ORIGINAL ARTICLE

Levels of Glycated Albumin and C-Reactive Protein as Risk Markers of Coronary Artery Disease in Type-2 Diabetics

FARKHANDA GHAFOOR[1], AMBER MALIK[2], SAIMA NAZ MOHSIN[3], *SAULAT SIDDIQUE[4]*

ABSTRACT

Aim: To determine the clinical usefulness of glycated albumin and hs-CRP levels for predicting CAD using logistic regression model in patients with type-2 diabetes

Methods: The cross-sectional study was conducted in the Department of Cardiology and PMRC Centre NHRC, SZM Complex Lahore from November 2011 to August 2012 with the assessment of GA and hs-CRP levels was conducted in relation to the lumen narrowing levels amongst 90 subjects who were undergoing coronary angiography. Study participants with < 30% lumen diameter narrowing were considered normal (Group 1) whereas with lumen diameter 30–50% were considered mild CAD (Group II), with 51-70% moderate CAD (Group III) and greater than 70% severe CAD (Group IV).

Results: The association between glycated albumin levels, hs-CRP and lumen narrowing diameter was found significant in all groups by applying ANOVA ($p<0.01$). The post HOC Tukey's test also showed significant relationship between all groups ($p<0.01$) except for hs CRP between group III and group IV (p 0.857).

Conclusion: Both glycated albumin and hs-CRP tests can be used to assess the levels of risk for CAD with type-2 diabetes and other accompanying risk factors.

Keywords: Coronary artery disease, Type-2 diabetes, Glycated albumin, hs-CRP

INTRODUCTION

Diabetes has been recognized as an important risk factor for coronary artery disease (CAD). Diabetic patients are at 2-fold increased risk of cardiovascular mortality compared to non diabetics.[1-3] Early detection and screening of CAD with simple tools has become important for the diabetic population, as if myocardial ischemia develops, it may lead to poor prognosis at later stage.[4-6] In pathogenesis of diabetic complications, glycated albumin appears early which plays critical role in process of atherogenesis by including inflammatory mediators in vessel wall, as well as the proliferation and migration of vascular smooth muscle cells, leading to adverse effects on vascular biological functions. The glucose reacts with amino groups of circulating or vessel wall proteins in hyperglycemic milieu to form a Schiff base, which produces Amudori-type early glycation products, in hours and weeks. Some of the Amadori products continue to undergo a complex series of chemical rearrangements for weeks and months, and finally resulting in the formation of glycation end-products (AGEs)[7,8,9,10].

The arteriosclerosis and acute cardiovascular events have also shown association with CRP in type-2 diabetics without any previous history of cardiovascular disease[3,10,11] hs-CRP has proven a useful biomarker for extent and severity of atherosclerotic lesions and a predictor for subsequent events.[12] Various studies have observed glycated serum protein and CRP as risk factors.[13,14] Increased levels of inflammatory markers are seen more in South Asians compared to white population;[13] this association has never been studied in Pakistani population. This study was aimed to determine the clinical usefulness of glycated albumin and hs-CRP levels for predicting CAD using logistic regression model in patients with type-2 diabetes.

SUBJECTS AND METHODS

The study was approved by the Institutional Review Board of SZMC (IRB No. 1004). During the study period (November 2011 to August 2012), diabetic patients, who were scheduled for angiography in the department of Cardiology, Shaikh Zayed Hospital Lahore, were informed of the objectives of the study. Ninety patients who agreed to be participants were enrolled and written informed consent was obtained from them. Type 2 diabetic patients who had additional co morbidity like rheumatoid arthritis or any other inflammatory disease, of lungs, liver or kidney, steroid use etc., were excluded from the study. Presence of risk factors, including duration of diabetes, medication history, smoking, hypertension and family history of CAD and diabetes were recorded on designed study proforma. Five ml of venous blood was obtained from each study participant, serum was separated, aliquoted and stored in two aliquots at –20°C until analysed. One aliquot was used to determine glycated serum protein and the other for hs-CRP. Coronary angiography was

[1,3]PMRC Research Centre, NHRC Shaikh Zayed Medical Complex, Lahore, [2,4]Department of Cardiology, Shaikh Zayed Medical Complex, Lahore

Correspondence to Dr. Farkhanda Ghafoor e-mail: fghafoor99@hotmail.com

performed by a single skilled interventional cardiologist. Study participants were divided into two groups, normal (lumen diameter narrowing <30%) and CAD (lumen diameter >30%), whereas the CAD group was further divided into sub groups based on lumen diameters which were measured by trained cardiologists, blinded to the lab. results.

Total albumin levels and glycated albumin were determined by commercially available enzyme immunoassay kit (Glycaben Exocell). The amount of glycated albumin was expressed as a relative percent determined from glycated and total albumin in the sample. The hs-CRP levels were determined by using high sensitivity ELISA kit (Biocheck Laboratories, USA) with linear range 0.62 to 119.3mg/L.

Assay performance: Sample handling and assay conditions were strictly observed for all procedures according to manufacturer's instructions. For precision of assay, calibrated micro/multichannel pipettes from Gilson were used throughout the assay procedure and washing was performed with automatic plate washer. Analysis of glycated Alb and hs-CRP were carried out using Softmax Statistical Package. For diagnostic assurance of results all investigations were carried out with 6-7 standards points and 3 quality control pools in each assay batch.

The study data were analysed using SPSS version 15.0. The outcome of study included quantitative and qualitative factors, which were correlated for association using Chi square and t test. The association was initially assessed between the normal and CAD groups: However the data of subgroups were tested using ANOVA and Post Hoc Tukey's Test for significance.

RESULTS

Patients who were enrolled in study were grouped on the basis of coronary artery lumen diameter narrowing. Participants with < 30% lumen diameter narrowing were considered normal (Group 1), with 30–50% as mild CAD (Group II), with 51-70% as moderate CAD (Group III) and greater than 70% as severe CAD (Group IV). The mean age was similar to one another (56.8±9.0, 57.6±6.8, 57.9±5.3 and 58.2±8.3), in all groups i.e. I-IV respectively (p>0.05). The lumen narrowing diameters were almost similar in gender distribution in relation to various groups except in group IV, where the females had higher lumen narrowing than males (Table 1).

However subjects in this group were in small numbers in both genders and the difference may not be significant. In majority of study participants (group I) the duration of diabetes was ≤5 yrs in relation to lumen diameter (<30%). However group IV showed an unusual high frequency (83.3%) with shorter duration of diabetes. History of hypertension progressively rose from 22.2% to 58.3% in group I to group IV. Smoking history presented the same pattern, with increase from 0% to 41.7% in group I to group IV respectively. Family history of diabetes was present in 14.8% in group I, 32% in group II and 76.9% in group III, whereas in group IV it was 41.7%. History of CAD in the family was seen in 7.4% in group I, 12% in group II and 19.2% in group III and 41.7% in group IV (Table 1).

The association between Glycated Albumin levels and lumen narrowing diameter was found significant, using ANOVA, and Post HOC Tukey's test between all the groups (p<0.01). Similarly the hs-CRP levels and the lumen narrowing of four groups was found significant using ANOVA (p<0.01), whereas post HOC Tukey's test showed significance between the groups (p<0.01) except between group III and group IV (p 0.857) respectively. The increase in mean glycated albumin was observed from 2.18% to 5.33 % in group I to IV respectively. Mean hs-CRP followed a similar trend of increase from 3.5 mg/L in group I to 13.3mg/ml in group IV. A striking spurt was noticed for 5.1mg/L in group II to 12.9 mg/L in group III (Table 1 & Fig. 1). The levels of both glycated albumin and hs-CRP between the four groups showed high significance levels (<0.001) except for hs-CRP of group III and group IV.

Table 1: Distribution of Factors and Lumen Diameter

Lumen narrowing Diameter	Group-I (n=27) 30%		Group-II (n=25) 30–50%		Group-III (n=26) 50-70%		Group-IV (n=12) >70%	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Gender								
Males	11	40.7	13	52.0	14	53.8	4	33.3
Females	16	59.3	12	48.0	12	46.2	8	66.7
Duration of diabetes								
> 5 Years	5	18.5	12	48.0	14	53.8	2	16.7
≤ 5 Years	22	81.5	13	52.0	12	46.2	10	83.3
History of								
Smoking	-	-	2	8.0	3	11.5	5	41.7
Hypertension	6	22.2	10	10.0	14	53.8	7	58.3
Family history								
Diabetes	4	14.8	8	32.0	20	76.9	5	41.7
CAD	2	7.4	3	12.0	5	19.2	5	41.7
	N	x	N	x	N	X	N	X
Mean glycated albumin %	27	2.2	25	3.3	26	4.0	12	5.3
Mean hs CRP	27	3.5	25	5.1	26	12.9	12	13.3

The levels of glycated albumin and hs-CRP have uniformly shown a positive correlation (r= +0.99 & +0.88 respectively). The regression value for every 13% increase in lumen narrowing accounted for one mean variation unit in Glycated Albumin. For hs-CRP the regression value was more sensitive, as for every

5% increase in lumen narrowing, there was one mean unit rise in hs CRP narrowing. The association between Glycated Albumin levels and lumen diameter was found significant in all groups by applying ANOVA and post Hoc Tukey's test ($p<0.01$). Similarly the hs-CRP levels and the lumen narrowing in the four groups was significant using ANOVA ($p<0.01$), whereas the post Hoc Tuckey's test showed significance in all groups ($p<0.01$) except between group III and group IV (p 0.857).

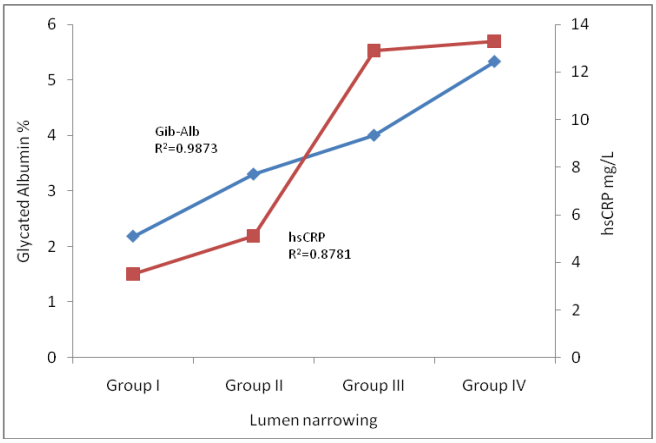

Fig 1: Levels of Glycated Albumin and hs-CRP in the four groups of increasing Coronary Luminal Narrowing Diameter

DISCUSSION

Coronary artery disease (CAD) is one of the major vascular complications in patients with type 2 diabetes. Studies have shown that up to 30% of diabetic patients with CAD had silent ischemia, mainly caused by autonomic neural dysfunction. The proportion may be as high as 60% in those at high risk.[2,3] Therefore early detection and routine screening of CAD with simple tools is important and desirable for diabetic population.[1,3,4] Among several predictive biomarkers for CAD in diabetic patients, hs-CRP and glycated albumin appeared to be useful in clinical practice. In type 2 diabetics serum levels of hs-CRP >10mg/L are reported to be associated with a 2.6 fold increase at risk of CAD when compared with those with hs-CRP <10mg/L[15].

The high levels of hs-CRP reflect inflammatory status in type 2 diabetes regardless of glycemic control which merits hs-CRP to be an independent biomarker for predicting CAD. Glycated albumin too was reported as an independent risk factor for CAD in patients with type 2 diabetes with an odds ratio of 3.46 (95%, CI 1.78-6.72, $p<0.001$)[15].

The aim of the present study was to determine the clinical usefulness of Glycated Albumin and hs-CRP levels, and to compare with coronary artery lumen narrowing diameter in diabetic patients. The results of this study has shown positive correlation of Glycated Albumin and hs-CRP with coronary artery luminal narrowing r=0.99 and 0.88 respectively (Fig 1).

A study in China reported the adverse effects of Glycated Albumin in type -2 diabetics and suggests that it accelerates atherosclerosis. If levels of glycated albumin were $\geq$19%, it acts as an independent predictor for presence of CAD (OR 2.9 $p<0.001$) and if $\geq$ 21%, there was 2-3 fold increase in risk of progressing to 3- vessel disease when compared with serum glycated albumin levels <21%, indicating that inhibition of in vivo glycated albumin was important for the prevention of CAD in patients with type 2 diabetes mellitus.[16] In the current study progressive increase in glycated albumin with increase in lumen narrowing was seen: every 13% increase in lumen narrowing accounted for one mean variation unit in glycated albumin. The relation between hs-CRP levels and extent of atherosclerosis is a controversial issue. Some studies have reported a correlation between hs-CRP levels and extent of atherosclerosis[17,18], whereas others have not found such a correlation.[19]

Reports from women's health study (WHS) have also shown that hs-CRP is a strong predictor of future cardiovascular events in women (RR 4.4, 93% CI 2.2-8.9) with no history of hyper-lipidemia, hypertension, smoking diabetes, or family history of CHD.[20,21] Another study also demonstrated that hs-CRP can identify individuals at increased risk of developing future coronary events who otherwise would be missed if only lipid measurements were used.[10] The results of this study have shown a correlation between lumen narrowing and hs-CRP: hs-CRP levels increased with lumen diameter narrowing. Lumen diameter narrowing >70% was seen in twice as many females than males. A study elsewhere reported that in diabetics with unstable angina and AMI before reperfusion therapy, CRP levels were found to be not significantly different to those in patients with stable CAD (5.96±2.6 vs 4.35±2.6 mg/L; $p=0.12$).[22]

Tobacco addiction has been reported as a principal contributor to the development of coronary artery disease (CAD) and its consequences, including sudden cardiac death, acute myocardial infarction, and heart failure. In fact 30% of all CAD deaths are attributed to smoking.[23]

Continued cigarette smoking significantly elevates the risk of sudden cardiac death in patients with CAD and stresses the importance of complete smoking cessation in risk factor modification of patients with healed MI and/or stable Angina pectoris[24].

In the current study, the frequency of cigarette smoking increased progressively by (0-41%) and was

seen more in diabetics with lumen diameter narrowing of >70%. In this study, factors like hypertension, family history of diabetes and of CAD, have also shown progressive increase from group I to group IV (Table 1).

Diabetes mellitus and coronary artery disease constitute an ominous clinical combination. Rates of morbidity and mortality as a result of cardiovascular complications are high in patients with type 2 diabetes mellitus.[25] Screening for silent coronary artery disease, to detect the disease in an early stage and to be able to initiate early appropriate treatment, is an important focus of clinical investigation.[25]

CONCLUSION

Increased serum levels of glycated albumin and hs-CRP are associated with the presence and severity of CAD, and can be useful in screening patients with type-2 Diabetes Mellitus. However, larger studies are required for more solid recommendation.

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