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Methylglyoxal level in Type 2 Diabetes with Acute Myocardial Infarction and its Association with Systemic Hypertension

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ABSTRACT

Background: The chronic hyperglycemia in type 2 diabetes mellitus (T2DM) is characterized by formation of a variety of toxic α-oxoaldehydes among which the methylglyoxal (MG) damages low density lipoproteins raising the possibility of atherogenesis upto fourfold is one proposed mechanism. The atheroma may then cause the coronary artery disease i.e. myocardial infarction and ischemic disease.

Objective: To study the methylglyoxal levels in type 2 DM with acute myocardial infarction (AMI) compared normal controls and to assess its predictive significance.

Study Design: comparative case control study

Place and Duration of Study: This study was conducted at the Diabetic clinics of Isra University Hospital & other tertiary care hospitals in Hyderabad from _____.

Materials & methods: Thirty normal controls (Group. I) and thirty type 2 diabetics with acute myocardial infarction (Group. II) were studied according to inclusion and exclusion criteria. 5.0 ml of blood was transferred into citrated bottles. Sera were obtained by centrifugation at 4000 rpm for 10 minutes and were frozen at -20 ^{0}C. The blood glucose (BS) level was detected by glucose oxidase method. MG was measured by the ELISA assay. Student`s t-test, Chi square test and Spearman`s correlations was used for the continous & categorical variables and linear association respectively. Data was collected on a proforma. Informed consent was taken from the participants. Study protocol was approved by the ethics committee of the institute. The Data was analyzed using SPSS version 17.0. A p-value of $\leq$0.05 was taken statistically significant.

Results: The male and female ratio was noted as 0.57:1 and 1:2.7 and age of 51.9±5.0 and 53.5±6.8 years in controls and type 2 diabetics with acute myocardial infarction respectively. The random blood sugar was noted as 112.6±16.8 and 304±73.8 (mg/dl) in both groups respectively (p= 0.0001). Very high levels of BS (90%) indicate that most of patients are reluctant to glycemic control. Very high levels of MG were observed in T2DM with acute MI compared with normal healthy controls; 87.7±44.2 vs. 9.19±1.29 ng/ml. (p=0.0001). Hypertension was observed in 19 (63%) of diabetics and drug non-compliance was common; 26 (86%). A powerful Spearman`s correlations of MG was observed with of the BS, SBP and DBP (p = 0.0001).

Conclusion: The present study provides evidence that MG is a predictor of acute myocardial infarction and elevation of systemic blood pressure in type 2 diabetics, suggesting its clinical usefulness as a biomarker for diabetic macroangiopathy.

Key Words: Diabetes mellitus, Methylglyoxal, Myocardial infarction, Diabetic macroangiopathy, systemic blood pressure.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in individuals with type 2 Diabetes mellitus (T2DM), for which 65% of deaths are attributable to heart disease or stroke. Hyperglycemia is encountered in up to 50% of all ST-elevation myocardial infarction (STEMI) patients, whereas previously diagnosed T2DM is present in only 20% to 25% of STEMI patients.[1] When admission glucose level exceeds 200 mg/dL, chances of mortality increases in diabetics with acute myocardial infarction (AMI). Admission glucose has been identified as a major independent predictor of both in-hospital congestive heart failure and mortality in STEMI.[2] The prognosis is poorer in patients with T2DM that suffers a myocardial infarction compared with people without diabetes mellitus. In patients with AMI the underlying mechanism of increased mortality associated to glucose levels are poor understood. Acute phase hyperglycemia and diabetes are both associated with adverse outcomes in AMI, with higher reported incidences of congestive heart failure, cardiogenic shock, and death. However, the association between hyperglycaemia and adverse outcomes is not confined to patients with diabetes, indicates no clear mechanism. Hyperglycemia, therefore, is seen as an epiphenomenon that is associated with poor outcomes only because adrenergic stress is closely related to the extent of myocardial injury. The possible mechanisms that influence the increased risk in diabetes for cardiovascular events include, insulin resistance, changes in endothelial function, dyslipidemia, chronic inflammation and release of mediators of inflammation, procoagulability and impaired fibrinolysis.[3] The chronic hyperglycemia in T2DM is characterized by formation of a variety of toxic α-oxoaldehydes among which the methylglyoxal (MG) damages low density lipoproteins raising the possibility of atherogenesis upto fourfold compared to general adult population.[4] The atheroma may then cause the coronary artery disease i.e. myocardial infarction and ischemic disease, stroke, and peripheral arterial disease.[5] These observations strongly suggest the role of MG in diabetic vascular complications. The aim of present study was to assess the MG level in T2DM subject suffering from acute myocardial infarction in comparison with normal healthy controls.

MATERIALS AND METHODS

A comparative case control study was conducted at the Diabetic clinic of Isra University Hospital and other tertiary care hospitals of Hyderabad over a period of six months Normal volunteer healthy controls (Group. I) (n=30) and diagnosed cases of T2DM with AMI (Group. II) (n=30) were selected through non-probability purposive sampling. Diabetics with acute myocardial infarctions age of >40years and <65 years were included in the present study. Diabetics with acute myocardial infarctions having ischemic heart disease, renal failure, chronic systemic illnesses e.g. pulmonary tuberculosis, Rheumatoid arthritis, etc; alcoholics and smokers were excluded from the study. Diabetes mellitus was defined as post parendial level of ≥200 mg/dl or fasting blood sugar level of ≥ 126mg/dl.[6] BMI was calculated from the weight and height by formula; BMI=Weight (kg)/Height (m^2).

Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg.[7] Systemic BP was recorded with a mercury sphygmomanometer after the patient had taken 5 minutes rest. For each subject, the average of two readings was recorded in supine and standing position. The blood samples were drawn through venepuncture under aseptic condition using standard methods of blood sampling. 5.0 ml of blood was transferred into citrated bottles. The blood glucose level was detected by glucose oxidase method on Spectrophotometer Hitachi 902 (Roche diagnostics, USA).

Centrifugation of Blood samples: The blood was centrifuged at 4000 rpm for 10 minutes to obtain serum. The serum samples thus obtained were frozen at -20 ^{0}C.

Assay for Methylglyoxal level: Enzyme-Linked immunosorbent assay (ELISA) technique was employed for measurement of methylglyoxal according to the standard technique.[8]

The consent was taken from all the willing participants. The data was collected on pre-structured proforma. The study was approved by ethics committee of the institute. The continuous variables were analyzed Student`s

t- test (independent samples), results were presented as Mean ± Std.Deviations. While the categorical variables were analyzed using Chi square test and presented as frequencies and percentages. The strength of association of MG with BS, SBP, DBP was analyzed using Spearman`s correlations. The Data was analyzed using SPSS version 17.0 for Windows (Chicago, Illinois, USA). A p-value of ≤0.05 was taken statistically significant.

RESULTS

Thirty type 2 DM subjects with acute myocardial infarction (T2DM-AMI) were studied to analyze the blood sera for methylglyoxal (MG) levels. The male and female subjects in Groups 1 & 2 were 11 (36.6%) vs.22 (73.3%) vs. and 19 (63.3%) vs. 8 (26.6%) respectively. The age noted was 51.9±5.0 and 53.5±6.8 years in controls and T2DM-AMI subjects respectively (p= 0.06). The BMI, random blood sugar, systolic BP, diastolic BP and hypertension of study population are shown in Table. I. Subjects in both groups were age and BMI matched. Very high levels of MG were observed in T2DM with acute MI compared with normal healthy controls; 87.7±44.2 vs. 9.19±1.29 ng/l. (p=0.0001) (Table. 3) (Grpahs I).

Table No. 1: Demographic characteristics of study population (n=60)

	Group I (Controls) n=30	Group II (T2DM-AMI) n=30	*p-value
Age (years)	47.9 ± 5.0	53.5 ± 6.8	0.001
BMI † (kg/m²)	24.8± 4.0	24.65 ± 3.32	0.80
BS □ (mg/dl)	112.6 ± 16.8	304 ± 73.8	0.001
Systolic BP (mmHg)	118.3± 10.9	157.6±23.29	0.001
Diastolic BP (mmHg)	75.6 ± 6.6	95.6 ± 10.05	0.003
Hypertension	-		
Drug noncompliance	-	26 (86%)	-

†Body mass index Blood sugar *p-value ≤ 0.01

Table No.2: Gender Distribution among study population (n=60)

	Patient Distribution		
	Group I (Controls) n=30	Group II (T2DM-AMI) n=30	Total
Male	11	22	33
Females	19	8	27
Total	30	30	60

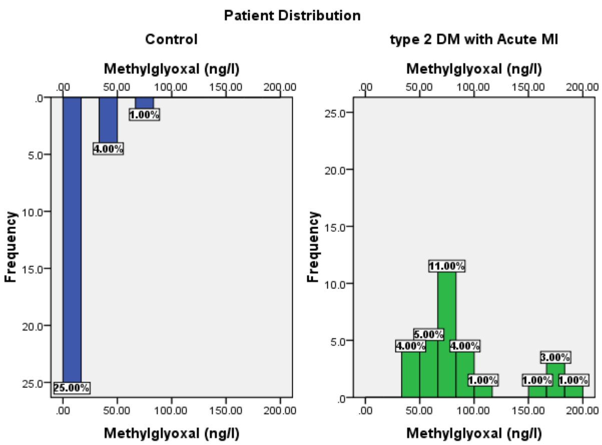
The BS was noted as 112.6±16.8 and 304±73.8 (mg/dl) in both groups respectively (p= 0.0.001). (Table I.) Very high levels of BS (90%) indicate that most of patients are reluctant to glycemic control. BS values as high as 544 mg/dl were observed in diabetics. drug non-compliance which was 26 (86%) Hypertension was observed in 19 (63%) of diabetics. A powerful Spearman`s correlations of MG was observed with of the BS, SBP and DBP (p = 0.0001) (Table. 4)

Table No.3. Methylglyoxal levels (ng/dl) among study population (n=60)

	Group I (Controls) n=30	Group II (T2DM-AMI) n=30
Mean	9.19	87.7
Std. deviation	1.92	44.4
95% Confidence interval	1.99 - 16.3	71.17-104.3
Range	0.81-81.2	36.2 -195.7
Interquartile range	0.58	32.39

Table No.4: Spearman's correlation of Methylglyoxal

Parameter	Correlation coefficient (r)	p=value
Blood sugar	0.802	0.0001
Systolic blood pressure	0.707	0.0001
Diastolic blood pressure	0.698	0.0001



Graph No.1: Methylglyoxal level between controls and type 2 diabetics with acute myocardial infarction

DISCUSSION

Ischemic heart disease continues to gain prevalence as a cause of disability and death and is costly in terms of patient morbidity and mortality as well as financial resources utilized in acute and chronic treatment.[9] The specific molecular mechanisms underlying why diabetes mellitus directly increases ischemic heart disease risk remain elusive.[10,11,12] Evidence suggests elevated MG levels may play a role in the development of a number of diabetic complications.[13] A previous study provided evidence that protein glycation is a new mechanism through which MG aggravates cardiac reperfusion injury after myocardial infarction.[9] This study provides for the first time evidence that MG is a predictor in T2DM of elevation of systemic blood pressure and MI suggesting its clinical usefulness as a biomarker for diabetic macroangiopathy.(Table. III & IV) Hyperglycemia increases MG and advances macroangiopathy. Therefore, MG is believed to increase the subsequent advancement of macroangiopathy in T2DM like acute myocardial infarction.[14,15] Our contention that MG predicts the development of diabetic macroangiopathy in type 2 diabetics is in good agreement with previous observations[9,28] that chronic hyperglycemia, a factor contributing to the development of macroangiopathy, dramatically increases the production of MG. A previous study by Beisswenger et al[16] demonstrated that the biguanides lower plasma MG in diabetic patients as are being widely used in diabetic subjects. MG has indeed been linked to the progression of hypertension in diabetic models through increases in vascular resistance, insulin resistance, and salt sensitivity and by the retention of body fluid volume.[17,18,19] Previous studies by us and others have demonstrated that administration of MG induces a rise in BP in experimental animals, which is significantly suppressed by administration of angiotensin receptor blockers or *N*-acetyl cysteine (an antioxidant agent).[18,20,21] In blood vessels under diabetic conditions, MG primarily accumulates in endothelial cells, increases oxidative stress and induces vascular disorders.[22] Moreover, MG increases the salt sensitivity. [19,23,24] These are better explanations as to why the levels of MG should predict systemic blood pressure. Mori et al [25] observed previously that MG induces hypertension and cardiorenal injury in Dahl salt-sensitive rats with a normal diet through the angiotensin II–mediated oxidative stress pathway. These previous observations support our findings of highly elevated MG in diabetics with myocardial infarction. Despite an early loss of glycemic differences, a continued reduction in microvascular risk and emergent risk reductions for myocardial infarction and death from any cause were observed during 10 years of post trial follow-up in the United Kingdom Prospective Diabetes Study-80.[26,27] Early and rigorous blood glucose control thus has either a metabolic memory effect or a legacy effect of suppressing the onset of vascular disorders for extended periods. The possible mechanism of such effects remains unclear, although Holman et al[27] suggested that increased formation of AGEs may play an underlying role.30 The increased levels of MG observed in individuals with diabetes mellitus are not merely the result of short-term changes in glucose or MG but may reflect long term alterations to tissue proteins. In this context, it is of interest that MG, a precursor for AGEs, at the baseline is an independent risk factor for the percentage changes after 5 years of intima-media thickness, pulse wave volume, and BP.[28,9] Elucidation of the effects of MG and other AGE precursors upon the ischemic heart, and the involved underlying mechanisms, could yield improved preventative and therapeutic treatment of the diabetic heart at risk for and undergoing ischemic injury, respectively.[9] MG could be a target for future study to elucidate the biochemical mechanisms of such a legacy effect.[9] The cause effect relationship of methylglyoxal with acute myocardial infarction cannot certainly be made in cross sectional studies. As this was a cross sectional study conducted in outpatients department in which diverse interventions and treatments might have interfered as confounders, hence the findings cannot be generalized to other settings. We are of the opinion that elaborated longitudinal studies should be conducted to make guidelines for MG levels in diabetics and then interventions are made in proper direction to overcome the problem.

CONCLUSIONS

We report very high levels of MG in type 2 diabetics with acute myocardial infarction. The present study provides evidence that MG is a predictor of acute myocardial infarction and elevation of systemic blood pressure in type 2 diabetics, suggesting its clinical usefulness as a biomarker for diabetic macro-angiopathy.

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