EVALUATION OF CORRELATION BETWEEN METHYLGLYOXAL AND OXIDATIVE STRESS IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background: Diabetes mellitus (DM) is a cluster of metabolic disorder with various complications that significantly impact patient's health outcomes. Hyperglycemia associated with DM can increase oxidative stress which is hypothesized to play a significant role in the development of DM and its complications. When the oxidative stress increases in diabetes, methylglyoxal (MG), a strong and hazardous reactive metabolite that is derived from glucose and fructose metabolism is accumulated in blood, further increases oxidative stress and also affects insulin secretion from pancreatic β-cells. This study aimed to evaluate the relationship between MGO and oxidative stress with DM. **Method:** Total 130 participants were included in the study and divided into two groups as healthy control group (65) and diabetic group (65) irrespective of gender. Diabetic patients with complications were excluded from the study.

Glucose level, HbA1c,methylglyoxal(MG), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GRx) were estimated and compared among the groups. **Results:** Diabetic group exhibited significantly increased fasting and post-prandial glucose levels and HbA1c compared to healthy controls. MG and MDA levels were significantly higher, while GPx and GRx activity were significantly reduced in a diabetic group as compared to healthy control group. Elevated post-prandial glucose showed positive association with elevated HbA1c and MG levels. **Conclusion:** Highly significant increase in MG levels and oxidative stress markers and positive correlation between MG and indices of glycaemic control emphasized the importance of MG and oxidative stress in pathogenesis of diabetes.

Keywords: Methylglyoxal, Type 2 diabetes mellitus, Oxidative stress.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by sustained hyperglycemia leads to many health complications. It is classified into two major types such as Type 1 and Type 2 DM, on the basis of the pathogenic process that leads to chronic hyperglycemia.[1] Type 1 DM is associated with insulin deficiency and its severity depends on the degree of dysfunction of beta cells of islets of pancreas and predominance of insulin resistance. Type 2 DM is a most common type of DM which is characterized by variable degree of insulin resistance, impairment of insulin secretion and excessive hepatic glucose production.[1]DM and its complications is the significant cause of morbidity and mortality throughout the world.[2] Other less common types of diabetes are gestational diabetes, maturity-onset diabetes of the young (MODY), latent autoimmune diabetes in adults (LADA) and secondary forms of diabetes.[3] Glycation is a non-enzymatic reaction between glucose and free amino groups on proteins, lipids and nucleic acids results in the formation of advanced glycation end-products (AGE) that stimulates the pathological changes in DM and its complications.[4,5].

Over the past 20 years, there has been a sharp rise in the number of people diagnosed with diabetes worldwide.6 The negative consequences of persistently increased plasma glucose levels on the various body systems varies from cell to cell, hence is has grown as pandemic disease.. Many factors including population ageing, economic development and accompanying changes in lifestyle, more effective treatments and higher survival rate have contributed to the rising prevalence of diabetes.[3]

The main contributing factor in DM is chronic hyperglycemia, which impairs endothelial cell function. One theory explaining how hyperglycemia results in diabetic complications is an increase in AGE (advanced glycation end products) formation inside the cells.[7] As a result of intracellular hyperglycemia, the triose phosphates like glyceraldehyde 3- phosphate and dihydroxyacetone phosphate

accumulates in endothelial cells which leads to the formation of extremely reactive dicarbonyl molecule MG(methylglyoxal).[7]

All mammalian cells produce methylglyoxal (MG), a characteristic member of oxaldehyde family of chemicals, both intracellularly and by non-enzymatic means. The majority of MG is generated from the glycolytic pathway's triose phosphate intermediates; glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.[4]

Oxidative stress is an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Peroxides and free radicals produced by disturbances in the normal redox state of cells can harm all cell constituents, including proteins, lipids, and deoxyribonucleic acid (DNA).[8] Chemically, oxidative stress is linked to either a rise in the generation of oxidizing species or a sharp decline in the efficiency of antioxidant defenses like glutathione.[8] A cell can overcome minor perturbations and return to its initial state, therefore the effects of oxidative stress depend on the extent of these alterations. Severe oxidative stress can cause cell death , moderate oxidation can trigger apoptosis, while more intense stress may cause necrosis.

Although the biochemical and molecular pathways behind the emergence of diabetes complications are better understood, its complex etiology still poses many unanswered concerns. This study was conducted to evaluate the correlation between methylglyoxal and oxidative stress associated with Type 2 DM and its complications . Despite many significant clinical advances in treatment of Type 2 DM ,there remains a gap in knowledge that can allow the physicians to predict and intervene to prevent or to treat diabetes prior to the occurrence of irreversible damage due to diabetic complications. Furthermore, there is a shortage of clinically relevant and measurable biomarkers of diabetes that can help in this process. Commonly used methods such as determination of fasting , postprandial blood glucose levels and measurement of HbA1c for the diagnosis of diabetes only allow the physicians to see a snapshot in time.

AIM : Evaluation of correlation between methylglyoxal and oxidative stress in Type 2 Diabetes mellitus.

OBJECTIVES:

1. To determine the blood levels of fasting and postprandial glucose and glycated hemoglobin (HbA1c) in patients of Type 2 DM.

2. To measure serum methylglyoxal (MG), malondialdehyde (MDA) and antioxidant enzyme levels for the assessment of oxidative stress in Type 2 DM.

3. To evaluate the correlation between methylglyoxal and oxidative stress in Type 2 DM.

MATERIAL AND METHODS

This is prospective, observational study was carried out in the Department of Biochemistry of Dr. D. Y. Patil Medical College, Hospital and Research centre, Pimpri, Pune from July 2016 to June 2018.

Patients were obtained from the wards of Medicine, Surgery, Nephrology, Intensive Care Unit and from Ophthalmology Out Patient Department with prior permission of respective head of the departments.

Prior approval was obtained from Institutional Ethics Committee for Human Research,

Sample size was determined considering the prevalence of Type 2 DM (9.1%) in rural population of India.

Total 130 participants were enrolled in this study and equally divided into two groups as follows;

1) **Group 1 – Healthy Control Group**
2) **Group 2 – Diabetic group**

Inclusion Criteria:

- Newly diagnosed patients of Type 2 DM with impaired glucose tolerance or insulin resistance.
- Healthy volunteers who are willing to participate in the study.

Exclusion Criteria:

- Patients taking any antioxidant therapy.
- Patients not willing to participate in the study.
- Patients without diabetic complications.

METHOD OF DATA COLLECTION:

Written informed consent was obtained for participation in the study and for blood collection from the study participants. Their personal information, medical history, present complaints, treatment history, past history, family history and any other related information was recorded.

Total 5 ml venous blood was collected and distributed in fluoride bulb (1 ml) , citrate bulb(1 ml) and in plain bulb(3ml) for further investigations.

BIOCHEMICAL INVESTIGATIONS:

All the routine tests were performed on Fully Automated Biochemistry Analyzer using following methods:

a. Fasting and Postprandial Blood Glucose(mg/dl) – Glucoseoxidase- Peroxidase method

b. Glycated Haemoglobin(%) - High Performance Liquid Chromatography

c. Malondialdehyde (n.mol/ml) - Wilbur K.M.et al

d. Superoixide dismutase(units/ml) – Marklund et al

e. Glutathionine peroxidase (units/L) – Paglia and Valentine.

f. Glutathionine Reductase (units/L) – Inger Carberg and Beng Mamervik

g. Methylglyoxal (n.mol/L) – Racker's method

STASTICAL ANALYSIS:

The categorical variables were presented as frequencies and percentages and were compared between the group with Chi-square test.

The continuous variables were presented as mean +/- SD, and the difference in the means between the groups was compared by independent student 't' test.

Pearson's correlation coefficient (r-value) was used to find out correlation between the parameters of the study groups.

Interpretation was done according to r-values as follows:

r=0.00-0.19 depicts very weak correlation

r=0.20-0.39 depicts weak correlation

r=0.40-0.59 depicts moderate correlation

r=0.60-0.79 depicts strong correlation,

r=0.80-1.00 depicts very strong correlation

The cut-off points were plotted for biochemical parameters for different groupsusing receiver operating characteristics (ROC) curve analysis. It is used to assess the overall diagnostic performance of the test and to compare the performance of two or more tests.

Student t test was used to compare the means between two groups. The p vaule of <0.05 was considered significant at 95% confidence interval and ANOVA was used to compare the means among the groups.

Statistical analysis was carried out using Statistical Program for Social Sciences (SPSS) software version 24.0.

OBSERVATIONS AND RESULT:

Table 1 and **Graph 1** shows mean and standard deviations (SDs) of age distribution in both the groups.

Table 1: Age distribution

Group	Group 1		Group 2		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**p Value**
Average Age(years)	30.14	8.23	47.94	6.74	**<0.0001***

There was a significant difference (p<0.0001*) in mean age between both the groups.

Graph 1: Mean and standard deviations (SD) in age distribution

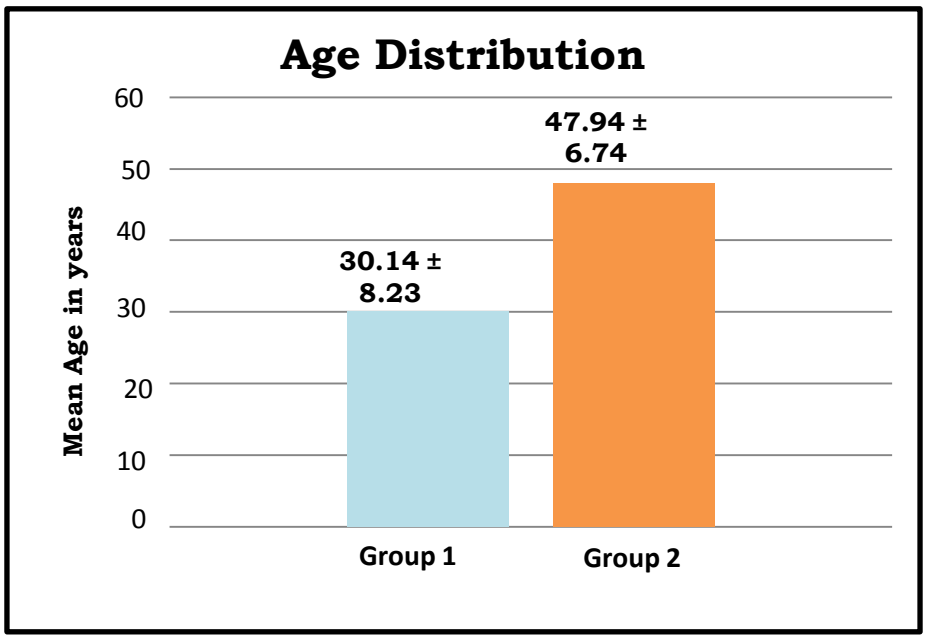
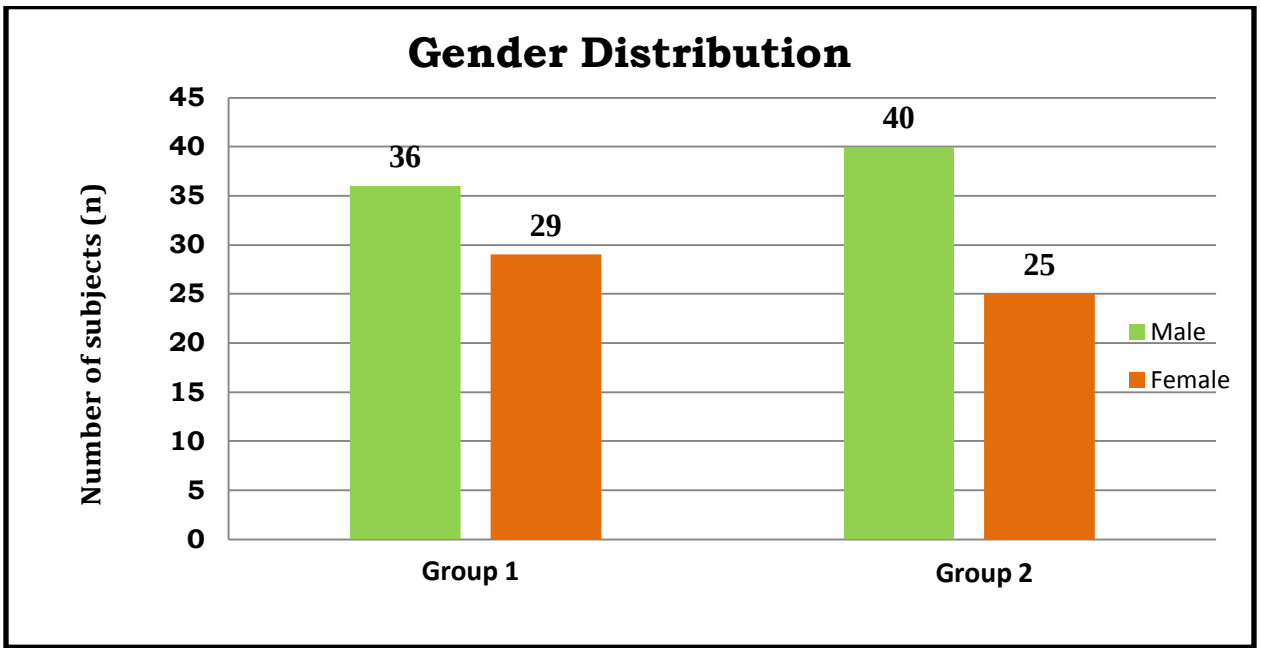


Table 2 and Graph 2 shows gender distribution between the study groups.

Table 2: Gender distribution

Gender	Group 1	Group 2	p Value 0.8137
Male	36	40	
Female	29	25	
Total	65	65	**130**

Graph 2: Gender wise distribution of patients between the groups.



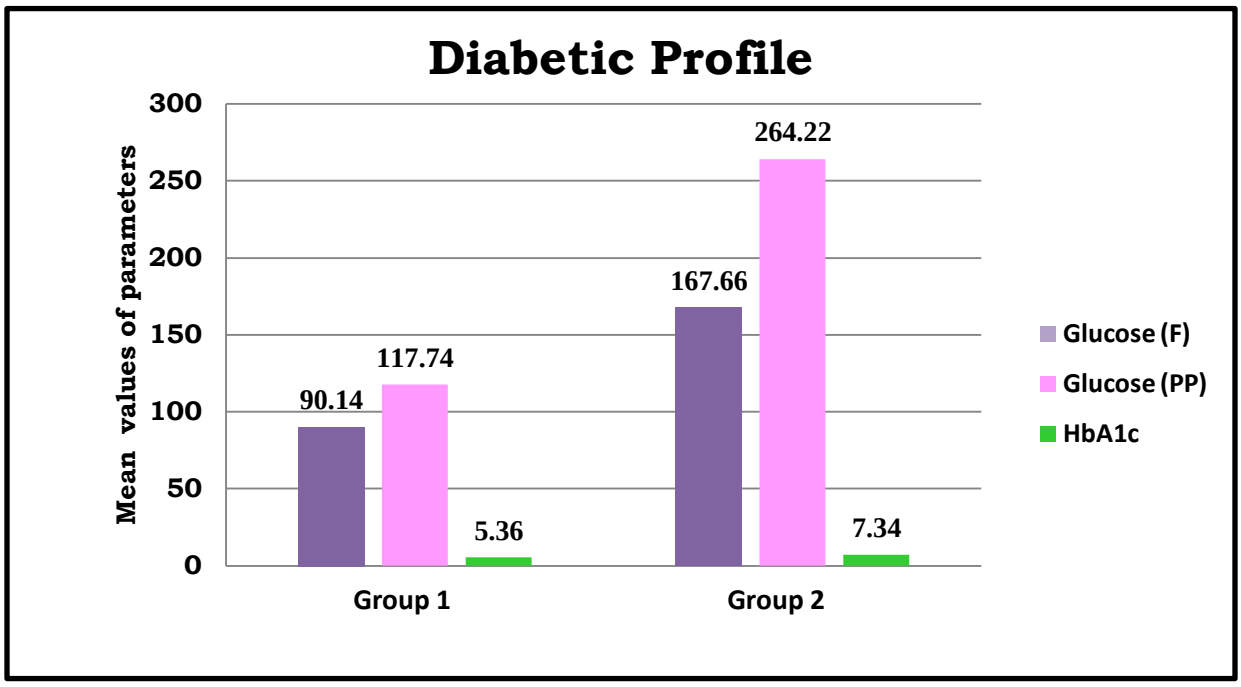
Comparatively there were more males (n=76) in both study groups than females (n=54). Therewas no significant (p=0.8137) difference in the gender wise distribution of participants amongthe groups.

Diabetic Profile : Fasting, Postprandial Glucose & Glycated Hemoglobin Levels (Table 3)

The mean ± SD values of fasting, PP glucose and HbA1c in both study groups with respective p values are shown in **Table 3** and presented graphically in **Graph 3**.

Table 3: Mean ± SD values of fasting, PP glucose and HbA1c

Parameters	Group 1		Group 2		P value
	Mean	SD	Mean	SD	
Glucose (F)	90.14	78.59	167.66	14.93	<0.0001*
Glucose (PP)	117.74	9.96	264.22	22.58	<0.0001*
HbA1c	5.36	0.19	7.34	0.30	<0.0001*



Graph 3: The mean values of fasting, PP glucose and HbA1c.

The fasting and PP glucose values were significantly ($p<0.0001$) increased in Group 2 as compared to Group 1. Similarly, HbA1c values were also increased in Group 2 as compared to Group 1.

Oxidative Stress & Antioxidant enzyme status estimation:

The levels of serum methylglyoxal (MG), malondialdehyde (MDA), and antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRx) were estimated in both study groups to determine the oxidative stress and antioxidant enzyme status..

The mean ± SD values of MG, MDA and antioxidant enzymes with respective p values are shown in **Table 4** and presented graphically in **Graph 4**.

Table 4 : Oxidative stress and antioxidant enzyme status

Parameters	Group 1		Group 2		P value
	Mean	SD	Mean	SD	
MDA	3.56	0.47	4.40	0.57	<0.0001*
SOD	3.70	0.54	3.64^{NS}	0.26	<0.0001*
GPx	284.49	27.54	195.21	30.84	<0.0001*
GRx	197.88	24.81	164.02	6.25	<0.0001*
MG	72.66	1.29	126.37	11.55	<0.0001*

Graph 4:

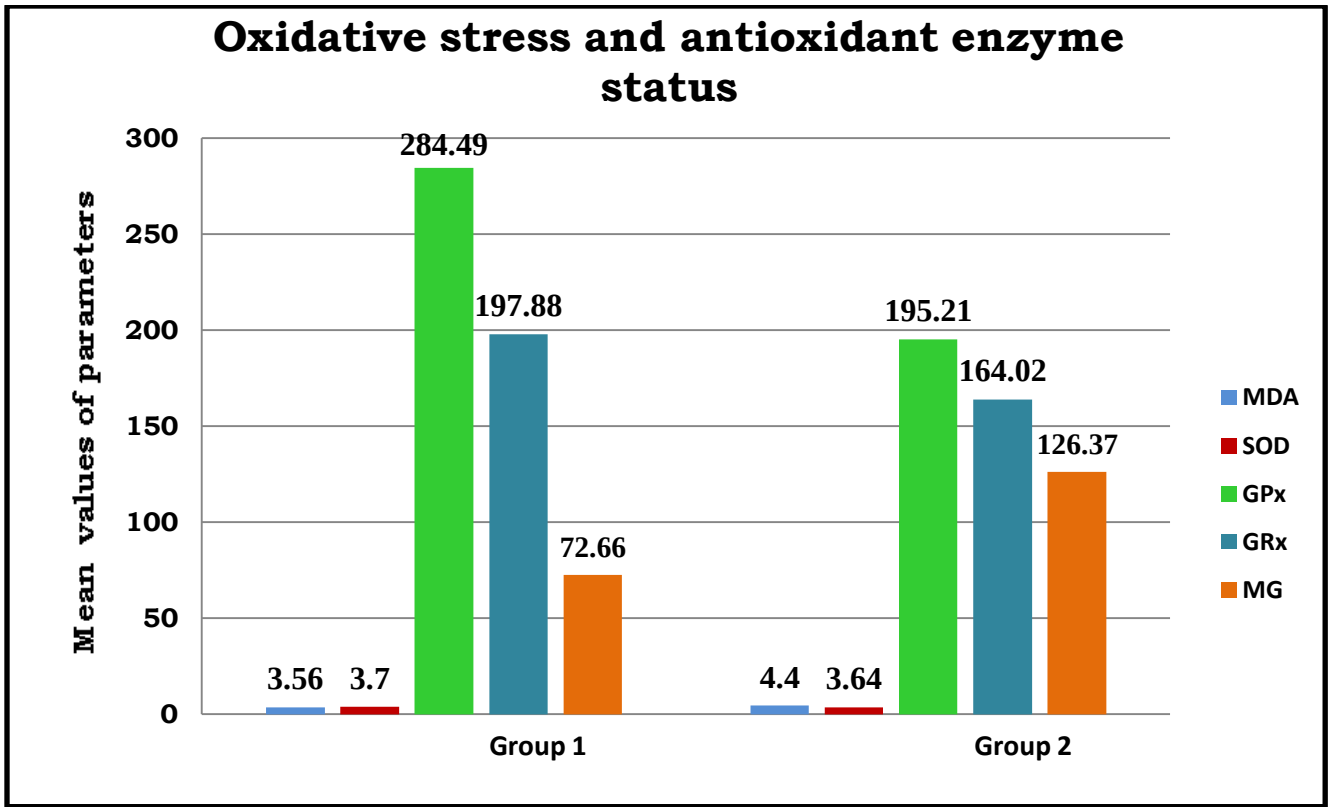


Table 4 shows that the levels of MG and MDA were significantly ($p<0.0001$) higher in Group 2 as compared to Group 1. Antioxidant enzymes including GPx and GRx were significantly lowerin Group 2 than in Group 1. There was no significant ($p=0.4211$) difference noted between these parameters in both the groups. These observations were represented in **Graph 4.**

Correlation between biochemical parameters: The correlation between all biochemical parameters used in this study for Group1and Group 2 are shown in **Table 5** and **Table 6** with respect to their 'r' and 'p' values.

Table 5: Correlation between biochemical parameters in Group 1

Parameters		Glucose (F)	Glucose (PP)	GPx	GRx	HbA1c	MDA	MG	SOD
Glucose (F)	r		0.088	-0.082	0.161	0.077	-0.176	0.049	0.14
	P		0.4859	0.5174	0.2008	0.5434	0.1605	0.701	0.267
Glucose (PP)	R	0.088		0.094	-0.163	-0.047	-0.051	0.056	0.068
	P	0.4859		0.457	0.1948	0.7087	0.6842	0.6602	0.5895
GPx	R	-0.082	0.094		-0.356	0.062	0.056	0.032	-0.081
	P	0.5174	0.457		0.0036	0.626	0.6593	0.8025	0.52
GRx	R	0.161	-0.163	-0.356		-0.13	-0.009	0.033	-0.041
	P	0.2008	0.1948	0.0036		0.3034	0.9411	0.7913	0.7484
HbA1c	R	0.077	-0.047	0.062	-0.13		0.068	-0.082	0.02
	P	0.5434	0.7087	0.626	0.3034		0.5887	0.5159	0.8753
MDA	R	-0.176	-0.051	0.056	-0.009	0.068		0.003	-0.265
	P	0.1605	0.6842	0.6593	0.9411	0.5887		0.9832	0.0033
MG	R	0.049	0.056	0.032	0.033	-0.082	0.003		0.18
	P	0.701	0.6602	0.8025	0.7913	0.5159	0.9832		0.1504
SOD	R	0.14	0.068	-0.081	-0.041	0.02	-0.265	0.18	
	P	0.267	0.5895	0.52	0.7484	0.8753	0.0033	0.1504	

Among healthy subjects,we found significant negative correlation of MDA with SOD (p=0.0033).While we do not find any other parameters were related to each other in these groups.

Table 6: Correlation between biochemical parameters in Group 2:

		Glucose (F)	Glucose (PP)	GPx	GRx	HbA1c	MDA	MG	SOD
Glucose (F)	R		0.077	0.069	0.047	-0.081	0.063	0.121	-0.028
	P		0.5445	0.5826	0.7126	0.5205	0.6203	0.3388	0.8241
Glucose (PP)	R	0.077		0.153	0.229	0.351	-0.034	0.299	0.006
	P	0.5445		0.2233	0.0661	0.0041	0.7865	0.0155	0.9639
GPx	R	0.069	0.153		-0.018	-0.031	-0.085	-0.065	0.028
	P	0.5826	0.2233		0.8872	0.8069	0.4988	0.6057	0.8261
GRx	R	0.047	0.229	-0.018		0.047	0.059	0.308	0.107
	P	0.7126	0.0661	0.8872		0.7102	0.6424	0.0124	0.3982
HbA1c	R	-0.081	0.351	-0.031	0.047		-0.068	0.143	0.092
	P	0.5205	0.0041	0.8069	0.7102		0.5927	0.2553	0.4654
MDA	R	0.063	-0.034	-0.085	0.059	-0.068		-0.06	-0.015
	P	0.6203	0.7865	0.4988	0.6424	0.5927		0.6326	0.9053
MG	R	0.121	0.299	-0.065	0.308	0.143	-0.06		-0.027
	P	0.3388	0.0155	0.6057	0.0124	0.2553	0.6326		0.83
SOD	R	-0.028	0.006	0.028	0.107	0.092	-0.015	-0.027	
	P	0.8241	0.9639	0.8261	0.3982	0.4654	0.9053	0.83	

In Group 2, significant positive association of Glucose PP with HbA1c (p=0.0041) and MG (p=0.0155) was noted. Furthermore, a significantly (p=0.0124) positive correlation was observed between MG and antioxidant enzyme GRx. Other remaining parameters were not significantly related to each other in both the groups.

ROC Curve analysis :

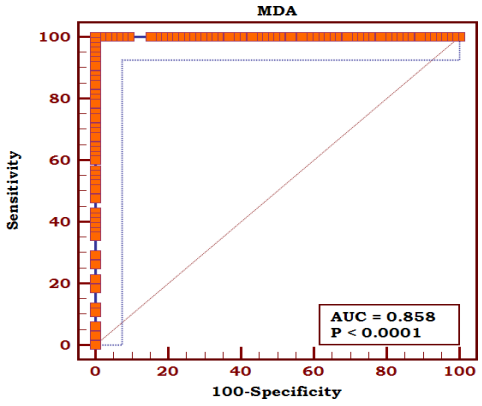
It was done for the studied parameters to find the area undercurve (AUC) and respective cut-off values in the group.

The AUC ± SE (standard error) with respective p values and cut off values in Group 2 for MDA, SOD, GPx , GRx and MG parameters are shown in **Table 7** and represented graphically with ROC curves in **Graphs: 7A,7B,7C,7D and 7E** .

Table 7 : ROC curve analysis and cut off values of parameters in diabetic patients (Group 2)

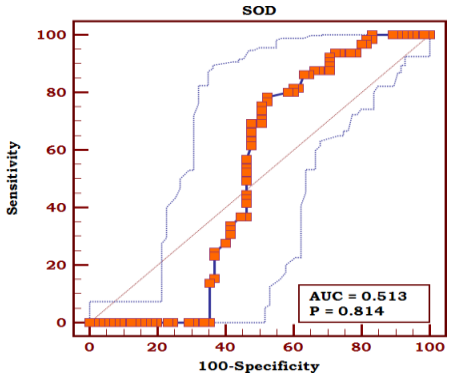
Parameters	ROC curve analysis			Cut off values				
	AUC ± SE	95% CI	P value	Cut off value	Sensitivity	95% CI	Specificity	95% CI
MDA	0.858 ± 0.0312	0.786 - 0.913	<0.0001	>4.22	60	47.1 - 72.0	95.38	87.1 - 99.0
SOD	0.513 ± 0.0558	0.424 - 0.602	0.814	>4	00	0.0 - 5.5	64.62	51.8 - 76.1
GPx	0.982 ± 0.0084	0.942 - 0.997	<0.0001	≤244.3	98.46	91.7 to 100	92.31	83 to 97.5
GRx	0.943 ± 0.0228	0.888 - 0.976	<0.0001	≤173.5	96.92	89.3 - 99.6	86.15	75.3 - 93.5
MG	0.999 ± 0.000	0.972 - 1.000	<0.0001	>74.8	100	94.5 - 100.0	100	94.5 - 100.0

Graph 7A: ROC curve of MDA in Group 2



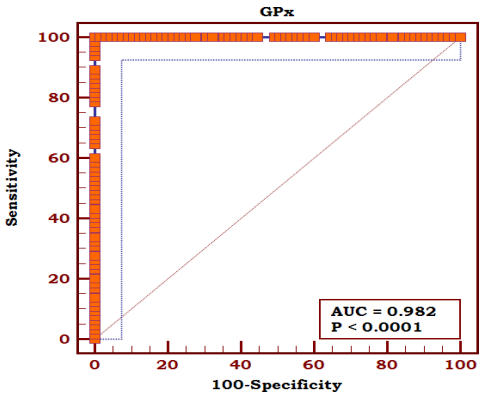
Sensitivity: 60
Specificity: 95.38
Cut-off value: > 4.22

Graph 7B: ROC curve of SOD in Group 2



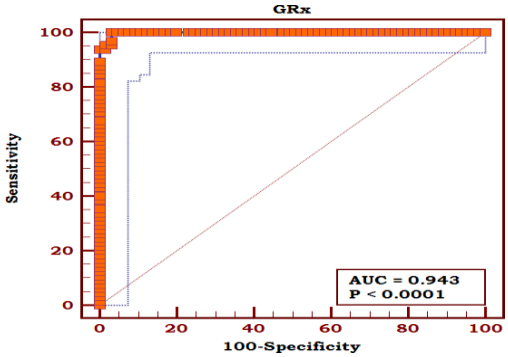
Sensitivity: 00
Specificity: 64.62
Cut off value: >4

Graph 7C : ROC curve of GPx in Group 2



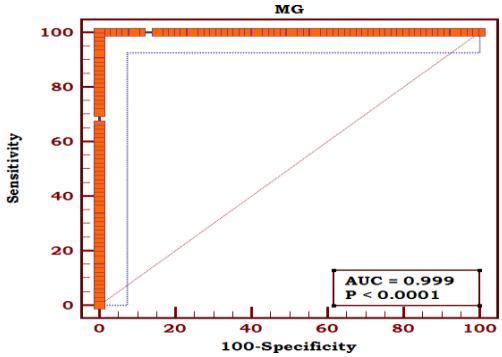
Sensitivity: 98.46
Specificity: 64.62
Cut off value: ≤ 244.3

Graph 7D: ROC curve of GRx in Group 2



Sensitivity: 96.92
Specificity: 86.15
Cut off value: ≤ 173.5

Graph 7E:ROC curve of MG in Group 2



Sensitivity: 100.00
Specificity: 100.00
Cut off value: > 74.8

Overall, the results suggest that there are complex relationships between various biochemical parameters in diabetic group (Group 2).

DISCUSSION

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin or the body cannot effectively use that insulin. MG is a strong reactive metabolite which is derived from the metabolism of glucose and fructose and its increased levels are found to play an important role in the development of DM and its cardiovascular complications.[9] Uncontrolled DM leads to increased production of ROS which affects the biomolecules such as nucleic acids, lipids , proteins etc .leading to cell damage in DM. Multimodal mechanisms leading to DM shows strong association [10]

Oxidative stress results from an imbalance between radical-generating and radical scavenging systems, i.e. increased free radical production or reduced activity of antioxidant defenses or both. [11]

The necessity of this study is to determine the relationship between MG and oxidative stress in terms blood levels of different parameters of diabetes and oxidative stress involved in DM. Diabetes is a progressive metabolic disorder ,hence, study of metabolites and their role in prediction and progression of DM should be monitored and metabolic flux should be studied using various novel viable biomarkers candidates such as Methylglyoxal, AGEs and RAGE and this knowledge can be exploited to study their role in diabetes.

This study was done to evaluate the relationship between methylglyoxal and oxidative stress in diabetes.

In diabetic group, (N=65), 40 were males and 25 were females and in healthy control group (N=65), 36 were males and 29 were females. There was no significant difference in the means of ages of study groups. Also there was no significant difference in gender distribution between the study groups.

The levels of fasting and postprandial glucose and HbAlc was significantly higher ($P<0.0001$) among diabetic patients as compared to healthy control group.

Nelli et al also found higher levels of HbAlc in diabetic patients and they stated that, it was due to excessive glycosylation of hemoglobin .We also agree with these results.

The levels of MG and MDA were significantly higher among diabetic patients ($P<0.0001$) compared to healthy control group in this study. Additionally, the antioxidant enzymes GPx

and GRy ,were significantly lower (P<0.0001) in diabetic patients than healthy control group. There was no statistically significant decrease (P=0.42111) in levels of SOD as compared to healthy control group. In our study, we observed a significant association between various biochemical parameters related to glucose metabolism, oxidative stress and antioxidant enzyme activity in diabetic patients. We found a significant positive correlation between postprandial glucose (PP glucose) levels and with glycated hemoglobin (P=0.0041) and methylglyoxal (P=0.0155).

Reactive oxygen species (ROS) are produced constantly inside the cells as a consequence of nutrient catabolism. The balance between ROS production and its elimination maintains the cell redox homeostasis and biological functions, avoiding the occurrence of oxidative distress which is responsible for irreversible oxidative damage in DM. A fundamental players in the maintenance of this fine balance is reduced glutathione (GSH) required for the scavenging of ROS and methylglyoxal (MG). MG is a cytotoxic compound, formed constitutively as byproduct of nutrient catabolism, particularly glycolysis and detoxified in a GSH-dependent manner by glyoxalase pathway.[12]

Production of ROS overcomes the antioxidant fortifications of the cells. This occurs due to the rich production of oxidants or reduction of antioxidant fortification of the cells .As a result,several cellular constituents can be injured. The crucial cellular marks are DNA, lipids and proteins. Nelli et al found a significant reduction in the levels of antioxidant enzymes CAT, GPx and SOD in Type 2 DM, which is similar to this study.[10]

Studies of Waggiallah H et al observed increased oxidative stress in diabetes patients which is in parallel with our study. [13] They also noted highly significant levels of GSH, GPO and fasting blood glucose and HbA1c in diabetic patients in comparison with healthy controls which is similar to our findings in this study.

De Mattia et al mentioned in their study that, reduction of these antioxidant enzyme in Type 2 DM is due to decrease in GSH concentration which leads to an increase in oxidative stress. However, it is important to note that elevation of blood glucose level generates oxidative stress, which could contribute to increase in glutathione utilization.[14]

Thornalley PJ et al found the increased level of MG and decreased levels of GRx. Glyoxal and Methylglyoxal, as they are detoxified by the glyoxalase system with reduced glutathione as co-factor. The concentration of reduced glutathione may be decreased by oxidative stress and by decrease in situ glutathione reductase (GRx) activity in DM. A reduced concentration of

reduced glutathione may predispose diabetic patients to oxidative damage and to **α-**oxaldehyde-medaited glycation, by decreasing in situ glyoxalase-I activity. These findings are also similar to our study. **[15]**

Zhaoliang Fei et al in their study observed the decreased levels of SOD in diabetic patients as compared to healthy controls which is consistant with our study. SOD could connect the imbalance between ROSs and antioxidant scavengers and downstream effects. Further, SOD can also improve hyperglycemia induced oxidative stress, apoptosis and inflammation. **[16]**

In the study of Fumiaki Kimura et al, serum concentrations of free radical scavenging enzymes, EC-SOD are found to be significantly higher in patients with Type 2 DM as compared with healthy control subjects. They stated that, it is probably due to the combined effect of several factors. Evidence suggests that reduced tissue binding of EC-SOD may be a major cause of this increase. **[17]**

Kumavat et al showed that, increased oxidative stress , associated with alteration in antioxidant enzyme activities and increased lipid peroxidation (MDA levels) in Type 2 DM. These findings are aiso consistent to this study. **[18]**

Morau et al reported that , patients with Type 2 DM, has elevated levels of MG. Elevated MG has a major role in cardiovascular complications. Recent clinical studies, however found that Type 2 DM patients who were treated to maintain glycemia below the diabetes definition threshold (HbAlc <6.5%), still develop diabetic complications. This suggests that, the additional insulin and glucose-independent mechanisms could be involved in disease initiation as well as its progression.**[19]**

Sakamoto et al reported that in DM, there is increased oxidative stress and intracellular metabolic disorders owing to insulin resistance, increased blood glucose level and chronic inflammation. **[20]**

Blum J et al found about 50% of SOD in electrolytes of diabetic patients is glycated , resulting in low activity of SOD, similar to this study. The decrease in SOD activity may lead to an increase in the level of superoxide radicals which will cause the inactivation of GPX. **[21]**

Attman PO et al stated that, HbAlc can provide an accurate and reliable method to routinely assess the relative level of diabetes control. This finding was supported by an evidence based study which showed a graded relationship between HbAlc and the risk of nephropathy in Type 2 DM. Lipoprotein abnormalities are more pronounced in patients with high HbAlc levels.**[22]**

Grover J.K et al observed that, diabetic patients had much higher glucose levels and decreased insulin level when compared with normoglycaemic subjects. The increase in blood glucose level and decreased insulin level depends upon the degree of β – cell destruction. We also found the similar results in this study. **[23]**

Moussa S.A. et al mentioned the significant increase in the levels of HbA1c and fructosamine with a concomitant decrease in the level of plasma insulin in the diabetic patients as compared to normoglycemic subjects, in their study. They also found significantly higher MDA levels in diabetic patients as compared to controls. These findings are also similar to this study. They found higher enzymatic activities of GPx and GRx in diabetics than in healthy subjects but the difference was not significant, These findings are not consistent to our findings which shows significant decrease in the levels of antioxidant enzymes GPx and GRy in diabetic patients as compared to healthy controls. **[24]**

Hence , the present study is conducted to evaluate the relationship between MG and oxidative stress in Type 2 DM. This can help in understanding the critical relationship between highly specific biomarker i.e. MG of oxidative stress and DM that can lead to early detection of DM. Furthermore, this study may help in determination of other important oxidative stress biomarkers and the status of antioxidant enzymes in DM that can provide valuable insights into the pathogenesis of future diabetic complications and also can help in development of effective therapeutic strategies.

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CONCLUSION

The necessity of this study is to evaluate the relationship between methylglyoxal and oxidative stress involved in diabetes mellitus. Increased levels of fasting, postprandial and glycated hemoglobin and oxidative stress biomarkers such as methylglyoxal and malondialdehyde, along with decreased levels of major antioxidant enzymes like superoxide dismutase, glutathione peroxidase and glutathione reductase, indicates strong correlation between these biomarkers and DM. This study proves the importance of methylglyoxal in Type 2 DM. Antioxidant status is proportionately declined along with inclined levels of methylglyoxal in diabetic group highlighting the inverse relationship between them. Based on these observations, it can be concluded that, free radicals (ROS) mediated cellular damage in diabetic patients is correlated with methylglyoxal levels irrespective of their hyperglycemic status. Further, estimation of serum methylglyoxal level as a biomarker may give valuable guidelines to understand the prognosis of diabetes and pathophysiology of its complications. Further studies are required to prove the role of these biomarkers in diabetic complications and to evaluate its clinical application for greater therapeutic benefits. Therefore, we have planned extended studies to explore this correlation further in patients with diabetic complications.

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