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The Role of Serum Methylglyoxal and Glyoxalase 1 on Diabetic Peripheral Neuropathy

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ABSTRACT

Diabetic neuropathy (DN) is a characteristic microvascular complication linked to diabetes. It affects about 50% of those who have this ailment. It has been revealed that Advanced Glycation End Products (AGEs) play a role in the development of diabetic neuropathy. Patients diagnosed with type 2 diabetes (T2DM) have higher quantities of methylglyoxal (MG), particularly harmful precursor of AGEs. One of the numerous defensive mechanisms responsible for MG metabolism and prevention of the generation of AGEs is the glyoxalase system. The cytotoxic byproduct methylglyoxal (MG) is removed by the detoxifying enzyme glyoxalase 1 (GLO1) via converting it to D-lactate, which is not harmful to tissues. This study intends to unveil the role of serum methylglyoxal and glyoxalase 1 on the onset of diabetic peripheral neuropathy (DPN). The present research was designed as a case-control study; including 160 individuals (males and females) aged (30-60) years. The subjects were divided into three groups: group one includes 40 type 2 diabetic patients with peripheral neuropathy, group two includes 40 type 2 diabetic patients without peripheral neuropathy and group three includes 80 apparently healthy as the control. Blood specimens were collected from all patients conducted at the National Diabetes Center, Mustansiriyah University in Baghdad, Iraq. Serum was used to measure glyoxalase 1 and Methylglyoxal levels using Enzyme-Linked Immunosorbent Assay (ELISA) technique. Whole blood with ethylene diamine tetraacetic acid (EDTA) was employed to measure glycated hemoglobin (HbA1c) levels with standard biochemical technique (the CLOVER system). Anthropometric measurements, such as height, weight, BMI and duration of diabetes were assessed. Individuals in control group showed higher serum level of glyoxalase 1 (26.4±4.06 ng/ml) than either patients with DPN (13.61±2.22 ng/ml) or patients with T2DM without peripheral neuropathy (15.25±1.68 ng/ml) with highly significant differences. In contrast, patients with DPN demonstrated higher level of methylglyoxal (29.45±6.06 ng/ ml) than either patients with T2DM without peripheral neuropathy (23.76±5.66 ng/ml) or controls (14.04±4.46 ng/ml) with highly significant differences. In the context of Discrimination between Diabetic Peripheral Neuropathy and Diabetes Mellitus without Peripheral Neuropathy, glyoxalase 1 has an area under curve (AUC) of 0.776, 95% confidence interval [CI] been 0.671-0.882. The test's sensitivity and specificity were 73% and 72%, respectively, at the cut-off value of serum glyoxalase 1 = 13.94 ng/ml. The area under curve for methylglyoxal was 0.758, 95% confidence interval was 0.654-0.863, p < 0.001. The test's sensitivity and specificity were 63% and 70%, respectively; at a cut-off value of serum methylglyoxal = 27.57ng/ml. In the context of discrimination between DPN and controls, the area under curve for glyoxalase 1 was 0.997, 95% confidence interval was 0.992-1.0, p<0.001. Sensitivity and specificity were 99% and 97%, respectively, at a cut-off value of serum glyoxalase 1 = 19.45 ng/ml. The area under curve for methylglyoxal was 0.977, 95% confidence interval was 0.965-0.997, p<0.001. Sensitivity and specificity were 93% and 90%, respectively, at a cut-off value of serum methylglyoxal = 20.81 ng/ml. The findings demonstrated that decreased glyoxalase 1 levels and elevated serum methylglyoxal levels are contributing factors to the development of diabetic peripheral neuropathy in the group of patients under study.

INTRODUCTION

Diabetic neuropathy (DN) is a prominent microvascular consequence associated with diabetes, and persistent hyperglycemia in diabetic patients is known to increase the risk of developing diabetic microvascular problems (Gouveri and Papanas 2022). It is particularly important to note that DN is the main contributor to the development of diabetic foot ulcers, which can lead to lower-extremity amputations (Bönhof, Herder, *et al.,* 2019). Because type 2 diabetes hyperglycemia causes glucose toxicity by increasing the flow of glucose through the glycolytic pathway, excessive glycolysis leads to an accumulation of dihydroxyacetone phosphate because of inadequate triose phosphate isomerase activity and an increase in the synthesis of methylglyoxal (MG) (Perrone, Giovino, *et al.,* 2020). MG is a highly reactive substance that is mostly created during glycolysis and lipid peroxidation and it is a key precursor in the creation of advanced glycation end products (AGEs) (Maessen, Stehouwer, *et al.,* 2015; Schalkwijk and Stehouwer 2020). AGEs are heterogeneous compounds created via the non-enzymatic glycation of proteins, lipids, or nucleic acids during hyperglycemia. The accumulation of AGEs in peripheral nerves has recently been suggested to be an additional risk factor for the development of diabetic neuropathy (DN). This buildup of AGEs in peripheral nerves raises the reactive oxygen species (ROS), which in turn triggers neural inflammation and impairs axonal transport (Papachristou, Pafili, *et al.,* 2021). The detoxification of methylglyoxal, as well as other endogenous hazardous metabolites into innocuous compounds, is accomplished by a widely expressed and well-preserved glyoxalase enzyme network, such as D-lactate (He, Zhou, *et al.,* 2021; Morgenstern, Katz, *et al.,* 2020; Rodrigues, Borges, *et al.,* 2020). This system, which utilizes reduced glutathione (GSH) composed of the two key enzymes glyoxalase 1 and glyoxalase 2, is active in all mammalian cells' cytoplasm (Sousa Silva, Gomes, *et al.,* 2013; Antognelli,

Ferri, *et al.,* 2017). Hemithioacetal, the main substrate for GLO1, is created in the first stage by the combination of reduced glutathione (GSH) with MG. Hemithioacetal is transformed into S-D-lactoylglutathione by GLO1. The hydrolysis of S-D-lactoylglutathione is catalyzed by glyoxalase 2 (GLO2) in the following step, creating D-lactate and recycling GSH. The rate-limiting stage for the detoxification of MG is the GLO1-reaction (Rabbani and Thornalley 2014). Although various investigations in diabetic human subjects and animal models have demonstrated that glycation has a role in diabetic neuropathy, there haven't been any reports among Iraqi citizens that highlight the significance of MG and glyoxalase 1 in diabetic peripheral neuropathy. The purpose of this study was to assess how the imbalance in the serum MG-GLO1 levels contributes to the development of diabetic peripheral neuropathy (DPN).

MATERIALS AND METHODS

A case-control study was carried out in the National Diabetes Center at Mustansiriyah University in Baghdad, Iraq, from April 2022 to November 2022. 160 participants within the ages of 30 to 60 years were included in this study. The participants were divided into three groups: group one includes 40 type 2 diabetics with peripheral neuropathy, group two includes 40 type 2 diabetics without neuropathy and group three includes 80 apparently healthy controls Every patient's medical history, including their gender, age, duration of their diabetes, BMI, and any prior histories of other diseases, was noted. The Toronto Clinical Neuropathy Scoring System (TCSS) was used to evaluate peripheral neuropathy. It consists of three parts: the symptoms, the sensory test, and the reflex scores. The maximum number of points is 19. Peripheral neuropathy is identified as having a score $\geq$ 6 (Bril, Tomioka, *et al.,* 2009). Both diabetes patient groups with and without peripheral neuropathy underwent clinical examinations and biochemical testing, such as HbA1c measurements. Body Mass Index was calculated by using the

(weight in kilograms/height in square meter) equation (Nuttall 2019). Five milliliters of blood were taken from the participants for biochemical analysis, and two milliliters of that blood were put into an EDTA tube to measure glycated hemoglobin (HbA1c). Blood HbA1c levels were measured by the CLOVER A1c system (Ibrahim, Baban *et al.,* 2019). Serum methylglyoxal and glyoxalase 1 levels were estimated by using ELISA technique (sandwich method).

Exclusion Criteria:

The study excluded patients or control who taking medications that cause peripheral neuropathy as well as smoking individuals and alcoholism.

Statistical Analysis:

SPSS software version 25.0 was used to perform the statistics. The quantitative description was summarized using mean and standard deviation, and three groups were compared using analysis of variance. Categorical variables, which were presented as numbers and percentages, were evaluated using the chi-square test. Serum glyoxalase 1 and methylglyoxal were evaluated utilizing the Receiver Operating Characteristic Curve (ROC) in the context of discriminating between diabetic patients, diabetics with peripheral neuropathy, and healthy controls. A statistically significant difference was determined to exist when the p-value was less than 0.05.

RESULTS

Patients in the DPN group had a mean age of 49.1 ± 5.44 years, which did not significantly differ from diabetic patients' mean ages of 48.27 ± 7.2 years. However, the two groups differed significantly from controls (42.06 ± 6.21 years). Although the female ratio was higher in the DPN group (80%) than in either the T2DM group (60%) or controls (62.5%), the differences were not significant. Similar to age, the BMI did not differ significantly between diabetic patients with and without peripheral neuropathy; however, both groups had significantly higher BMI than controls ($p< 0.001$).

As a marker for diabetic control, HbA1c was higher in patients with DPN (10.56 ± 2.64%) than diabetic patients (8.79 ± 2.53%) which in turn higher than controls (4.77 ± 0.38%) with a high significance. The median duration of T2DM in patients with and without DPN was 7.0 years and 4.0 years, respectively with a highly significant difference (Table 1).

Table 1: Demographic characteristics of the population under study.

Variable	Diabetic peripheral neuropathy (n=40)	Diabetes mellitus without peripheral neuropathy (n=40)	Controls (n=80)	p-value
Age, years				
Mean±SD	49.1±5.44[a]	48.27±7.2[a]	42.06±6.21[b]	**<0.001**
Range	39-60	30-60	33-57	
Gender				
Males	8(20%)	16(40%)	30(37.5%)	0.101
Females	32(80%)	24(60%)	50(62.5%)	
BMI, Kg/m²				
Mean±SD	31.66±4.9[a]	30.59±3.5[a]	26.8±2.85[b]	**<0.001**
Range	23.65-44.5	22.3-35.5	20.5-32.5	
HbA1c				
Mean±SD	10.56±2.64[a]	8.79±2.53[b]	4.77±0.38[c]	**<0.001**
Range	7.0-15.6	6.1-15.5	4.0-5.8	
Disease duration, y				
Mean±SD	8.26±4.69	5.25±4.38		
Median	7.0	4.0	--------------	**0.001**
Range	1-18	1-22		

Different small letters indicate significant differences, BMI: Body mass index, S.D: Standard deviation.

Individuals in control group showed higher serum level of glyoxalase 1 (26.4±4.06 ng/ml) than in either the patients with DPN (13.61±2.22 ng/ml) or patients with T2DM (15.25±1.68 ng/ml) with highly significant differences (Fig. 1). In contrast, patients with DPN demonstrated higher levels of methylglyoxal (29.45±6.06 ng/ ml) than either patient with T2DM (23.76±5.66 ng/ml) or controls (14.04±4.46 ng/ml) with highly significant differences (Fig. 2 and Table 2).
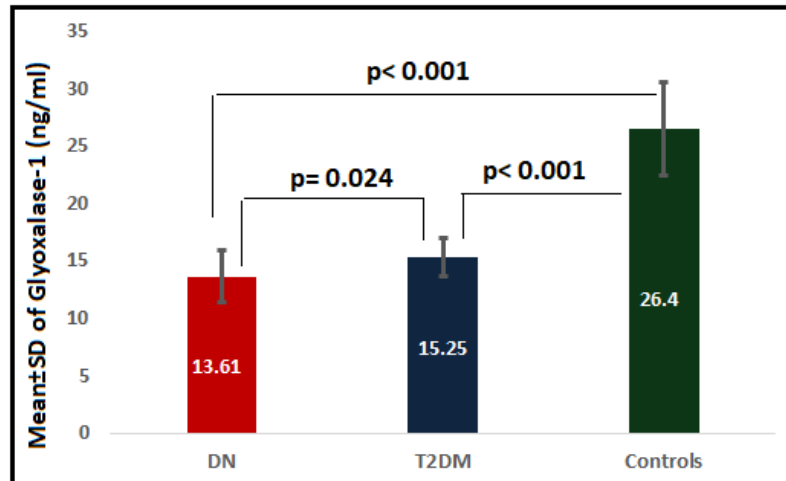

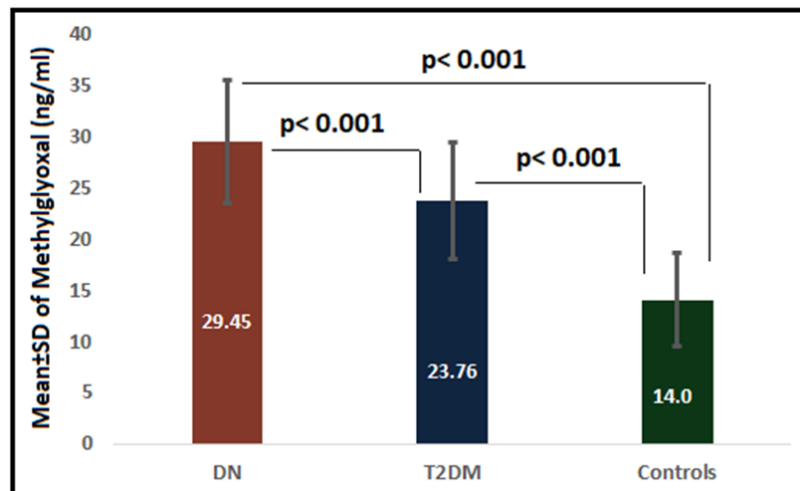**Fig. 1:** Mean serum levels of glyoxalase 1 in DN, T2DM and controls.

Fig. 2: Mean serum levels of methylglyoxal in DN, T2 DM and control.

Table 2: Serum levels of glyoxalase 1, methylglyoxal in different groups.

Variable	DPN (n=40)	T2DM without neuropathy (n=40)	Controls (n=80)	p-value
Glyoxalase 1, ng/ml				
Mean±SD	13.61±2.22[a]	15.25±1.68[b]	26.4±4.06[c]	<0.001
Range	10.58-19.8	12.91-18.08	19.13-43.28	
Methylglyoxal, ng/ml				
Mean±SD	29.45±6.06[a]	23.76±5.66[b]	14.04±4.46[c]	<0.001
Range	19.97-37.98	16.21-33.83	6.6-23.5	

Different small letters indicate significant differences.

Glyoxalase 1, and methylglyoxal were evaluated for their diagnostic value using a Receiver Operating Characteristic (ROC) curve.

In the context of Discrimination between Diabetic Peripheral Neuropathy and Diabetes Mellitus without Peripheral Neuropathy, glyoxalase 1 has an area under curve of 0.776, 95% confidence interval was 0.671-0.882. Sensitivity and specificity of the test at a cut-off value of serum glyoxalase 1 = 13.94 ng/ml were 73% and 72%, respectively (Fig. 3).
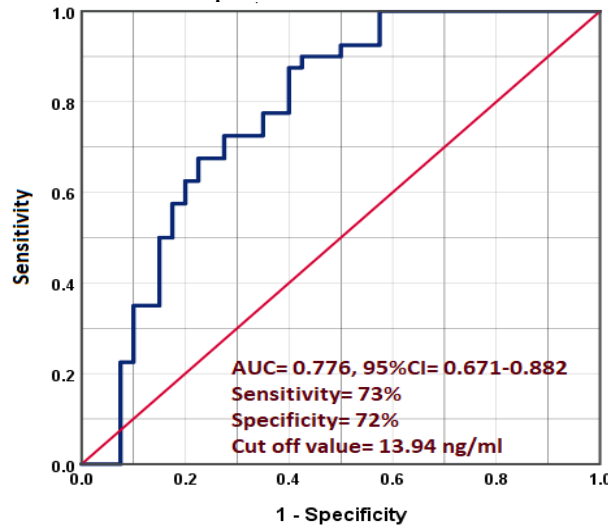

AUC= 0.776, 95%CI= 0.671-0.882
Sensitivity= 73%
Specificity= 72%
Cut off value= 13.94 ng/ml

Fig. 3: Receiver operating characteristic curve for glyoxalase 1 in the context of discrimination between diabetic peripheral neuropathy and diabetes mellitus without peripheral neuropathy.

The area under curve for methylglyoxal was 0.758, 95% confidence interval was 0.654-0.863, $p < 0.001$. Sensitivity and specificity were 63% and 70%, respectively; at a cut-off value of serum methylglyoxal = 27.57ng/ml (Fig. 4 and Table 3)
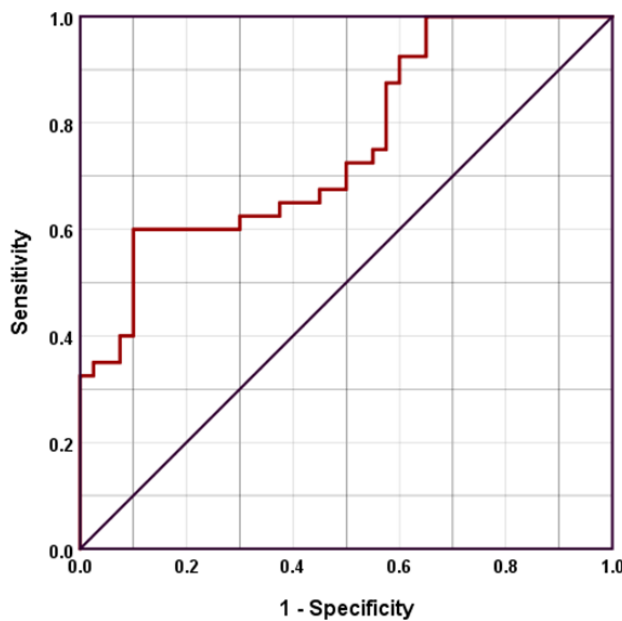

Fig. 4: Receiver operating characteristic curve for methylglyoxal in the context of discrimination between diabetic peripheral neuropathy and diabetes mellitus without peripheral neuropathy.

Table 3: Diagnostic value of glyoxalase 1, methylglyoxal in the context of discrimination between diabetic peripheral neuropathy and diabetes mellitus without peripheral neuropathy.

Markers	AUC	Sensitivity	Specificity	Cut off value
Glyoxalase 1	0.776	73%	72%	13.94 ng/ml
Methylglyoxal	0.758	63%	70%	25.57 ng/ml

In the context of discrimination between DPN and controls, the area under curve for glyoxalase 1 was 0.997, 95% confidence interval was 0.992-1.0, $p<0.001$. Sensitivity and specificity were 99% and 97%, respectively, at a cut-off value of serum glyoxalase 1 = 19.45 ng/ml (Fig. 5).
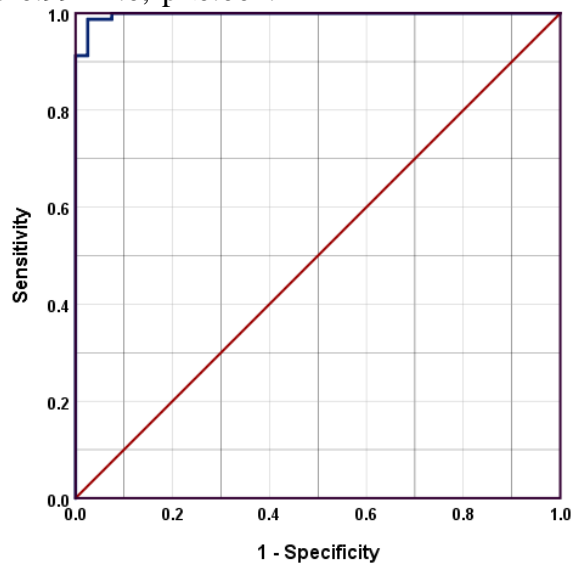

Fig. 5: Receiver operating characteristic curve for glyoxalase 1 in the context of discrimination between diabetic peripheral neuropathy and controls

The area under curve for methylglyoxal was 0.977, 95% confidence interval was 0.965-0.997, $p<0.001$. Sensitivity and specificity were 93% and 90%, respectively, at a cut-off value of serum methylglyoxal = 20.81 ng/ml.
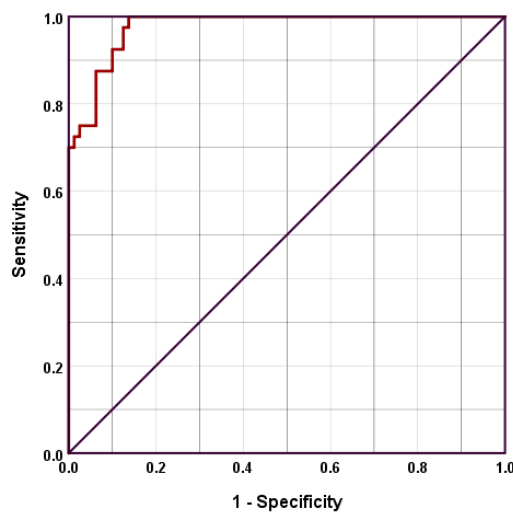

Fig. 6: Receiver operating characteristic curve for methylglyoxal in the context of discrimination between diabetic peripheral neuropathy and controls.

Table 4: Diagnostic value of glyoxalase 1 and methylglyoxal in the context of discrimination between diabetic peripheral neuropathy and control.

Markers	AUC	Sensitivity	Specificity	Cut off value
Glyoxalase 1	0.997	99%	97%	19.45 ng/ml
Methylglyoxal	0.977	93%	90%	20.81 ng/ml

DISCUSSION

The most popular microvascular consequence of diabetes mellitus is diabetic peripheral neuropathy (Román-Pintos, Villegas-Rivera, *et al.,* 2016). In the pathophysiology of type 2 diabetes and the vascular consequences of diabetes, hyperglycemia has been linked to the synthesis and buildup of methylglyoxal (MG), a highly reactive bicarbonyl molecule. MG is a key precursor of DNA and protein glycation that does not require enzymes, which causes the development of advanced glycation end products (AGEs) (Schalkwijk and Stehouwer 2020). Recent researches have highlighted the significance of AGEs and their precursors, MG, in the development of diabetic problems in both animal models of diabetes and diabetic patients (Brings, Fleming *et al.,* 2017; Groener, Oikonomou, *et al.,* 2019). The glyoxalase system is required for advanced glycation end products (AGEs) detoxification (Aragonès, Rowan, *et al.,* 2021).

Anthropometric Measurements and Clinical Characteristics of Study Groups:

In the current study, there are substantial differences in the age and BMI between the two categories—DPN, T2DM, and control. The average age of the patients in the DPN group was not statistically different from that of the diabetic patients, but the two groups differed significantly from controls. Although the female ratio was higher in the DPN group (80%) than in the T2DM group (60%) or controls (62.5%), the differences were statistically insignificant. Similar to age, the BMI of diabetic patients with and without DPN did not differ substantially; nevertheless, both groups had considerably higher BMIs than controls (p<0.001). Zhou R *et al.,* found that type 2 diabetes mellitus patients who were both general and abdominally obese had a higher probability of developing an incident DN, regardless of sex (Zhou, Li *et al.,* 2021). As a marker for diabetic management, the present study revealed that HbA1c was higher in patients with DPN than diabetic patients without neuropathy which in turn was higher than healthy controls with highly significant differences. This result is in agreement with the results obtained by Hunaif I *et al.,* who found a relationship between the HbA1c level and the degree of diabetic neuropathy in Type 2 DM (Hunaifi, Agustriadi, *et al.,* 2021). On the other hand, the present study showed that the median duration of T2DM in patients with and without DPN was 7.0 years and 4.0 years, respectively with a highly significant difference. The current study is consistent with recent work done by Jaya Kumar, who demonstrated that having diabetes for a longer period of time and having a higher HbA1c level significantly increased the risk of neuropathy (Jaya Kumar and Ponnu 2022).

Serum Levels of Glyoxalase 1 and Methylglyoxal in the Study Groups:

The human glyoxalase system, which is situated in the cytoplasm or nucleus, has the responsibility for detoxifying MG, a highly reactive bicarbonyl molecule created during the metabolism of carbohydrates, lipids, and proteins, but mostly during the metabolism of carbohydrate (He, Y.,*et al.,* 2021 and Rodrigues, T., *et al.,* 2020). Excessive MG formation exacerbates oxidative stress by causing mitochondrial malfunction and the generation of reactive oxygen species (Rabbani, Xue, *et al.,* 2016). It is expected that methylglyoxal synthesis and accumulation will be higher in neuronal tissue because glucose is this tissue's main energy source (Groener, Oikonomou, *et al.,* 2019). In the present study, Individuals in control group showed higher serum level of glyoxalase 1 than either diabetic patient with or without Peripheral neuropathy. In contrast, patients

with DPN demonstrated higher level of methylglyoxal than patients with T2DM which in turn was higher than controls with highly significant differences. In patients with DPN, this result showed a negative significant correlation between glyoxalase 1 and methylglyoxal. Previous studies revealed that diabetes has been linked to decreased GLO1 activity and expression (Maessen, Stehouwer, *et al.,* 2015; Rabbani and Thornalley 2019). The most recent study showed that methylglyoxal (MG), an AGEs precursor, and advanced glycation end products (AGEs) were significant factors in establishing the connection between diabetes and vascular injury (Schalkwijk, Micali, *et al.,* 2023). The present study is in agreement with other study done by Hamoudane M et al. which found an association between decreased blood concentration of glyoxalase I and diabetic consequences (Hamoudane, Amakran *et al.,* 2015). Also, the current study agreed partially with the most recent study established by Harkin C *et al.,* which demonstrated that patients with T2DM had considerably higher levels of methylglyoxal and glyoxal in their urine and serum than the controls (Harkin, Cobice, *et al.,* 2023). Other previous studies using animal models showed that diabetic mice with lower levels of GLO1 in the peripheral nerve tissue developed neuropathy, and they discovered that lower levels of GLO1 also caused similar increases in plasma MG concentrations (Jack and Wright 2012). Additionally, the current study is in agreement with the ADDITION-Denmark study that showed a higher level of methylglyoxal was identified as a risk factor for incident DSPN (Andersen, Witte, *et al.,* 2018). In contrast, the present study showed disagreements with other study done by Hansen *et al., which* found no association between serum methylglyoxal and DSPN in type 2 diabetic patients with the long-term disease (Hansen, Jensen *et al.,* 2015).

The clinical importance of MG and the glyoxalase system in health and disease is currently better understood than it was in 1994. Early developments toward GLO1-based therapeutics have shown promise.

Conclusion

It is evident that MG buildup impairs microvascular function by triggering neurodegenerative disorders and diabetic microvascular consequences. The strategy that maintains the MG/GLO1 balance is essential to ensuring the containment of MG levels beneath the hazardous threshold. The present state of knowledge about MG buildup along with decreased GLO1 levels in diabetes, as well as how both of these variables affect the development of diabetic peripheral neuropathy, is the primary purpose of the study.

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