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# Methylglyoxal Level In Type-2 Diabetes Mellitus Patients: A Prospective, Randomized and Case Control Study at Tertiary Care Hospitals, Hyderabad, Pakistan

**1. Mumtaz Ali Qureshi 2. Nabila Soomro 3. Ghulam Murtaza Talpur**

1. Dept. of Biochemistry, Isra University Hyderabad, Sindh, Pakistan 2. Medical Center, SSGC, Latifabad Unit 3. Hyderabad, Sindh, Pakistan

## ABSTRACT

**Objective**: To study the methyglyoxal (MG) levels in type 2 diabetes mellitus (T2DM) subjects compared with normal controls and to evaluate relationship of MG with blood sugar, systolic and diastolic blood pressure.

**Study Design:** comparative case control study

**Place and Duration of Study:** This study was conducted at the Diabetic clinics of Isra University Hospital for a period of six months from.

**Subjects & methods**: Thirty normal controls (Group. I) and thirty T2DM (Group. II) were studied according to inclusion and exclusion criteria. 5.0 ml of blood was transferred into citrated bottles. Serum was obtained by centrifugation at 4000 rpm for ten minutes and were frozen at -20$^0$C. The blood glucose level was detected by glucose oxidase method. MG was measured by the ELISA assay. Student`s t-test, Chi square test and Pearson`s correlations were used for the continous & categorical variables and linear association respectively. The Data was analyzed using SPSS version 17.0. A p-value of ≤0.05 was taken statistically significant.

**Results**: Very high levels of MG were observed in type 2 diabetics compared with controls; 45.60±37.24 vs.1.29±0.30 ng/l (p=0.0001). The male and female in groups I & II were 19 (63.3%) vs.11 (36.6%) and 20 (66.6%) vs. 10 (33.3%) respectively. Mean (±SD) of age was 47.9±5.0 and 49.3±6.6 years in both groups respectively (p= 0.67). Majority of diabetics; 23 (76.6%) were having very high levels of blood sugar. More alarming situation was of anti-diabetic drug non-compliance which was noted in 19 (63.3%). Hypertension was observed in 17 (56.6%) of diabetics. A positive linear correlation of MG was observed with RBS (r=0.70, p = 0.001), SBP (r=0.33, p = 0.01) and DBP (r=0.35, p=0.006).

**Conclusions**: We report elevated methyglyoxal levels in type 2 diabetes mellitus patients compared with controls. A positive linear correlation was observed with sytemic hypertension and blood glucose level.

**Key Words**: Diabetes mellitus, Hyperglycemia, Methylglyoxal.

## INTRODUCTION

The chronic hyperglycemia in type 2 diabetes mellitus (T2DM) is characterized by formation of a variety of toxic α-oxoaldehydes which in turn react with amino groups of proteins; eventually leading to the formation of the advanced glycation end products (AGEs).[1] Among toxic α-oxoaldehydes, the methylglyoxal (MG) is most abundant and most studied in DM patients.[1] The MG is also known as the pyruvaldehyde and or 2-oxopropanal ($CH_3$-CO-CHO or $C_3H_4O_2$). The MG contains 3 carbons and possesses 2 carbonyl groups located on the first and second carbons; hence it is a dicarbonyl compound.[2,3] The MG is produced mainly from glycolysis intermediates such as glycerylaldehy de-3-phosphate (G-3-P) and di-hydroxyactone phosphate (DHAP). Other minor sources of MG are from acetone formation of fatty acid metabolism, lipid peroxidation and threonine metabolism.[3]

The MG has been linked to both the micro- and macrovascular complications of T2DM; like diabetic neuropathy, nephropathy, retinopathy, atherosclerosis, coronary artery disease and myocardial infarction.[4]

It is reported that the MG damages low density lipoproteins in T2DM patients raising the possibility of atherogenesis upto fourfold compared to general adult population.[4] The atherogeneicity is the most understood underlying lesion of macrovascular diabetic complications.[5] The atheroma may then cause the coronary artery disease i.e. myocardial infarction and ischemic disease, stroke, and peripheral arterial disease[6]. Taken together these reports into consideration, it strongly suggests the role of MG in diabetic vascular complications.

As currently Pakistan has much burdened of T2DM[7] and its related complications, and unfortunately there have been a few studies examining whether raised plasma MG levels are an independent risk factor that may predict the progression of diabetic vascular complications. The aim of present study was to assess the MG level in normal healthy controls and T2DM subject and it was hypothesized that the MG levels are independently elevated in diabetic subjects.

# SUBJECTS AND METHODS

A comparative case control study was conducted at the Diabetic clinic of Isra University Hospital and other tertiary care hospital of Hyderabad over a period of six months. Normal volunteer healthy controls (Group. I) (n=30) and diagnosed cases of T2DM (Group. II) (n=30) were selected through non-probability purposive sampling. T2DM subjects were selected through inclusion and exclusion criteria. Subjects of age of >40years and <65 years were included in the present study. T2DM subjects 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 Random blood sugar (RBS) level of ≥200 mg/dl or fasting blood sugar level of ≥ 126mg/dl.[8] 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.[9] 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. 5.0ml 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.[3]

The informed written consent was taken from all the participants. They were further informed that they can withdraw from study any time if not willing or feeling worrisome. The data was collected on pre-structured proforma. The study was approved by ethics committee of the institute. The quantitative variables were presented as Mean ± SD and range. Frequency and percentages were presented for categorical variables i.e. gender. The data was analyzed using Student`s independent samples t-test. Chi square test was used for the categorical variables. Pearson`s correlations was used for the association of MG with BS, SBP, DBP. 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

The demographic characteristics of study population are shown in Table. I. The current study of Methylglyoxal level (MG) in T2DM subjects revealed very high levels of MG compared with normal healthy controls; 45.60±37.24 vs. 1.29±0.30 ng/l. (p=0.0001) (Table. I). The male and female subjects in Groups I & II were 19 (63.3%) vs.11 (36.6%) and 20 (66.6%) vs. 10 (33.3%) respectively. Mean (±SD) of age was 47.9±5.0 and 49.3±6.6 years in both groups respectively (p= 0.67). The controls and T2DM subjects were BMI matched; 24.8±4.0 vs. 24.94±5.2 kg/$m^2$ (Table I.). The RBS was noted as 112.6±16.8 and 204±75.8 (mg/dl) in both groups respectively (p= 0.0.001). (Table I.) Most of T2DM subjects; 23 (76.6%) were having very high levels of RBS which indicates that most of patients were not aware about the glycemic status. More alarming situation was of anti-diabetic drug non-compliance which was noted in 19 (63.3%). Hypertension was observed in 17 (56.6%) of diabetics.

A positive linear correlation (Pearson`s correlations) of MG was observed with of the BS, SBP and DBP by using bi-variate method which revealed correlation co-efficient (**r**) of r=0.70 (p = 0.001), r=0.33 (p = 0.01) and r=0.35 (p = 0.006) respectively. (Table 2) (Graphs 1, 2 and 3).

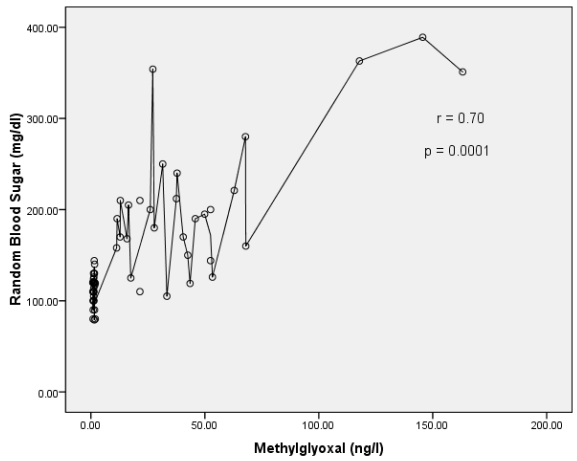
**Table No.1: Demographic characteristics of study population (n=60)**

| | Group I (Controls) n=30 | Group II (DM) n=30 | *p-value |
|---|---|---|---|
| **Age (years)** | 47.9 ± 5.0 | 49.3 ± 6.6 | 0.67 |
| **Male** | 19 (63.3%) | 11 (36.6%) | 0.001 |
| **Female** | 20 (66.6%) | 10 (33.3%) | 0.001 |
| **BMI † (kg/$m^2$)** | 24.8± 4.0 | 24.94 ± 5.2 | 0.91 |
| **BS □ (mg/dl)** | 112.6 ± 16.8 | 204 ± 75.8 | 0.001 |
| **Methylglyoxal level (ng/dl)** | 1.29 ± 0.30 | 45.60 ± 37.24 | 0.0001 |
| **Systolic BP (mmHg)** | 118.3± 10.9 | 134.8±25.9 | 0.008 |
| **Diastolic BP (mmHg)** | 75.6 ± 6.6 | 83.6 ± 11.05 | 0.01 |
| **Hypertension** | - | 17 (56.6%) | - |
| **Drug noncompliance** | - | 19 (63.3%) | - |

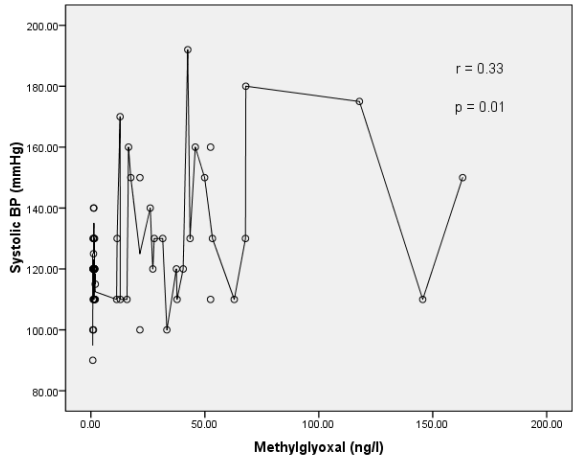
**†Body mass index Blood sugar *p-value ≤ 0.05**

**Table No.2: Pearson's correlation of Methyglyoxal**

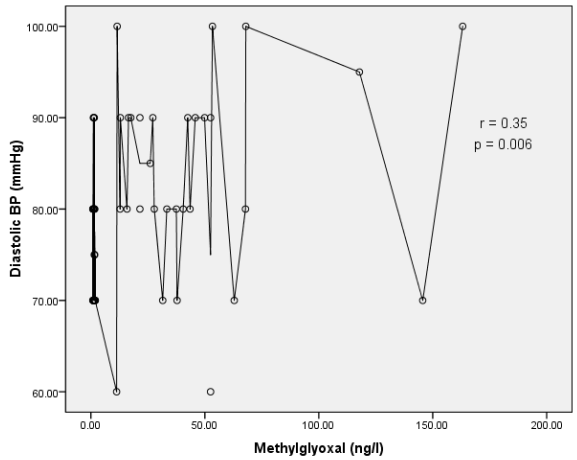
| Parameter | Correlation coefficient (r) | p=value |
|---|---|---|
| **Blood sugar** | 0.70 | 0.0001 |
| **Systolic blood pressure** | 0.33 | 0.01 |
| **Diastolic blood pressure** | 0.35 | 0.006 |




**Graph No.1: Pearson's correlation of methyglyoxal with blood sugar**




**Graph No.2: Pearson's correlation of methyglyoxal with Systolic blood pressure**




**Graph No.3: Pearson's correlation of methyglyoxal with diastolic blood pressure**

# DISCUSSION

We report major differences in the methyglyoxal (MG) levels in the controls and T2DM (Table. I). The MG is one of the AGEs producer in subjects suffering from chronic hyperglycemia. Many studies[1,2,3] have been conducted focusing on the MG and AGEs in patients with T2DM.

Our results of MG levels in T2DM subjects are comparable to the previous studies mentioned in the literature.[1,10,11] Elevated blood MG levels have been reported in T2DM subjects in a recent study.[12] Even in type 1 DM subjects, the MG derived "hydroimidazolone" has been reported to be higher compared to non-diabetic controls.[13] Recently a prospective study was conducted to evaluate the impact of MG and glucose on mononuclear cells and reported elevated levels of MG parallel to a rise in blood sugar levels. It was reported that the MG levels in diabetics were significantly elevated compared to non-diabetics[11]. These findings are in close agreement with our present work. Many recent studies[11,12,13] show that the MG induces apoptosis in body cells like Schwann cells and renal tubular epithelial cells, and thus produces tissue injury leading to diabetic vascular complications.[11]

Dhar et, al; 2010 reported very high levels of MG in induced diabetic Sprague-Dawley (SD) rats. The MG was infused in SD rats and severe glucose intolerance was seen consequent upon.[10] The chronic MG infusion in SD rats through a minipump implanted subcutaneously for 28 days (60 mg/kg/day), to produce abnormal glucose metabolism. The biochemical abnormalities of glucose homeostasis and insulin release that were highly similar to the T2DM were observed.[10] Our findings of elevated MG in T2DM compared with normal controls are consistent with the results reported (Table I).[10]

Our findings also show positive correlation of MG with blood glucose levels, systolic and diastolic BP (Table. II) (Graphs. I-III) are highly consistent with the previous studies conducted on animals[10], or human beings.[11] A recent study from Japan, reported that the MG level was proved to be an independent risk factor for the systolic blood pressure, pulse wave velocity, and urinary albumin excretion.[1] Our findings of positive correlation of MG with Systolic blood pressure (SBP) are comparable finding with the previous study.[1] Our present study, shows a positive correlation of MG with SBP (r=0.33 and p=0.01). (Graph. 2). and also a positive correlation of DBP with the MG (r=0.35 and p=0.006) (Graph 3).

Our finding of MG and its association with SBP, DBP, BS and more elevated MG levels in T2DM are consistent with the findings reported in the litrature.[1,10,11] The positive association of MG with the hypertension has been reported by other studies also.[14,15] The accelerated formation of MG in T2DM subjects and its relationship with the hypertension has been mentioned in the literature.[17,18] The elevated MG levels in Wistar-Kyoto (WKY) rats and the raised blood pressure compared with the baseline, is also reported in one study.[17]

In recent study[16] It is reported that when the MG was incubated with human insulin in-vitro, the insulin was modified at β-chain at arginine residue and the MG-insulin complex thus formed, showed no insulin activity.[16]

Although we could not reached to evaluate the role of MG at the molecular level but our finding of elevated MG in type 2 diabetics is very important evaluate on detail T2DM subjects on clinical trials in future.

As this was a cross sectional study conducted in outpatients department so its findings cannot be generalized to hospitalized T2DM subjects having multiple disease problems. The cause effect relationship of MG and DM cannot certainly be made in cross sectional studies, hence we don't claim for this relationship.

# CONCLUSION

We report elevated methyglyoxal levels in type 2 diabetes mellitus subjects compared with controls. A positive linear correlatin of methyglyoxal was observed with systemic hypertension and blood glucose level. A positive correlation was observed with systemic hypertension and blood glucose level leading to diabetic macroangiopathy.

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**Address for Corresponding Author:**
**Dr. Nabila Soomro,**
Flat No. 504 New Kauser
Apartment near Teen Talwar Clifton Karachi
Email: drnabilamurtaza@yahoo.com