors revealed that LDL, ALT and AST are significantly associated with GA (Table 1). The data were further analyzed for male and female separately. Men's GA was observed to be significantly associated with HB1C only, while women's GA is highly associated with LDL only. Although GA is not affected by anemia and variant hemoglobin, it is affected in patients with dysfunction of albumin metabolism (Koga and Kasayama, 2009). GA depicts decreased values in relation to glycemia in patients with hyperthyroidism, nephrotic syndrome and glucocorticoid use in which albumin metabolism elevates. Meanwhile, GA presents superior values comparative to plasma glucose levels in patients with hypothyroidism and hepatic cirrhosis in which albumin metabolism decreases. Certainly, it has been demonstrated that GA was set inferior in relation to plasma glucose levels in hyperuricemic patients, hypertriglyceridemia, smokers and men with non-alcoholic fatty liver disease (NAFLD) with high ALT levels in whom chronic inflammation is suggested (Leite *et al.,* 2009; Targher *et al.,* 2010; Targher *et al.,* 2013).

Table 2: Pearson correlation between, fasting blood glucose and glycated albumin and biochemical parameters.

Variables	Hemoglobin 1C	Fasting Blood Glucose	Glycated Albumin
Hemoglobin 1C	1	0.637**	-0.134
Fasting Blood Glucose	0.637**	1	-0.116
Glycated Albumin	-0.134	-0.116	1
Cholesterol	0.007	-0.017	-0.010
Triglyceride	0.000	-0.078	0.013
Low Density Lipoprotein	0.110	0.067	0.201
High Density Lipoprotein	-0.105	-0.010	-0.015
Alanine Transaminase	-0.037	0.100	0.012
Aspartate Transaminase	-0.076	0.047	-0.104
Alkaline Phosphatase	0.005	0.001	-0.080
Total Bilirubin	-0.006	-0.099	-0.074
Albumin	0.089	**-0.067**	0.092
Backward Stepwise Linear Regression Model			
Variables included in the model	**Beta**	***t***	**Sig.**
(Constant)		.612	.542
Low Density Lipoprotein	0.211	2.021	.046
Alanine Transaminase	0.458	2.252	.027
Aspartate Transaminase	-0.446	-2.229	.029

**significant at 0.01 level of propability.

Covariation of the study parameters was investigated using factor analysis and principal components analysis (PCA). As shown in Figure 1, five factors were extracted using Varimax method of PCA. 69.13% of variance was explained by the extract factors. Table 3, shows the distribution of the current study parameters obtained from T2DM patients. GA and LDL were extracted in the same component, with factor loading of 0.575 and 0.773, respectively (Table 3). This confirms that GA was co-varied with LDL. This indicates that GA could be employed for screening high risk diabetic patients for early diagnosis of dyslipidemia and appropriate intervention with hypolipidemic drugs (Karachalias *et al.,* 2005). It is well known that glycation among a variety of proteins is augmented in diabetic patients compared with healthy subjects. Presently, amongst these glycated proteins, HbA1C is utilized as the gold standard clinical test of glycemic control in clinical settings for diabetes cure (Koga, 2014). However, HbA1C does not precisely show the real standing of glycemic control in some conditions where blood glucose varies throughout short

term, and in patients who have ailments such as variant hemoglobin and anemia. In contrast, another indicator of clinical glycemic control, GA, more precisely revealed variations in blood glucose during short term and also postprandial plasma glucose. Although GA is not affected by diseases of hemoglobin metabolism, it is influenced by dysfunctions of albumin metabolism (Joseph *et al.,* 2011; Lee *et al.,* 2013; Marigliano *et al.,* 2011; Qi *et al.,* 2012). These include the status of glycemic control changes during short term, diseases which cause iron deficiency anemia, postprandial hyperglycemia, chronic liver disease (liver cirrhosis), chronic renal failure (diabetic nephropathy), pregnancy, and variant hemoglobin. Fasting plasma glucose are altered by numerous factors like stress, acute illness, medication, venous stasis, posture, sample handling, food ingestion, prolonged fasting and exercise. These factors are also likely to affect the 2 hr oral glucose tolerance test. The same factors, however do not have any affects on HbA1c measurements (Furuya *et al.,* 2014; Speeckaert *et al.,* 2014).

Table. 3: Factor analysis.

	Number of Components and Factor Loading				
	1	2	**3**	**4**	**5**
Aspartate Transaminase (AST)	0.934				
Alanine Transaminase (ALT)	0.900				
Alkaline Phosphatase (ALP)	0.612				
Triglyceride		0.926			
Cholesterol		0.916			
Hemoglobin 1C (HB1C)			0.895		
Fasting Blood Glucose (FBG)			0.887		
Low Density Lipoprotein – Cholesterol (LDL)				0.773	
Glycated Albumin (GA)				0.575	
High Density Lipoprotein– Cholesterol (HDL)					0.779
Albumin					0.610
Total Bilirubin					0.489

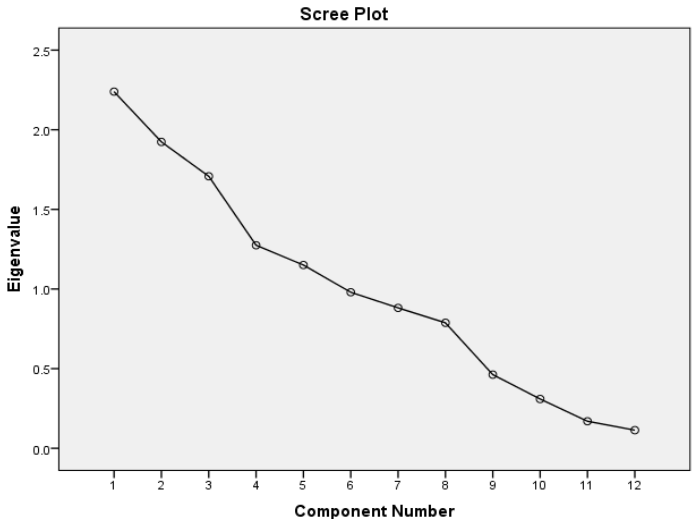

Fig. 1: Scree plot showed the number of factors extracted using factor analysis and principal component analysis.

CONCLUSION AND LIMITATIONS

The current study is the first of its kind in the Saudi population. Current findings also provide novel insights on the use glycated-albumin as clinical chemistry maker. However, our research had certain boundaries and limitations. Firstly, our subjects were from a uniform population, potentially restraining the generalizability of our findings. Secondly, we did not have information concerning pubescence condition which has helped us to evaluate the effects of pubescence on susceptibility to diabetes, and thirdly, we did not capture into our vision some potential confounders such as nutritional habits, physical activity and socioeconomic condition in our analysis. Additional research is required to establish GA as a better indicator of the diagnosis of DM.

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