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Value of Serum Glycated Albumin in Prediction of Coronary Artery Disease in Type 2 Diabetes Mellitus

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Abstract Coronary artery disease (CAD) is a major vascular complication of diabetes mellitus and reveals high mortality. Up to 30 % of diabetic patients with myocardial ischemia remain asymptomatic and are associated with worse prognosis compared to non-diabetic counterpart, which warrants routine screening for CAD in diabetic population. The purpose of this study was to evaluate the clinical value of serum glycated albumin level in predicting the presence of CAD in patients with type 2 diabetes. Ninety patients with type 2 diabetes were divided into four groups having the coronary artery disease with lumen diameter narrowing < 30 %, Group II with mild CAD contained those patients with lumen diameter narrowing 30-50 %, and Group III with Major CAD contained those subjects with lumen diameter narrowing 50-70 %. Finally group IV with severe CAD including the patients with lumen diameter narrowing > 70 %. Serum levels of glycated albumin was determined using ELISA as well as serum concentrations of glucose, lipids, were taken in questionnaire in all groups. Serum glycated albumin levels were significantly increased in diabetic patients with CAD.

Keywords Diabetes Mellitus, Coronary Artery Disease, Glycated Albumin, ELISA, Glycated Hemoglobin

1. Introduction

Diabetes mellitus is a group of metabolic disorder in which there is absolute or relative deficiency of insulin with resultant hyperglycemia, glycosuria, polyuria and polydipsia, which represent typical clinical manifestations. Diabetes can cause acute or chronic complications. The new classification system identifies three types of diabetes mellitus: type 1, type 2, "and gestational diabetes.

1.1. Diabetes Mellitus and Coronary Artery Disease

Diabetes mellitus (DM) is common associated with both micro vascular and macro vascular complications. Macro vascular complications manifest themselves as accelerated arteriosclerosis, clinically resulting in premature coronary artery disease (CAD), increased risk of cerebrovascular disease, and severe peripheral vascular disease.

Patient with type 2 diabetes mellitus (T2DM) have a two to four fold increase in the risk of CAD[1]. Several independent factors, e.g. insulin resistance, hyperglycemia, hypertension, dyslipidaemia, abdominal obesity and low-grade inflammation, have all been associated with this condition in subjects with type 2 diabetes[2].

Diabetic state also promotes the Amadori-modification of many circulating proteins, giving rise to concentrations of glycated proteins approximately one and a half to three times those found in non-diabetic persons that reflect integrated glycemia to which the protein has been exposed during its residence time in the circulation[3].

1.2. Glycated Albumin

Early stage reaction product of albumin or serum protein is called Glycated albumin or Fructosamine[4].

Glycated albumin exhibits potential atherogenic effects in various cell types, including mesangial, monocyte- macrophage, mesothelial, endothelial, and vascular smooth muscle cells[3].

Glycated albumin is the predominant circulating Amadori -type glycated protein in vivo and plays a major role in the development of diabetic vascular complications. An increased serum level of glycated albumin is associated with the presence and severity of CAD, and may be useful in screening patients with T2DM[5].

It is suggested that GA provides a significantly better measure to estimate glycemic control in HD patients with diabetes and that the assessment of glycemic control by HbA1c in these patients might lead to underestimation likely as a result of the increasing proportion of young erythrocyte by the use of erythropoietin[6].

1.3. ELISA

To evaluate the clinical utility of a highly specific monoclonal antibody directed against the glycated epitopes re-

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siding in human albumin, Cohen and Hud developed an ELISA using this antibody to measure glycated albumin in plasma samples from non-diabetic and diabetic individuals[7].

Relative percent concentration of glycated albumin in a sample is determined by dividing the microgram glycated albumin in the sample by the total microgram albumin in the sample.

2. Materials and Methods

2.1. Study Design and Study Population

It was a Cross Sectional Analytical study. Among the study population, 90 cases of type II diabetes were registered in the institute of biochemistry and biotechnology at the University of Punjab Lahore. Inclusion Criteria was that individuals with type II diabetes and undergoing diagnostic coronary angiography to find out the presence and extent of CAD were included in this study group and all individuals with type 1 diabetes rheumatoid arthritis and any other inflammatory diseases were excluded from the study.

2.2. Data Collection

90 cases of type II diabetes undergoing coronary angiography for the diagnosis of CAD were taken from the Punjab institute of cardiology. All the patients were angiographically confirmed for coronary artery disease. The demographic information like (name, age sex, height, and weight), history of present illness including history of diabetes and cardiac history were taken. The severity of CAD was based on lumen diameter narrowing as < 30 % (Minor CAD), 30-50% (mild CAD) 50-70 % (moderate CAD) and > 70 % (severe CAD) on visual assessment by experienced observer.

2.3. Sample Collection and Serum Separation

5 ml of venous blood was drawn from each selected subject using standard venepuncture techniques. The sterilized needles were used for the collection of blood from all the patients. The blood was allowed to clot at the room temperature for 2-3 hours. The clot was then removed while the supernatant was centrifuged at 4000 rpm.

2.4. Quantitative Determination of Glycated Albumin and Total Albumin

Glycated albumin and total albumin was determined in each samples by using commercially available Glycaben ELISA kit. Glycated albumin can be reported as an absolute concentration (mg / ml) or as a percent (%) of total albumin as calculated from the following equation using determined for each of sample:

% Glycated Albumin $_{sample}$ = 100% x Glycated albumin $_{sample}$ / Total Albumin $_{sample}$

3. Results

3.1. Composition of Study Groups

Out of 90 total subjects 42 (46.7 %) were males and 48 (53.3 %) were females. In Group I (minor CAD), 11 (40.7 %) were males and 16 (59.3 %) were females. In Group II (mild CAD), 13 (52 %) were males and 12 (48 %) were females. In the Group III (Major CAD), 14 (54 %) were males and 12 (46 %) were females. In the Group IV (sever CAD), 4 (33.3 %) were males and 8 (66.7 %) were females (Table I).

Mean age group and SD were calculated for each group. The mean age and SD of the group I (minor CAD) was 48 ±0.95. The mean age and SD of the group II (mild CAD) was 61 ±1.17. The mean age and SD of the group III (Major CAD) was 53 ±0.84 and of the group IV (severs CAD) was 55 ±0.87 (Table II)

Table I. Distribution of the study groups by sex

Sr. No.	Study groups	Males		Females		Total	
		n	%	n	%	n	%
1	Group I (minor CAD) Lumen diameter narrowing < 30 %	11	40.7	16	59.3	27	30.0
2	Group II (mild CAD) Lumen diameter narrowing 30-50 %	13	52	12	48	25	27.8
3	Group III (major CAD) Lumen diameter narrowing 50-70 %	14	54	12	46	26	28.9
4	Group IV (sever CAD) Lumen diameter narrowing > 70 %	4	33.3	8	66.7	12	13.3
5	Total	42	46.7	48	53.3	90	100

Table II. Distribution of the study groups by ages

Sr. no.	Study groups	Mean age (yrs)	±SD
1	Group I (Minor CAD)	49	0.95
2	Group II (Mild CAD)	60	1.17
3	Group III (Major CAD)	52	0.84
4	Group IV (Sever CAD)	55	0.87

Out of 90 subjects 33 (36.7 %) have duration of diabetes less than 5 years and 57(63.3 %) have duration more than 5 years. And 37 (41.1 %) have the family history of diabetes and 53 (58.9 %) have no family history of diabetes (Table III and IV) and 15 (16.70 %) heaving family history of CAD, 75 (83.3 %) were without family history (Table V).

Table III. Duration of diabetes

Sr. no.	Study groups	Duration of diabetes				Total
		< 5 yrs		> 5 yrs		
		n	%	n	%	
1	Group I (Minor CAD)	5	18.5	22	81.5	27
2	Group II (Mild CAD)	12	48.0	13	52.0	25
3	Group III (Major CAD)	14	53.84	12	46.16	26
4	Group IV (Sever CAD)	2	16.7	10	83.3	12
5	Total	33	36.7	57	63.3	90

Table IV. Family history of diabetes

Sr. no.	Study groups	Family history of diabetes				Total
		Yes		No		
		n	%	n	%	
1	Group I (Minor CAD)	4	14.8	23	85.2	27
2	Group II (Mild CAD)	8	32.0	17	68.0	25
3	Group III (Major CAD)	20	77.0	6	23.0	26
4	Group IV (Sever CAD)	5	41.7	7	58.3	12
5	Total	37	41.1	53	58.9	90

Table V. Assessment of smoking in each study group

Sr. no.	Study groups	Assessment of smoking in each study group				Total
		No		Yes		
		n	%	n	%	
1	Group I (Minor CAD)	0	0	27	100	27
2	Group II (Mild CAD)	2	8	23	92	25
3	Group III (Major CAD)	3	11.5	23	88.5	26
4	Group IV (Sever CAD)	5	41.7	7	58.3	12
5	Total	10	11.1	80	88.9	90

Among 90 subjects 10 (11.1 %) were smokers and 80 (88.9 %) were non-smokers and 37 (41.1 %) were hypertensive and 53 (58.9 %) were non-hypertensive (Table VI and VII).

Table VI. Assessment of hypertension in each study group

Sr. no.	Study groups	Assessment of hypertension in each study group				Total
		Yes		No		
		n	%	n	%	
1	Group I (Minor CAD)	6	22.2	21	77.8	27
2	Group II (Mild CAD)	10	40.0	15	60	25
3	Group III (Major CAD)	14	53.8	12	46.2	26
4	Group IV (Sever CAD)	7	58.3	5	41.7	12
5	Total	37	41.1	53	58.9	90

Table VII. Family history of CAD

Sr. no.	Study groups	Family history of CAD				Total
		Yes		No		
		n	%	n	%	
1	Group I (Minor CAD)	2	7.4	25	92.6	27
2	Group II (Mild CAD)	3	12.0	22	88.0	25
3	Group III (Major CAD)	5	19.2	21	80.8	26
4	Group IV (Sever CAD)	5	41.7	7	58.3	12
5	Total	15	16.7	75	83.3	90

3.2. Determination of Total Albumin and Glycated Albumi by ELISA

There were three types of information sources for the total albumin and glycated albumin ELISA i.e. the calibration of the standards, quality control samples and the test specimens. These three were subjected to the analytical procedure and some response (color development) was obtained.

After running the ELISA test concentration of total albumin and glycated albumin for standards were calculated (Table VIII and IX). Graphical representation is in Fig. 1 (A), (B).

Table VIII. Standards for total albumin

Standards No.	A	B	C	D
Conc. of total albumin (mg/dl)	0.00	11.570	25.795	49.735
Absorbance at 630nm	0.00	4.679	8.070	10.46

Table IX. Standards for glycated albumin

Standards No.	A	B	C	D	E
Conc. of glycated albumin (mg/l)	0.00	3.00	1.500	0.750	0.370
Absorbance at 450nm	0.00	1.720	1.341	1.120	0.945

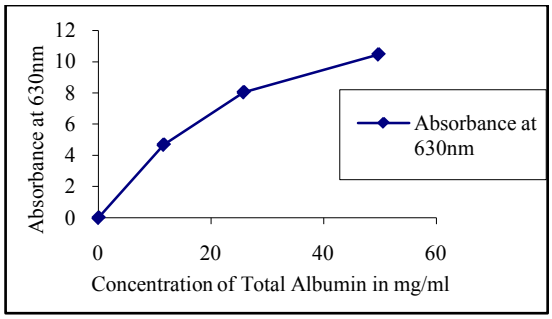

Figure 1(A). Standard curve for total albumin, whose absorbance is taken at 630 nm

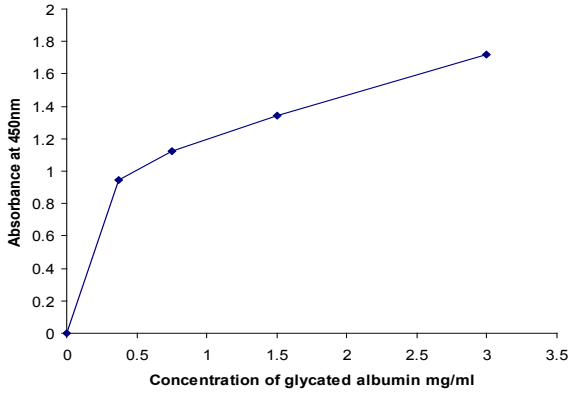

Figure 1(B). Standard curve for total albumin, absorbance is at 450 nm

The mean % age glycated albumin was calculated for each subject in each study group by using formula. The mean % age glycated in Group I was 2.18, in Group II was 3.3, in Group III 4.0, and in Group IV was 5.3 (Table X).

Table X. Values of % age glycated albumin

Sr. no.	Study Group	Mean of %age glycated albumin	±SD
1	Group I (Minor CAD)	2.1800	0.01000
2	Group II (Mild CAD)	3.3000	0.01000
3	Group III (Major CAD)	4.0000	0.01000
4	Group IV (Sever CAD)	5.3267	0.05508

The graphical analysis shows the relationship between severities of CAD with increase in % age glycated albumin (Fig. 2).

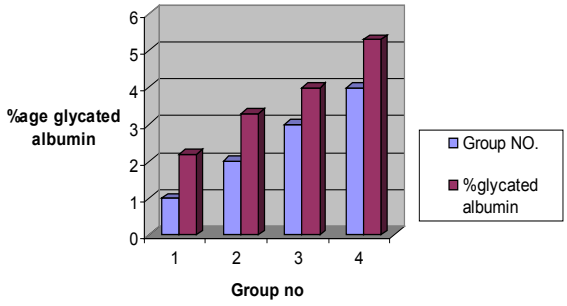

Figure 2. % age glycated albumin in each study group, groups are taken on x-axis and % age glycated albumin is on y-axis

3.3. Assessment of Mean BMI With % Age Glycated Albumin

Mean BMI = height /weight

In-group I (minor CAD) the mean BMI was 28.3, in-group II (mild CAD) the mean BMI was 27.4. In-group III (Major CAD) the mean BMI was 27.2 and in-group IV (sever CAD) the mean BMI was 18.2. The mean % age glycated albumin in the group I was 2.18, in the group II 3.3, in the group III 4.0 and in the group IV the mean % age glycated albumin was 5.3 (Table XI). There is negative association between mean BMI and % age glycated albumin (Fig. 3).

Table XI. Mean BMI for each study group

Sr no	Study Groups	Mean BMI	Mean %age Glycated albumin
1	Group I (Minor CAD)	28.3	2.18
2	Group II (Mild CAD)	27.4	3.3
3	Group III (Major CAD)	27.2	4.0
4	Group IV (Sever CAD)	18.2	5.3

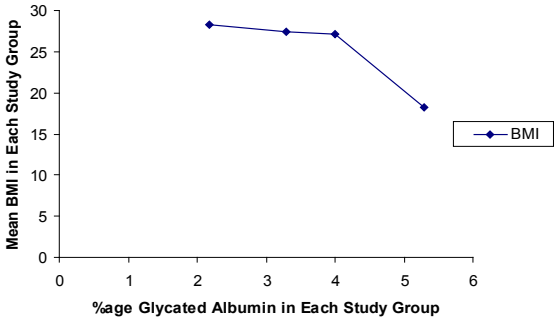

Figure 3. Assessment of mean BMI with % age glycated albumin, % age glycated albumin decreases with increase in BMI values

3.4. Association between Mean Triglycerides and Mean % Age Glycated Albumin

The mean % age glycated albumin and triglycerides level were calculated for each study group (Table XII). It was observed that % age glycated albumin level increases as the triglyceride level increases, providing a direct relationship between % age glycated albumin and TG (Fig. 4).

Table XII. Mean Triglycerides and mean %age glycated albumin

Sr No	Study groups	Mean % age Glycated Albumin	Mean Triglycerides mg/dl
1	Group I (Minor CAD)	2.18	102
2	Group II (Mild CAD)	3.3	136
3	Group III (Major CAD)	4.0	223
4	Group IV (Sever CAD)	5.3	340

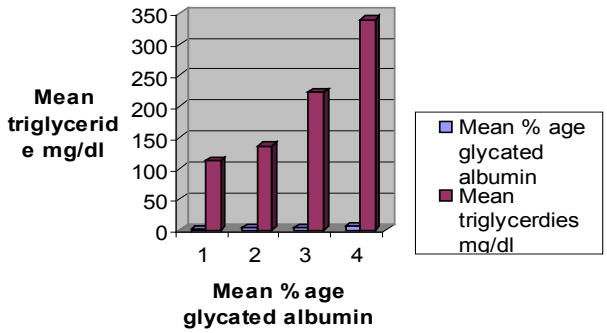

Figure 4. Association between mean triglycerides and mean % age glycated albumin

3.5. Association between Mean Glucose Level and Mean % Age Glycated Albumin

The mean % age glycated albumin and glucose level were calculated for each study group (Table XIII). It was observed that % age glycated albumin level increases as the glucose level increases, providing a direct relationship between %age glycated albumin and glucose (Fig. 5).

Table XIII. Mean glucose level and mean %age glycated albumin

Sr. no	Study groups	Mean %age Glycated Albumin	Mean Glucose mg/dl
1	Group I (Minor CAD)	2.18	192
2	Group II (Mild CAD)	3.3	201.3
3	Group III (Major CAD)	4.0	223
4	Group IV (Sever CAD)	5.3	240

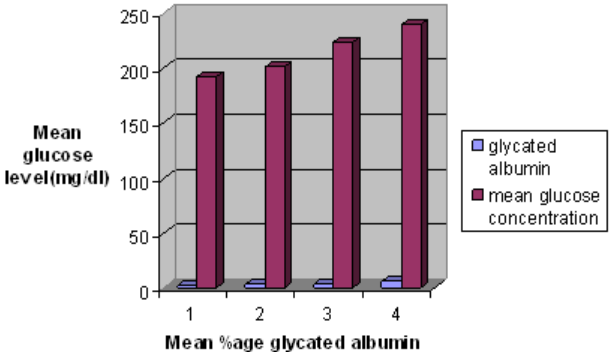

Figure 5. Association between glucose level and mean % age glycated albumin

4. Discussion

Diabetes has been recognized as an important risk factor for CAD, and diabetic patients are at 2-fold increased risk of cardiovascular mortality compared to their non diabetic counterparts[8]. Previous studies have demonstrated that silent myocardial ischemia, which is mainly caused by autonomic neural dysfunction, occurred in about 20 % to 25 % of diabetic patients, and the prevalence may be as high as 60 in those at high-risk[9].

All proteins in the body can be modified by non- enzymatic glycation. In diabetes mellitus the extent of the non-enzymatic glycation of proteins increases, compared with non-diabetic subjects, which may comprise at least a part of diabetic complications[10]. Among these modified proteins, measurement of HbA1c has been applied for clinical use in order to monitor chronic glycemic control in diabetic patients. But now we try to provide the better indicator of glycemic control in CAD patients which is Glycated albumin, is a kind of early-stage amadori-modified reaction products formed from Schiff's base adducts and has been implicated in the pathogenesis of diabetic complications and better indicator[11, 3].

Stable fraction of glycated hemoglobin (HbA1c) is routinely measured in the majority of patients with diabetes around the world; Since HbA1c reflects glycemic control over the preceding 2-3 months[12]. However HbA1c may not be suitable for evaluation of short term variation in glycemic control because of long life span of erythrocytes (120 days). Because the turnover of human serum albumin is much more rapid (half life of 15-20 days) than that of hemglobin, the measurment of glycated albumin (GA) provides an index of glycemic control over a short period of time than the measurment of HbA1c[13, 14].

There are several risk factors associated with coronary artery disease, such as family history of diabetes and coronary artery disease, smoking, hypertension, hyperglycemia, hypertriglyceridemia, BMI.

The prevalence of CAD increased with age and duration of diabetes[15] and in this study 57 (63.3 %) subjects have duration of diabetes more than 5 years and mean age is 55±0.87.

Cigarette smoking is a leading risk factor for CVD. Patients with diabetes who are smokers are doubly at risk[16]. Unfortunately, many patients continue to smoke despite having diabetes; for these patients, the benefits that can be derived from modifying other risk factors are mitigated. In this study, 80 (88.9 %) subjects were smokers.

High blood pressure accounts for 20 to 25 % of all CAD deaths. Subjects with hypertension have a two-fold higher risk of CAD[15]. In this study, Out of 90 subjects, 53 (58.9 %) patients were hypertensive.

Serum glycated albumin levels are low in obese[17]. The result of this study shows the negative association of % age glycated albumin with the BMI. And we found that serum GA levels are influenced by BMI in diabetic patients[18].

Hyperglycemia, leading to the formation of advanced glycation end products (AGE), on vascular function**[19].** Glycated albumin is a kind of Amadori-modified derivatives, accounting for about 80 % of the circulating glycated proteins in vivo. Hyperglycemia increases serum glycated albumin level, and the extent of increase reflects glycemia status of diabetic patients over a retrospective period[5]. Although relationship between diabetic atherogenesis and several common risk factors plus non traditional risk markers have been studied extensively and the data is having some controversies[20].

5. Conclusions

Previous studies showed that glycated albumin was more sensitive than glycosylated hemoglobin (HbA1c) in the evaluation of the severity of diabetes. The present study showed that glycated albumin was an independent risk factor for CAD in patients with type 2 diabetes, with and predicts the coronary artery disease in type 2 diabetes mellitus.

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