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The Use Of Serum Levels Of Glutathione-S-Transferase For The Detection Of Diabetic Nephropathy Among Libyan Diabetics.

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Abstract:
Introduction: *Diabetic nephropathy is one of the most common and serious complications of diabetes. Microalbuminuria (MA) is a crucial risk factor for diabetic nephropathy (DN). However, recent studies have challenged the use of microalbuminuria, as albumin excretion is more likely to revert to normal levels than to macroalbuminuria.*
Aim: *To investigate the serum levels of glutathione-s-transferase as a marker for the detection of DN among diabetic with and without DN, in combination with other biochemical parameters for checking renal function state.*
Material and methods: *This study involved 160 subjects from the Benghazi Medical Center and the diabetic clinic of Al-hyat private center of diabetes and Benghazi Center of Diabetes and Endocrine Disease, divided into 3 groups; DN group (60 subjects), diabetic "without DN" group (60 subjects), and non-diabetic "control" group (40 subjects). blood and urine samples were collected from study participants and tested urine albumin and GST-pi levels along with other biochemical tests.*
Results: *The mean ± SD of GST was non-significantly higher (11.3 ± 2.2 ng/ml) in the diabetic group than that of the DN group (10.8 ± 2.4 ng/ml) and the control (10.7 ± 2.2 ng/ml). Meanwhile, albumin levels were much higher in DN group (mean = 84.4 ± 59.4) than the diabetic group (10.7 ± 15.2) and the control (5.2 ± 1.3). Regarding kidney function tests; the means of blood urea, serum creatinine and uric acid were significantly higher in diabetic nephropathy group than the diabetic group and the control group.*
Conclusion: *The present study showed a nonsignificant elevation in serum levels of GST-Pi. The level of the enzyme was higher in diabetics without diagnosed nephropathy than that of DN group, this increase may be occurred as a compensation to the undiagnosed deterioration of kidney function. Future work using large number of samples are required to confirm the role of GST as an early biomarker of DN.*
Keywords: *Glutathione-s-transferase, GST, Diabetic nephropathy, Microalbuminuria*

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I. Introduction

Diabetes refers to a set of metabolic diseases characterized by sustained increase in blood glucose (hyperglycemia) due to impairments in insulin secretion, insulin action, or both of them. Chronic hyperglycemia of diabetes is linked to long-term damage, dysfunction, and failure of various body systems, including renal, visual, neural, and cardiovascular systems.

Diabetes represents a serious health problem in the whole world. In 2019, according to International Diabetes Federation (IDF), The number of people living with diabetes is about 463 million. Globally, the incidence of the disease is increasing rapidly, it was expected to reach the edge of 700 million people by the 2045 (Saeedi et al., 2019). In Libya, there is a shortage of the prevalence data of diabetes as suggested by a recent study (Younis et al., 2022). In 2017, there were about 442,500 subjects living with in Libya, from now until 2045, this figure will reach 762,500. The prevalence of diabetes among Libyan adults was 11.2% (Elmiladi, 2022).

Long-term hyperglycemia in T2 diabetes causes the release of reactive oxygen species (ROS) that finally leading to oxidative stress (OS) (Gupta et al., 2013) which is linked to either enhanced formation of oxidants or reduction in the antioxidant processes. The oxidative stress results in damage to the subcellular compartments and cellular proteins and enzymes. OS is causes an enhanced lipid peroxidation and insulin resistance that ultimately results in diabetic complications such as retinopathy, nephropathy and neuropathy (Maritim et al., 2003).

Diabetic nephropathy (DN) can be defined as a set of concomitant impairment that affecting the renal function of the kidneys in diabetics. The functional abnormalities include increased glomerular filtration, proteinuria, systemic hypertension, and ultimately kidney failure (Ayodele et al., 2004). Yuan et al., 2019 reported that About 35–50 % of diabetics may develop a form of DN. DN accounts for approximately 40 per cent of new end stage renal disease (ESRD) cases.

Antioxidants may improve the pathological conditions linked to OS and play a vital role in protecting cells from ROS (Lollinger, 1981). Glutathione S-transferase (GST) represent a family of enzymes that detoxify reactive electrophilic compounds resulted from OS as well as carcinogenic substances by conjugating them with reduced glutathione (Bessa et al., 2009). In normal physiological situations, there is a low concentration of GST in the blood due to normal cellular turnover. Therefore, assessing serum GST levels may help in monitoring the process of cellular damage (Barash et al., 2009). The aim of the present work is to assess the role serum GSTpi as a biomarker for diabetic nephropathy and its correlation with other biochemical markers in Libyan diabetics with and without DN.

II. Methods and materials:

Study population: This work was a retrospective study, a total of 160 subjects (**Figure 1)** were involved in this study, of which 60 people were diabetic patients suffering accompanying diabetic nephropathy, 60 people were diabetic patients with a normal kidney function (without diabetic nephropathy), and 40 healthy people (controls). All the study subjects were in the age group 50 to 70 years old. Half of the study subjects were males (80 subjects) and the other half was females (80 subjects as well).

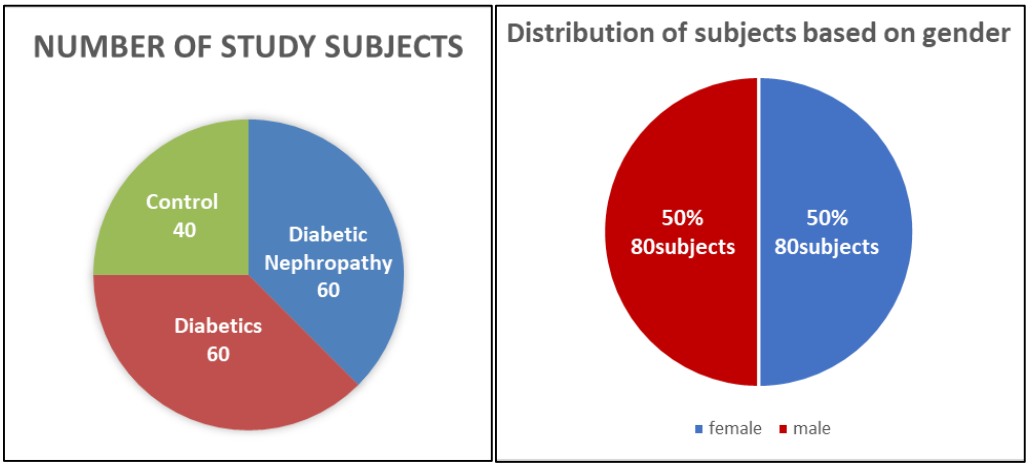

Figure 1: Distribution of study subjects based on numbers and gender among study groups.

Study investigations: The study subjects were tested for their levels of glutathione s-transferase along with glycated hemoglobin, and urine albumin (for microalbuminuria), all the study subjects were also tested for other parameters to ensure sample uniformity and eliminate the presence of other health conditions that might affect the validity of the results, these tests included kidney function tests such urea, uric acid, serum creatinine, sodium and potassium, and finally lipid profile tests.

GST levels (ng/ml) were determined using Human GST-Pi, ELISA Kit, this kit was manufactured by BT Lab of Shanghai Korainf Biotech Co. The COBAS INTEGRA 400 plus from Roche company was used for the assay of albumin and all other biochemical tests except sodium and potassium which were determined using Diestro 103AP Electrolyte Analyser is an In Vitro Medical Device that allows the measurement of electrolytes in whole blood, serum, plasma, and urine samples. It is capable to measure up to 5 electrolytes simultaneously: sodium, potassium, chloride, calcium, and lithium.

III. Results:

Comparing Urine Albumin levels among the study groups: The difference in means of albumin levels among the study groups was statistically high significant (p-value = 0.00). Albumin levels were much higher in DN group (mean = 84.4 ± 59.4) than the diabetic group (10.7 ± 15.2) and the control (5.2 ± 1.3).

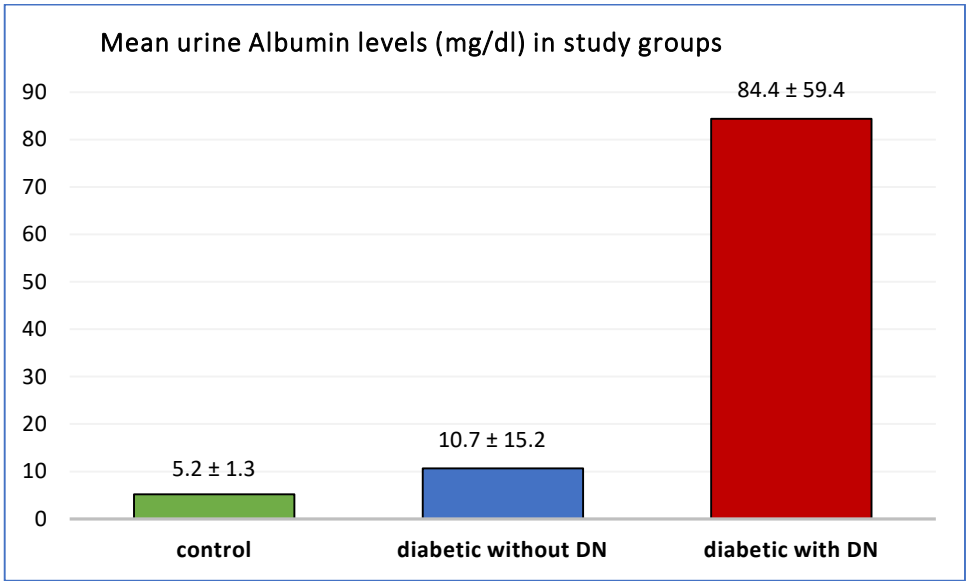

Figure 2: Mean ± SD of albumin levels (ng/ml) of study groups.

Comparing serum GST levels among the study groups: The difference in means of GST levels among the study groups was not statistically significant (p-value = 0.311) which is above the 0.05 significance level. The observed values (**figure 3**) of the mean ± SD of GST was non-significantly higher (11.3 ± 2.2 ng/ml) in the diabetic group than that of the DN group (10.8 ± 2.4 ng/ml) and the control (10.7 ± 2.2 ng/ml).

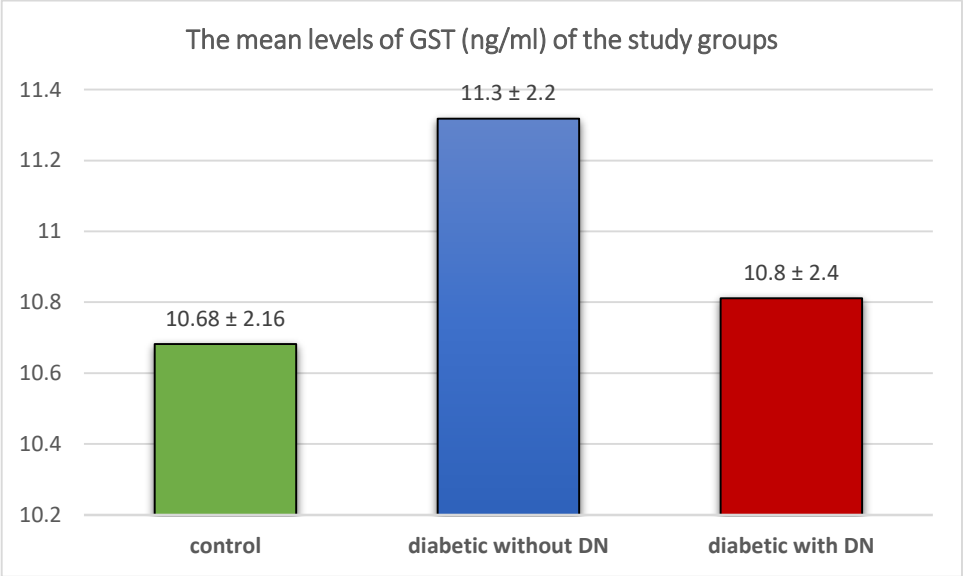

Figure 3: Mean ± SD values of GST levels in study subjects.

Comparing glycosylated Hb (HbA1c) levels among the study groups:
As clear from **Figure 4**, The mean blood level of HbA1c (7.9 ± 0.78 %) of the DN group and 8.02 %) for the diabetic group, both of them were higher (p-value = 0.00) than the HbA1c levels of control group (5.2 ± 0.3).

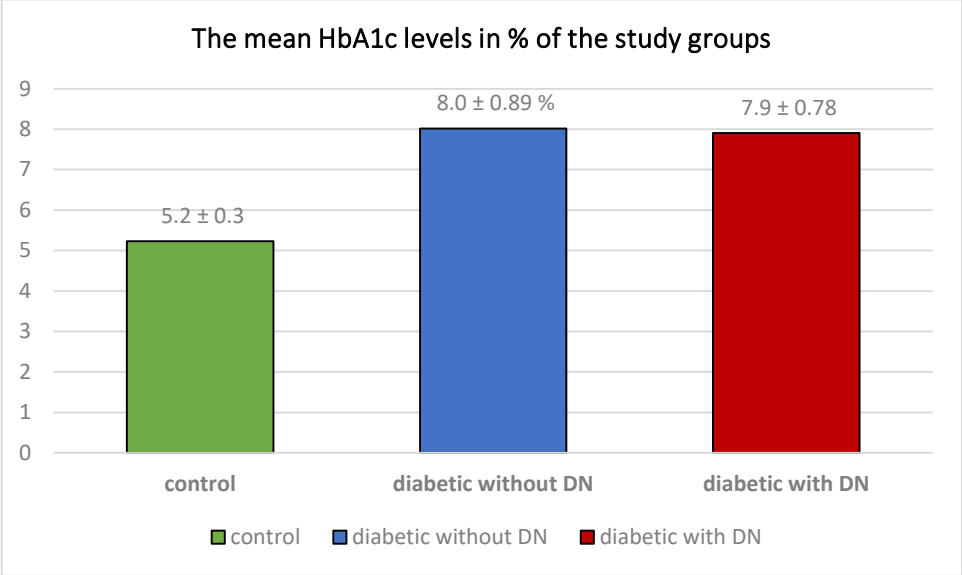

Figure 1: Mean ± SD values of HbA1c levels in study subjects

Comparing blood urea levels among the study groups:

The values of the mean ± SD of urea levels (28.7 ± 10.2 mg/dl) of the diabetic nephropathy was significant higher (p-value = 0.003) than the diabetics without DN group (23.6 ± 6.9 mg/dl), and the control (24.7 and 7.4 mg/dl).

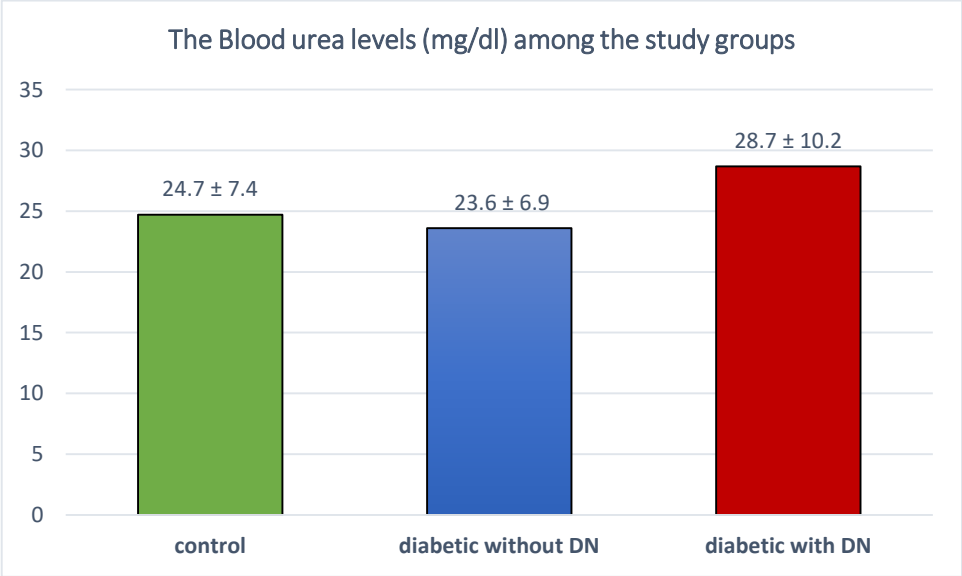

Figure 5: Mean ± SD values of urea levels in study subjects

Comparing serum creatinine levels among the study groups:

The mean ± SD of serum creatinine levels (0.95 ± 0.3 mg/dl) of diabetic nephropathy was significantly increased (p-value = 0.001) compared with the diabetic group (0.78 ± 0.17 mg/dl) and the non-diabetics (0.89 ± 0.2 mg/dl) group (control).

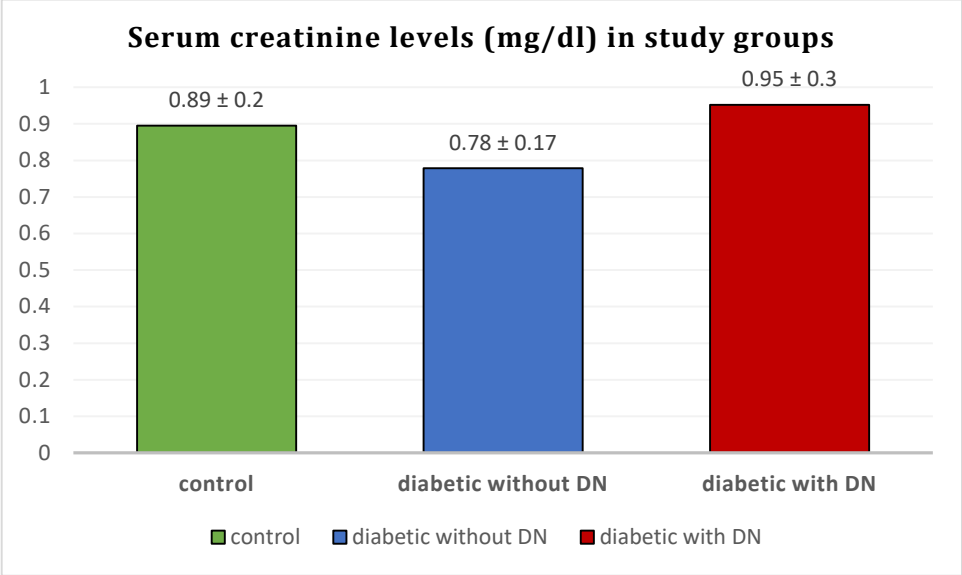

Figure 6: Mean and standard deviation of serum creatinine levels in study groups

Comparing serum uric acid levels among the study groups:

The mean ± SD of uric acid levels (4.97 ± 1.19 mg/dl) of diabetic nephropathy group was significantly increased (p-value = 0.001) compared with the diabetic group (4.08 ± 0.86 mg/dl) and the non-diabetics (4.5 ± 1.1 mg/dl) group (control).

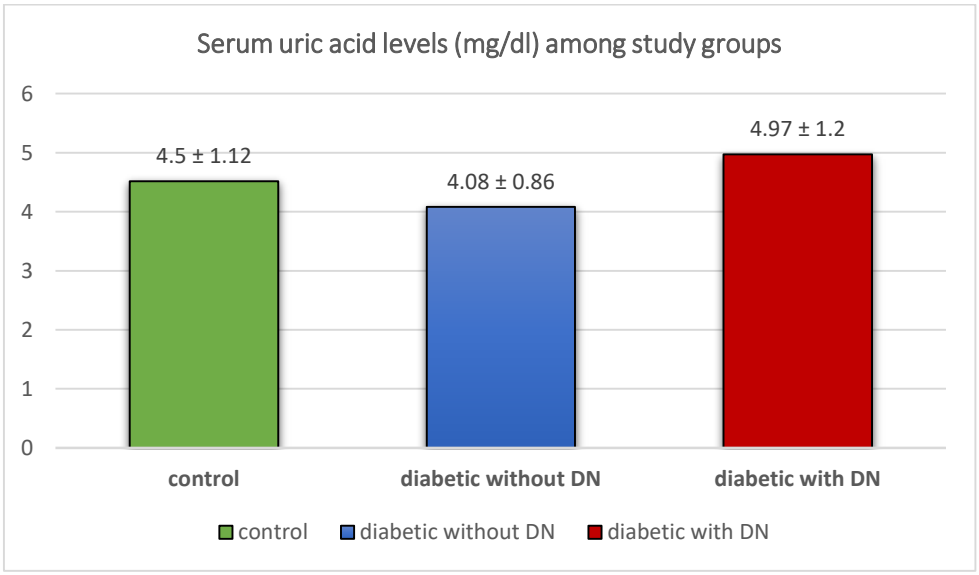

Figure 7: Mean and standard deviation of serum uric acid levels among study groups

Comparing lipid profile levels among the study groups:

Serum triglycerides (TG) levels among the study groups were highly significant (p-value = 0.00), The mean ± SD of serum TG (118 ± 33 mg/dl) of DN group was higher than serum TG (112 ± 25 mg/dl) of diabetics without DN group as well as for the control (91 ± 28 mg/dl). However, serum total cholesterol, LDL-C and HDL-C levels were not significant among the study groups p-values were equal to 0.222, 0.18 and 0.95 respectively as observed in **table 1** and **Figure 8**.

Table 1: The mean ± SD of the lipid profile parameters of the study groups

Groups	N	TC	TG	LDL-C	HDL-C
Control	40	163 ± 22	9 1± 28	98± 18	41.8± 6.9
Diabetic	60	164 ± 26	112 ± 25	99 ±24	42.3± 8
DN	60	155 ± 35	118 ± 33	91 ± 28	42.2 ± 8.7
P value	-	0.222	0.00*	0.18	0.95

The lipids were expressed in mg/dl, * means significant.

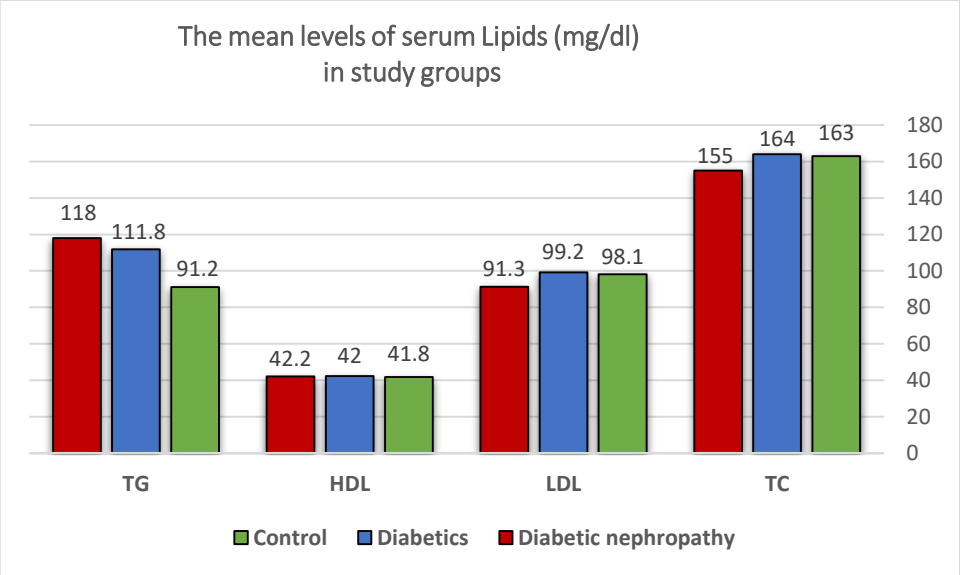

Figure 9: The mean ± SD of the lipid profile (mg/dl) parameters of the study groups.

Sodium and potassium:

The difference in means of sodium levels in the study groups was not statistically significant (p-value = 0.616)**.** On the other hand, the mean level of serum potassium (4.1 ± 0.3 mmol/l) of the DN group was highly significant (p-value = 0.007) lower than that of the diabetic (4.25 ± 0.29 mmol/l) and the control (4.27 ± 0.29 mmol/l).

Table 2: The mean ± SD of sodium and potassium (mmol/l) in the study groups.

Groups	**N**	**K^+**	**Na^+**
Control	40	4.3 ± 0.3	140 ± 1.9
Diabetic	60	4.2 ± 0.3	138 ± 16
DN	60	4.1 ± 0.3	139 ± 2.1
P value	-	0.007*	0.616

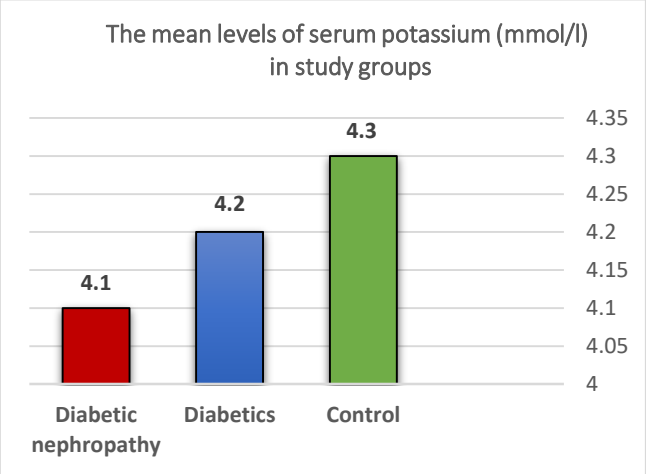

Figure 10: The mean potassium (mmol/l) in the study groups.

Correlation coefficient: In the diabetic nephropathy group although albuminuria level was increased there was no significant correlation between serum levels of GSTpi with other parameters except for potassium which was positively correlated to GSTPi levels. On the other hand, albuminuria levels show a positive correlation with HbA1c and uric acid, which means that when blood glucose and uric acid increase, they directly affect kidney function indicated by increasing albuminuria levels in urine.

IV. Discussion

Diabetic nephropathy (DN) is the most common complication of diabetes and considered as the major etiology of chronic kidney disease (CKD). The incidence and progression of DN is linked to OS. Identifying new biomarkers for the early detection of the disease may have may aid in the early treatment and may delay the progression of the DN to late stage of the renal disease (Bessa et al., 2009).

Reactive oxygen species are produced during metabolism. The abnormal elevation of ROS is implicated in OS. The role of OS as cause of diabetic complication such as DN was investigated by many clinical studies (Maritim et al., 2003, Tesauro et al., 2015). The major roles of Glutathione S transferase (GSTs) enzymes is to protect the cells against ORS such as H_2O_2 and to detoxify foreign compounds such as drugs. The enzymes can perform these roles by conjugation of reduced glutathione (GSH) to toxic hydrophobic compounds. This reaction facilitates toxins inactivation and renal elimination of a large number of toxins (Robertson et al., 2003). GSTs are considered as important components of phase II of xenobiotic metabolism. Therefore, measuring the levels of GSTs may provide information about the factors underlying the progress of diseases.

In the present study, we measured the concentration of serum GST-Pi which is the major isoenzyme in red blood cells (GST-P1-1,21) which accounts for greater than 95% of the erythrocyte GST (e-GST) pool (Singhal et al., 1999). Our study observed non-significant increase in GST levels in diabetics with and without DN compared to the control. However, the levels of GST in DN group were lower compared to type 2 diabetics. These finding were comparable to the recent study of Sharma et al., 2016 reported a significantly elevated GST activity in type 2 DM and diabetics with DN compared to control. On the other hand, Verma et al., 2013 observed lower GST level in diabetics of both genders and the lowest activity was in older ages (more than 60 years). This study concluded that investigating e-GST activity may detect subjects who are prone to develop type 2 DM.

Sharma et al., suggested that elevated GST activity caused by of increased production of reactive oxygen species (ROS) as a result of sustained high blood glucose levels. In accordance, Giebułtowicz and colleagues; reported that increased GST activity in diabetic patients was a compensation to resist OS. The author also concluded that GST activity rise was correlated to glycated Hb and also linked to the severity degree of diabetes.

Our findings were also in the same line with the study of Noce et al., 2014, who found a significant increased activity of GST in DN patients compared to diabetics and healthy controls. On the contrary, Bessa et al., 2023, reported that GST activity and glutathione (GSH) level were significantly decreased in diabetic subjects with and without nephropathy as compared to non-diabetics. Meanwhile, the biomarker of oxidative stress; malondialdehyde (MDA) levels were significantly elevated in diabetic groups with and without nephropathy as compared to control. The author concluded that rise in MDA occurred due to reduced antioxidant enzymes such as GST. The latest findings were supported by the study of Mistry et al., 2020, which reported an increase in OS as a result of hyperglycemia, evidenced by an increase in the markers of lipid peroxidation and a significant reduction in antioxidants such as GSH and superoxide dismutase.

In the present work, urine albumin levels were markedly increased (p-value = 0.00 compared to the diabetic and non-diabetic groups. In addition, a significantly elevated serum urea, creatinine, uric acid compared

to control. These findings were consistent with those of Sharma and colleagues, who found arise in kidney function tests as well as a reduced estimated glomerular filtration rate (GFR). Our work also found that GST levels was positively correlated with potassium levels which means that serum GST levels was partly linked to kidney function. In agreement, Dessì et al. 2012, found a positively correlated GST activity with the severity rate of chronic kidney disease evidenced by "kidney disease outcome quality initiative". The suggestion that GST polymorphisms may affect the risk of developing diabetes, DN as well as the levels of advanced glycation end products (AGEs), was supported by recent research by Pavlovic et al., 2023, the author also reported that carriers of GSTO2 variant were more susceptible to increased risk for developing DN.

The current study observed a nonsignificant increase in serum levels of GST-Pi. The increase was higher in diabetics without diagnosed nephrotic disease compared to DN group. This finding was in agreement with Noce et al., 2014, who concluded that serum elevated e-GST activity in undiagnosed-nephropathic diabetics may be resulted from undiagnosed decrease in kidney function. Future work is needed to confirm this suggestion which may open the door to depend on GST-Pi as biomarker for the detection of the early stages CKD among diabetic subjects.

V. Limitations:

The research work to show the protective ability of GST among diabetic with or without nephropathy require more investigations which could not achieved during this study due to lack financial support. Examples of these are, Malondialdehyde (MDA), which has been considered as a marker of lipid peroxidation caused by free radical, ferric reducing ability of plasma (FRAP) which give an account of the total serum antioxidants (sharma et al., 2016) and finally reduced glutathione (GSH) which is the secondary substrate of GST enzyme and one of the important antioxidant in human body, all those parameters in combination with serum GST could definitely analyze the role of serum GST as a biomarker for the early detection of DN among diabetics.

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