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Decreased Erythrocyte Glyoxalase 1 (GLO1) Activity in Patients with Diabetes with Reduced Estimated Glomerular Filtration Rate

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Abstract

Background: The glyoxalase enzymes are located in the cytosol of all cells, including erythrocytes, and prevent Advanced Glycation End products (AGEs) production through the detoxification of the Methylglyoxal (MGO). The present study was made to evaluate the GLO1 activity in diabetic patients and it relationship with estimated Glomerular Filtration Rate (eGFR).

Patients and Methods: GLO1 activity was measured spectrophotometrically in erythrocytes of 123 participants: 35 healthy subjects and 88 patients with diabetes. Biochemical parameters were measured and eGFR was calculated using the MDRD (Modification of Diet in Renal Disease) formula.

Results: We found no difference in GLO1 activity in patients with diabetes compared to healthy subjects. However GLO1 activity tended to be reduced in diabetic patients with loss renal function. A significant decrease was shown in patients with moderate to severe loss renal function. GLO1 activity was correlated with eGFR, creatinine and urea. Multivariate analysis showed that GLO1 activity was independently associated with eGFR.

Conclusion: GLO1 activity was related with loss renal function in patients with diabetes according glomerular filtration rate.

Keywords: Glyoxalase 1; Diabetes mellitus; Glomerular filtration rate

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Abbreviations

AGEs: Advanced Glycation End Products; BMI: Body Mass Index; DM: Diabetes Mellitus; GLO1: Glyoxalase Enzyme; HTA: Hypertension; MGO: Methylglyoxal; GFR: Glomerular Filtration Rate

Introduction

Diabetes is the most important disease in the wild words including Type 1 diabetes, Type 2 diabetes as known as Diabetes Mellitus (DM), and gestational diabetes [1-3]. DM is defined by chronic hyperglycemia and affected sugars metabolism caused by impaired insulin secretion [4]. Overweight and obesity are two risk factors or metabolic syndrome for developing DM. Indeed, obesity is characterized by excess body fat which is harmful to health, thus generating significant oxidative stress than chronic inflammation [5]. DM, as chronic hyperglycemia, promotes protein glycation and leads to the formation of Advanced Glycation End products (AGEs).

AGEs are formed by prolonged duration of hyperglycemia in diabetics and they have long-term toxicity in the body. Indeed, AGEs come from the attachment of sugar to a protein, an amino acid, or a lipid. These toxic products accumulate in all the organs leading to the activation of its RAGE receptors. A high number of publications have reported the AGEs involvement in the development of diabetes complications such as nephropathy, retinopathy, and atherosclerosis [6-8]. These products are not only present, but they also contribute to the severity of the pathology [9,10]. The pathophysiological mechanisms of the increase in these products are still unidentified, but the

formation of these products is done through the precursors of AGEs, also known as highly reactive dicarbonyl stress, the α-oxoaldehydes, such as the Methylglyoxal (MGO) has a key role in detrimental effects on cellular function and has a key factor in vascular complications leading to oxidative stress. MGO is metabolized to lactate or acetol [11]. The MGO was detoxified by the glyoxalase system [12]. The glyoxalase system has two enzymes, glyoxalase 1 (EC 4.4.1.5, S-D-lactoylglutathione lyase; GLO1) and glyoxalase 2 (EC 3.1.2.6, D-hydroxyacylglutathione hydrolase; GLO2) [12]. Reduced glutathione is an essential cofactor. GLO1 catalyzes the conversion of the hemithioacetal to the thioester S-D-lactoylglutathione. The GLO2 enzyme catalyzes the hydrolysis of S-D-lactoylglutathione to form the lactate. Reduced glutathione is important for the detoxification of reactive dicarbonyls, especially methylglyoxal [13]. Therefore, we aimed to go deeper in the relation between renal function impairment and the MGO system in patients with type 2 diabetes. So, we measured the enzyme activity of glyoxalase 1 in patients with diabetes according their renal function using estimated glomerular filtration rate.

Materials and Methods

Study population

In a cross-sectional study, we recruited 123 participants (88 with type 2 diabetes) between 2019 and 2021 from CHU Taher Sfar in Mahdia-Tunisia. Data included age, weight, and height, history of diseases, smoking, and alcohol consumption. Patients were asked if they used any medication, and blood was taken. Plasma and erythrocytes cells were stored at -80°C. This study was approved by the ethics committee.

Assessments of biochemical parameters

All the analyzes of the biochemical parameters were carried out in the biochemistry department of the CHU Taher Sfar of Mahdia, These parameters were measured directly after collecting blood samples using enzymatic kits. Estimated Glomerular Filtration Rate (eGFR) was calculated by the MDRD (Modification of Diet in Renal Disease) formula.

Measurement of GLO1 activity

GLO1 activity was measured according to Thornalley et al. [14]. Briefly, hemithioacetal was produced by incubation of MG (20 mM) and GSH (20 mM) for 30 min in an appropriate volume of sodium phosphate buffer (100 mM, pH 6.6) at 37°C. The GLO1 activity was calculated and was expressed in Units/mL. One unit was defined as the amount of enzyme that catalyzes the formation of 1 µmol of S-D lactoylglutathione/min under the mentioned assay conditions.

Statistical analysis

Statistical analyzes are carried out by SPSS analysis software. Data were given as mean or median in the case of non-normally distributed data. Group comparisons were performed using the Student's t-test or Mann-Whitney test, and the correlation coefficient was estimated using the Pearson or Spearman rank-order correlation analysis. Multivariate analysis was performed, and subgroups comparisons were performed by ANOVA test. A P-value <0.05 was used.

Results

Clinical parameters and GLO1 activity between healthy and diabetic subjects

Clinical parameters and GLO1 activity are shown in Table 1. Patients with diabetes had duration of diabetes between 5 and 17 years and had a high Body Mass Index (BMI) which indicates moderate obesity in most patients. Patients with diabetes showed 48% of hypertension, and 31% of hyperlipidemia. In addition, a significant decrease of renal function, including serum creatinine and eGFR, was shown in patients with diabetes. However, GLO1 activity did not differ between the healthy subjects and patients with diabetes.

Table 1: Clinical parameters between controls and diabetic patients.

Characteristics	Controls (n=35)	Patients (n=88)	*P*
Age (years)	57 ± 12	61 ± 9	NS
Male gender, n (%)	16 (45%)	36 (40)	NS
Duration of diabetes (years)	-	12 (4-15)	-
Dyslipidemia, n (%)	-	28 (31)	-
Hypertension, n (%)	-	43 (48)	-
Blood glucose (mmol/L)	4.90 ± 1.80	12.60 ± 4.87	<0.001
Hemoglobin A1c (%)	5.80 ± 0.50	9.50 ± 2.18	<0.001
Total cholesterol (mmol/L)	4.44 ± 1.00	4.73 ± 1.26	NS
Triglyceride (mmol/L)	1.23 (0.81-1.38)	1.74 (1.03-2.40)	<0.001
Creatinine (mmol/L)	65 ± 14	74 ± 38	<0.05
Urea (mmol/L)	4.30 ± 0.91	6.47 ± 3.51	0.001
eGFR(ml/min/1.73 m²)	112 ± 30	96 ± 33	0.019
GLO1 activity (U/mL)	37.22 ± 6.69	34.08 ± 8.40	NS

Data are shown as the mean (SD) or median (range), or number (percentage)
NS: No Significant

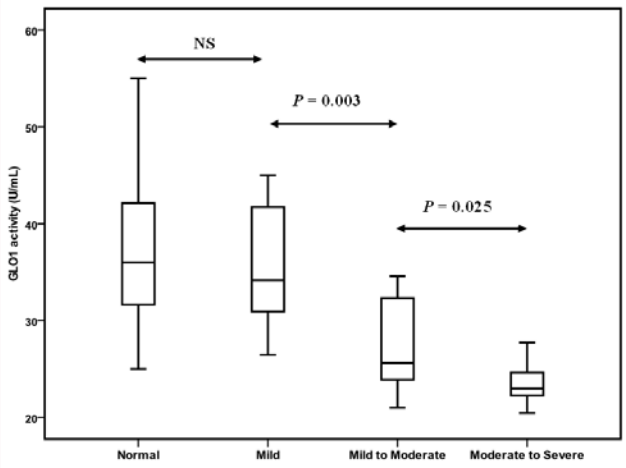

Figure 1: GLO1 activity in the subgroup diabetic patients according for the loss of renal function. (ANOVA analysis between the four subgroups: *P*<0.001).

Biochemical parameters and GLO1 activity according the loss of renal function

Clinical parameters and GLO1 activity in patient's subgroups according eGFR were shown in Table 2 and Figure 1. Patients with diabetes were classified in four subgroup as normal, mild, mild to moderate, and, moderate to severe according eGFR. Duration of diabetes, glucose, and HbA1c did not differ between subgroups. As expected, eGFR was deceased from normal to severe subgroups (P<0.001). For the GLO1 activity there was no difference between normal and mild group, however, a significant decrease was observed between mild to severe subgroups (P<0.001).

Correlation of GLO1 activity with eGFR and other variables

The GLO1 activity was correlated to eGFR (r=0.257; P=0.015)

Table 2: Biochemical parameters and GLO1 activity in diabetic patients' subgroups according eGFR.

Characteristics	Normal (n=45)	Mild (n=21)	Mild to Moderate (n=11)	Moderate to severe (n=11)
Age (years)	62 ± 7	60 ± 11	64 ± 7	60 ± 10
Duration-diabetes	10 ± 6	11 ± 6	16 ± 6	9 ± 4
Glucose (mmol/L)	12.3 ± 4.4	12 ± 5.6	13.3 ± 4.6	14.6 ± 6.1
HbA1c (%)	9.2 ± 2.2	9.4 ± 1.9	10.3 ± 2.1	9.8 ± 1.8
eGFR (ml/min/1.73 m²)	105 (91-115)	77 (69-83)	55 (51-56)	41 (18-43)**
GLO1 activity (U/mL)	37.5 ± 8.1	35.5 ± 5.9	27.5 ± 4.9	23.5 ± 2.2*

Data are shown as the mean (SD) or median (range), or number (percentage)
**Significantly decreased between each group; *P*<0.001
*Significantly decreased between Mild to severe group; *P*<0.001

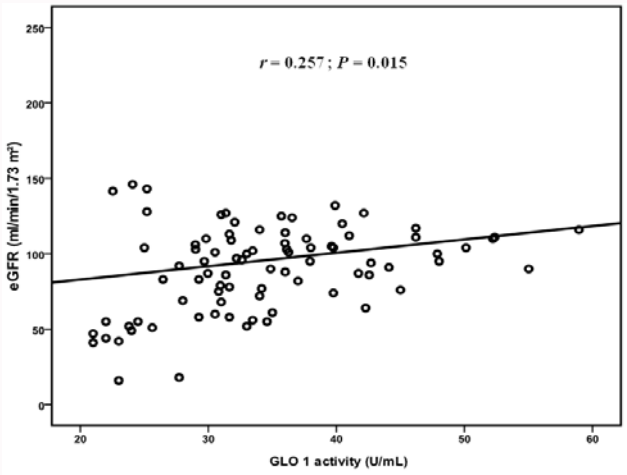

Figure 2: Correlation between GLO1 activity and the eGFR.

as shown in Figure 2. GLO1 activity was also correlated with serum creatinine (r= -0.328, p=0.002) and urea (r= -0.300, P=0.020,). Multivariate analysis showed that GLO1 activity was independently associated with eGFR (β=0.129, P=0.038). However, GLO1 activity did not show any correlation with glucose, HbA1c, cholesterol, and triglyceride.

Discussion

In this study, we examined the activity of GLO1 in patients with diabetes having normal to severe loss of renal function. According to our results, the GLO1 activity profile did not show a significant difference in healthy and patients. The GLO1 activity tended to be decreased with loss of renal function. We found a reduction of GLO1 activity in mild to severe loss of renal function, and were independently correlated to eGFR.

Most studies showed the role of AGEs and their interaction with their receptors, but there are a few studies about the relationships between glyoxalase system, as an antiglycation, and the loss of renal function. The first old study was done by Thornally et al. [14] showed no significant difference in the glyoxalase enzymes between patients with diabetes and controls. However, Thornally et al. [14] showed an increase of methylglyoxal and S-D-lactoylglutathione in diabetic patients *vs.* controls. Data concerning erythrocytes GLO1 activity in diabetes and diabetes complications are relatively scarce, and the results are controversial. Hamoudane et al. showed significantly lower GLO1 activity and glutathione levels in diabetic patients compared to controls. The levels of GLO1 activity were markedly lower in patients with diabetic complications, especially in diabetic patients with nephropathy [15]. In a study by Pacal et al. [16] GLO1 activity was significantly increased in diabetic patients compared to controls, and was higher in nephropathy patients in stages 1 to 2, and remained decreased in nephropathy patients in stages 3 to 4 [16]. Our present study confirms the findings of Thornally et al. [14], Pacal et al. [16], Sakhi et al. [17], and Peters et al. [18]. Furthermore, Peters et al. [18] found that GLO1 activity was lower in atherosclerotic carotid artery lesions, and the effects observed are related to the microenvironment of the damaged tissue. We hypothesize that GLO1 activity may affects also the microenvironment location in glomerular and its vascular tissues under chronic hyperglycemia that induce much production of AGEs precursors such as MGO and may inhibits GLO1 enzyme activity. This AGE accumulation has been closely associated with kidney diseases, and aging. Accumulating evidence demonstrates that the progression of renal tubular damage and tubular aging are often correlated with activation of the receptor for the AGE (RAGE)-AGE pathway or decreased activity of glyoxalase 1 [19].

To our knowledge, this is the first study showing the relationships between erythrocytes GLO1 activity and the estimated glomerular filtration rate in patients with diabetes with normal, mild, moderate and severe loss of renal function. The GLO1 activity decreased markedly with patients when they have moderate to severe loss of renal function. The direct pathogenic role of MGO/glyoxalase system in the development of diabetic nephropathy is strongly supported by animal experiments. Overexpression of GLO1 in diabetic rats reduced the production of AGEs, endothelial dysfunction, and also expression of early markers of kidney damage [20]. Interestingly, knockdown of GLO1 in non-diabetic mice induces kidney pathology very similar to diabetic nephropathy [21]. The reduced levels in GLO1 activity may result also from the deceased of glutathione levels but the most biomarker that affects GLO1 activity was the tissues accumulation of α-oxoaldehydes, especially MGO that are formed during cellular metabolic reactions [14]. Recently, it was well described in a review by Schalkwijk and Stehouwer [22] the involvement of the MGO in many diseases. Lowering the MGO levels can provide new therapeutic to reduce AGEs precursors and their accumulation [23-26]. Recent interesting studies are focused on GLO1 inducers as a new therapy [27-29].

Our study has obvious limitations. We have not measured MGO or MGO-derived AGEs due to the lack of technologies in our laboratory. Furthermore, healthy subjects and patients with moderate to severe loss of renal function subgroup showed small size samples.

Conclusion

GLO1 activity in erythrocytes was independently correlated in patients with diabetes having a decreased estimated glomerular

filtration rate.

Authors' Contribution

RS, HH, and AM: determined the GLO1 activity measurement, Clinical data, and wrote the manuscript. MK, SA, and AL contributed to the design and the concept of the study. HB measured the biochemical parameters. HZ: provided blood sampling. All authors read and approved the final manuscript.

Declaration

The protocol has been approved by the ethics committees at the CHU Hospital Tahar Sfar Mahdia. All participants signed the informed consent in writing before inclusion in the study.

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