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Evaluation of Insulin Resistance and Glutathione-S-transferase in Iraqi Patients with Type 2 Diabetes Mellitus and Diabetic Peripheral Neuropathy

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Abstract

Diabetes mellitus type 2 (T2DM) is a metabolic disorder that influences above 450 million individuals around the world. Type 2 diabetes is a lack of insulin due to pancreatic β-cell malfunction and insulin resistance. This study aimed to detect insulin resistance using homeostasis model assessment (HOMA IR) and determined the correlation with glutathione-s-transferase (GST) activity in T2DM and neuropathy patients as a predictor of oxidative stress, which occurs when the oxidation-antioxidant equilibrium is disrupted. Reactive oxygen species causes vascular injury and a series of inflammation. In the present study, the results show there is no significant difference in diabetic patients (DM) and neuropathy patients (NU) versus healthy people in insulin resistance ($p > 0.05$). GST activity significantly differs between the patients and healthy groups ($p \leq 0.05$). Moreover, this study has reported an improvement in insulin resistance and high activity of GST in the patient's group as a warning sign of excessive oxidative stress. There was no evidence revealing a link between insulin resistance and GST. The present study has demonstrated that HOMA-IR had a positive relationship with fasting blood sugar and insulin in the neuropathy group and diabetic group and a negative relationship with high-density lipoprotein (HDL).

Keywords: Type 2 Diabetes mellitus (T2DM), Diabetic Peripheral Neuropathy (DPN), Oxidative stress (OX), Glutathione-s-transferase (GST).

1. Introduction

People with diabetes mellitus have a metabolic disease that lasts the rest of their lives [1, 2]. Hyperglycemia brought on by issues with insulin secretion, insulin function, or a combination of these issues characterizes it [3]. HbA1c is a reliable diabetes diagnostic tool that was initially used in diabetes treatment in 1985 [4]. In March 2009, WHO convened and placed HbA1c as a diagnosis of diabetes based on available evidence [5]. Type II DM usually develops gradually because of obesity and other comorbidities that induce cells to become resistant to insulin's hormonal activity [6]. Insulin resistance was characterized by lower responsiveness of tissues targeted by insulin stimulation. Hyperinsulinemia is a condition that occurs when insulin resistance is accompanied by abnormal insulin production after a meal. Long-term hyperinsulinemia, interestingly, causes IR to be worse [7]. The ensuing insulin-resistant condition has systemic and tissue-level effects, affecting the subject's metabolic status. IR is a hallmark of early-onset T2DM that can occur in the main calorie storage areas, such as adipose tissue (AT), skeletal muscle, and the liver [8]. Peripheral neuropathies are illnesses of peripheral nerve cells and fibers that may be caused by different disorders. Cranial nerves, spinal nerve roots and ganglia, nerve trunks and divisions, and nerves in the autonomic nervous system are among these nerves [9]. The prevalence of diabetes mellitus has been widely related to oxidative stress. Previous studies have proven that the oxidative stress (OX) is a major factor in the development and progression of diabetes and its complications [10]. Antioxidant strategies may be useful in preventing or treating the disease, concerning the role of oxidative stress in the onset and progression of diabetes. In this regard, some types of fruits raise the level of glucose in the blood, and some fruits and vegetables are rich in antioxidant components. Several studies have reported the favorable benefits of various diets or meals in preventing diabetes [11]. These contents may serve as antioxidants, promote insulin secretion and sensitivity, and reduce the lipid levels in the blood [12]. Antioxidants can also help overcome oxidative stress. Endogenous antioxidant enzymes are: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase which have a role in oxidative stress defense (GST) [13]. GSTs are xenobiotic metabolizing enzymes through conjugation with reduced glutathione. GSTs play a vital function in cellular defense against reactive electrophiles and fatty acid hydroperoxides which are produced by the oxidative stress. As a result, GSTs aid cell detoxification by minimizing the tissue damage which caused by free radical attacks [14, 15]. The present study aimed to evaluate the insulin resistance and GST activity and highlighted if there is a relationship between these parameters in diabetic and neuropathy patients.

2. Materials and Methods

The present study is conducted at the Department of Chemistry, College of Sciences for Women / University of Baghdad, and at the National Diabetes Center for Treatment and Research at Mustansiriyah University. The study includes (120) subjects divided into three groups: Group 1 contains 40 healthy people as a control (20 males, and 20 females) with ages of (34- 66). Group 2 contains 40 subjects with type 2 diabetes mellitus (18 males and 22 females) with ages of (34-65). Group 3 has 40 subjects with type 2 diabetes mellitus with neuropathy (18 males and 22 females) in ages ranging (37-66). Peripheral neuropathy patients were evaluated by a neurologist at the National Diabetes Center using the Toronto Clinical Scale. From each participating subject (patient and control), about 10 mL of venous blood was collected using a 10 mL disposable syringe. The

blood was distributed into two parts; the first was dispensed in a gel tube to collect the serum (after clotting, blood was centrifuged at 3000 rpm for 10 minutes at room temperature, and then serum was separated, distributed into aliquots in an eppendorf tube, and stored at -20°C until assayed). The second part was drawn in an EDTA tube and analyzed for HbA1c assay. The patients were under the treatment with metformin and sulfonylurea drugs. Fasting serum insulin concentrations were determined by enzyme-linked immunoassay (ELISA) using a kit produced by CUSABIO company in the U.S.A. Fasting blood glucose, triglyceride, total cholesterol, and high-density lipoprotein were determined by Biolabo kit-France using Kenza (240TX) instrument. GSH (Sigma chemicals, U.S.A) was used to manually measure GST activity. Homeostasis Model Assessment (HOMA IR) is used to determine the insulin resistance by the formula:

HOMA IR= $\frac{Glucose \times Insulin}{405}$ [16].

Waist to hip ratio:

$WHR = waist\ cm \div hip\ cm$ [17].

Body Mass Index:

BMI = weight in (kilograms)/ height in (square meter) [18].

HbA1c was determined by high-performance liquid chromatography using HLC-723GX Tosoh from Japan.

Ethics approval: this survey was approved by the Scientific Committee of the College of Science for Women, and a verbal consent form was obtained from each participant enrolled in the study.

3. Data Analysis

The SPSS (version 26) application was used to conduct the statistical analysis. The data was presented as (mean ± SE) median. The ANOVA test was applied to find the difference between parameters in terms of T-test, (P-value), LSD, and correlation coefficient (r). Estimation by analyzing for linear regression was employed in the statistical test. The statistical significance was determined by the probability value, which was acknowledged as significant at ($p \leq 0.05$), and non-significant at ($p > 0.05$).

4. Results and Discussion

The mean values of BMI, W/H ratio, FBS, HbA1c, cholesterol, T.G, HDL, LDL-C, VLDL of the G1 control group and G2 diabetic group, and G3 neuropathy group listed in table 1 have shown a significant difference among G1 and G2, and G3 with ($p \leq 0.05$). The results agree with Nermina Babic *et al*. [19], Neeraj Chhari *et al*. [20], and Subarna Dhoj Thapa *et al.* [21]. Dyslipidemias, particularly hypertriglyceridemia and low levels of high-density lipoprotein cholesterol are common phenotypes associated with diabetes mellitus. Many studies have found an inverse relationship between HDL and total cholesterol content and also the risk of atherosclerotic vascular disease. Hydrophobic cholesteryl esters are entropically transferred to triglyceride-rich lipoproteins in the presence of hypertriglyceridemia, lowering cholesterol content in HDL and changing HDL molecular speciation [22]. Hyperglycemia-induced advanced glycation end products, oxidative stress, and inflammation all contribute to HDL dysfunction in

diabetes mellitus, which raises the chances of getting cardiac disease [23].

Table 1. A Comparison between the control and patients group regarding biochemical parameters

Parameters	Control Group (1) No. (40)	Diabetes Mellitus (DM) Group (2) No. (40)	Neuropathy Group (3) No. (40)	P-value	LSD between groups (1,2)	LSD between groups (1,3)	LSD between groups (2,3)
Age (year)	51.07 ± 1.15 (51)	52.08 ± 1.18 (53.5)	53 ± 1.18 (54.5)	0.513	0.549	0.249	0.579
BMI (kg/m^2)	23.92 ± 0.16 (24.03)	31.05 ± 0.73 (30.4)	31.05 ± 1.01 (28.92)	0.001**	0.001**	0.001**	1.00
W/H ratio	0.88 ± 0.01 (0.895)	0.95 ± 0.01 (0.94)	0.967 ± 0.014 (0.94)	0.001**	0.001**	0.001**	0.243
FBS (mg/dL)	92.07 ±1.61 (92.5)	176.05 ± 9.77 (157)	209.37± 12.22 (198.5)	0.001**	0.001**	0.001**	0.011*
HbA1C %	5.005 ± 0.05 (5)	8.56 ± 0.27 (8)	9.20 ± 0.30 (9)	0.001**	0.001**	0.001**	0.55
Cholesterol (mg/dL)	153.92 ± 1.66 (154.5)	169.01 ± 5.85 (172.5)	181.97 ± 5.45 (176.5)	0.001**	0.026*	0.001**	0.055
TG (mg/dL)	104.28 ± 3.26 (109.95)	140.62 ± 5.51 (34.85)	159 ± 11.72 (146.5)	0.001**	0.001**	0.001**	0.091
HDL-C (mg/dL)	48.24 ± 0.70 (47)	26.07 ± 1.21 (24.5)	26.02 ± 0.98 (25.5)	0.001**	0.001**	0.001**	0.972
LDL-C (mg/dL)	84.82 ± 1.68 (85.7)	114.81 ± 5.63 (122)	124.11 ± 5.98 (122.3)	0.001**	0.001**	0.001**	0.177
VLDL-C (mg/dL)	20.85 ± 0.65 (21.99)	28.12 ± 1.10 (28.4)	31.84 ± 2.34 (29.3)	0.001**	0.001**	0.001**	0.091

- Data were presented as (Mean ± SE) Median

- LSD: Least significant Difference

*Significant difference between means using ANOVA -test at 0.05 level.

**Highly Significant difference between means using ANOVA -test at 0.05 level.

The mean values of insulin and HOMA IR for all the studied groups in the current study are shown in **Table 2**. The mean values in this table revealed that there were significant differences in insulin levels (µIU/ml) between the patient groups with diabetes and neuropathy and the control group ($p \leq 0.05$). The current study is in agreement with the study of Rosemary C. Temple *et al*. [24]. Moreover, Christian Weyer *et al*. [25] have shown that during the shift from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT), the acute insulin response (AIR) to glucose decreased by 27%, demonstrating conclusively that insulin secretion abnormalities (significantly decrease) occur early in the progression of diabetes type 2. Free fatty acids (FFAs) are essential for the regular operation of the pancreatic beta-cell, as well as its ability to adjust for insulin resistance and failure in type 2 diabetes. Islet tissue deficient in fatty acids (FAs) loses glucose-stimulated insulin secretion (GSIS), a process that may be reversed quickly with exogenous FFAs nevertheless; saturated FFAs might impair insulin biosynthesis [26]. Insulin secretion is also consumed in excess over time, especially when combined with high blood sugar. This is because, in the natural history of this condition, postprandial hyperglycemia and dyslipidemia are typical

symptoms that affect the development of an insulin secretory deficiency [27]. HOMA IR in table 2 showed that there is no significant difference between the patient and control groups (p>0.05). This might be due to the fact that all patients (DM and Nu.) were under oral treatment. The results of this study are similar with a recent study that found that metformin, when administered as part of an anti-diabetic therapy in T2DM patients, improved glucose and lipid metabolism, decreased beta-cell activity, and enhanced insulin sensitivity [28]. With the addition of low-dose sulfonylurea to the treatment plan, the reduction in (IR) insulin resistance was considerably greater than without it. HOMA IR is a reliable test for detecting (IR) [29]. Akira Katsuki *et al*. [30] study concluded that HOMA IR is a reliable method for determining insulin resistance in patients under treatment with sulfonylurea, diet and exercises. According to a recent study, metformin improved insulin resistance by lowering the expression of miR223 in adipocytes. In insulin-resistant adipocytes against non-resistant adipocytes, and diabetic adipose tissue versus non-diabetics, MiR223 expression was significantly higher [31]. The impact of prolonged metformin treatment (a medicine that improves insulin sensitivity) on diabetic complications was helpful. Compared to the traditional group treated just with diet, metformin-treated individuals had a 32% lower risk of any diabetes-related outcome, including macrovascular problems. Compared with the control group, metformin patients had a 42% lower risk of diabetes-related death and a 36% decreased chance of dying from any causes [32]. Regarding GST, the mean values in (IU/L) for all the studied groups in the present study are shown in Table 2 and **Figure 1**

Table 2. Comparison of Insulin, HOMA IR and GST between control and patient groups

Parameters	Control Group (1) No. (40)	Diabetes Mellitus (DM) Group (2) No. (40)	Neuropathy Group (3) No. (40)	P-value	LSD between groups (1,2)	LSD between groups (1,3)	LSD between groups (2,3)
Insulin (µIU/ml)	3.14 ± 0.26 (2.75)	1.95 ± 0.19 (1.8)	1.69 ± 0.13 (1.4)	0.001**	0.001**	0.001**	0.375
HOMA IR	0.71 ± 0.06 (0.57)	0.82 ± 0.08 (0.7)	0.82 ± 0.065 (0.71)	0.46	0.291	0.271	0.966
GST activity (IU/L)	0.74 ± 0.68 (2)	2.89 ± 0.74 (2.54)	3.53 ± 0.85 (2.58)	0.029*	0.120	0.030*	0.825

- Data were presented as (Mean ± SE) Median

- LSD: Least significant Difference

*Significant difference between means using ANOVA -test at 0.05 level

**Highly significant difference between means using ANOVA -test at 0.05 level.

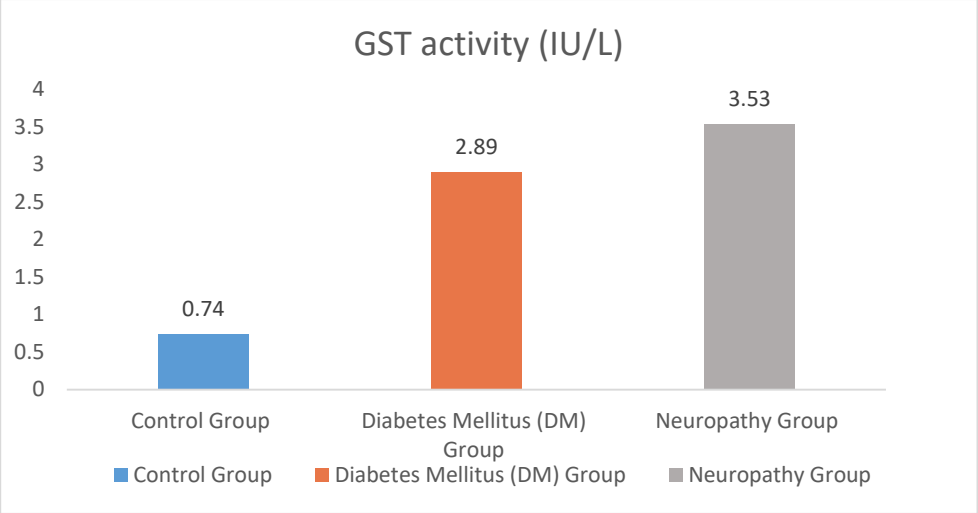

Figure 1. GST activity (IU/L) between groups

There is a difference in the mean between the control (0.74±0.68) and DM (2.89±0.74) and NU. Groups (3.53±0.85); the result suggested a significant difference in GST activities between patients and the control groups with ($p \leq 0.05$) LSD showed a significant difference between the control and neuropathy groups ($p \leq 0.05$) as shown in **Figure 1**

Antioxidant glutathione S-transferase is a multigene protein family that has a role in the metabolism of diseases causing electrophilic substrates, protecting cells from oxidative stress and monitoring cellular activation. The results of this study are in agreement with a previous study which showed a significant difference in GST activity between DM patients and control. There is strong evidence that oxidative stress (OS) plays a key role in diabetes development and complications [33]. Also, Mohini Sharma *et al.* [34] revealed that the highest GST activity in T2DM may represent a compensatory strategy in response to oxidative stress. On the contrary, Sarkar A. *et al.* [35] reported that there is no significant difference was found in GST between type 2 DM patients and healthy. In plasma and membrane proteins, there was autoxidation of unsaturated lipids and sugar-protein adducts, as well as sugars themselves; all created free radicals which were probable causes of oxidative stress and protein degradation in diabetes. As the oxidative stress increased the formation of free radicals and the weakening of the free radical inhibitors and scavengers would in turn, increase the oxidative stress leading to cell damage and death [36]. Regarding to the study by Mohammad Bagher Hashemi-Soteh *et al.* [37] the GSTT1-null genotype was considerably more common in diabetic neuropathy patients than in non-neuropathic individuals. The involvement of the GSTT1-null genotype in predisposing patients with T2DM was a higher risk factor for diabetic neuropathy.

Table 3 shows that (37.5%) of neuropathy patients and (65%) of diabetic patients are under metformin treatment, also (32.5%) of neuropathy patients and (5%) of diabetic patients are under (metformin + Daonil) treatment, and (12.5%) neuropathy patients are treated with glimepiride.

Table 3. The Medication used for the treatment of the patients' group.

Parameters		Diabetes Mellitus (DM) Group No. (40)		Neuropathy Group No. (40)	
		No.	%	No.	%
medicines used	Daonil	6	15	2	5
	Daonil -metformin	2	5	13	32.5
	Amaryl	3	7.5	3	7.5
	Amaryl -metformin	----	----	1	2.5
	Glibenglamied	1	2.5	1	2.5
	Glimepiride	----	----	5	12.5
	Metformin	26	65	15	37.5
	Metformin-vildagliptin	1	2.5	----	----
	Metformin-gliptinen	1	2.5	----	----

Table 4. Correlation between HOMA IR and other biochemical parameters

		HOMA IR		
		Control Group No. (40)	Diabetes Mellitus (DM) Group No. (40)	Neuropathy Group No. (40)
Age (years)	R	-0.033	0.032	0.056
	P	0.839	0.847	0.733
Weight (kg)	R	0.170	-0.063	0.009
	P	0.293	0.698	0.956
Length (cm)	R	0.231	-0.052	0.072
	P	0.152	0.750	0.660
BMI (Kg/m2)	R	-0.044	-0.051	-0.032
	P	0.786	0.755	0.844
W/H ratio	R	0.296	-0.163	-0.120
	P	0.063	0.316	0.459
FBS (mg/dL)	R	0.075	0.264	0.358*
	P	0.647	0.099	0.023
HbA1C %	R	-0.170	0.115	0.201
	P	0.296	0.481	0.214
Cholesterol (mg/dL)	R	-0.110	-0.111	0.076
	P	0.498	0.495	0.640
TG (mg/dL)	R	0.324*	0.173	-0.079
	P	0.042	0.285	0.541
HDL-C (mg/dL)	R	-0.330*	0.104	-0.315*
	P	0.038	0.524	0.047
LDL-C (mg/dL)	R	-0.095	-0.172	0.160
	P	0.558	0.290	0.323
VLDL-C (mg/dL)	R	0.324*	0.173	-0.099
	P	0.042	0.285	0.543
Insulin (µIU/ml)	R	0.982**	0.865**	0.704**
	P	0.0001	0.0001	0.0001
GST activity (IU/L)	R	0.347*	0.148	-0.155
	P	0.027	0.358	0.337

*Correlation is significant at the 0.05 level.

**Correlation is highly significant at the 0.05 level.

Table 4 shows a positive correlation between HOMA IR and insulin in DM(r=0.865**p≤0.05) and NU. Groups (r=0.704**p≤0.05) insulin has a major role in glucose homeostasis control due to its integrated activities on carbohydrates, protein, and lipid metabolism. The liver, muscle, and fat are the tissues where these glucoregulatory effects are most prominent. Although insulin has a wide range of effects, the phrase "insulin resistance" usually relates to insulin's impact on glucose homeostasis [38]. HOMA IR also also a negative relationship with HDL (r=-0.315*, p≤0.05). The results of this study was in agreement with the study by Boris Waldman *et al*. [39] who also found that HOMA-IR levels were negatively related to HDL-C levels in DM patients. Regarding the T2DM patients, abnormal fat metabolism is the primary factor of vascular complications. Studies on human islet cells and animals showed that exogenous HDL enhanced insulin production by enhanced reverse cholesterol transfer, reduction of LDL, and inflammation-induced death of pancreatic β-cells [40-41]. Moreover, HDL may have an impact on glucose homeostasis through processes such as insulin secretion, increased insulin sensitivity, and direct glucose intake by the muscle [42]. HOMA IR showed a positive relationship with FBS in neuropathy group (r=0.358*, p≤0.05) a little increase in insulin resistance have been noticed by the effect of elevated FBS due to insufficient insulin to handle glucose. A previous study found that increased glucose levels and/or fast glucose fluxes led to reduce the pain of tolerance. These results might have consequences for the pathogenesis and treatment of painful diabetic neuropathy in the future [43]. Insulin enhances the expression of the hexokinase gene in GLUT4 and therefore it helps glucose retention in cells [44, 45]. This procedure is essential because it improves the reduction of blood glucose levels after a meal [46]. Any reduction in these receptors reduces the insulin sensitivity significantly [47]. The present study has shown there is no relationship between the insulin resistance and GST activity in patient groups. The limitation of this study is the delay in obtaining samples for peripheral neuropathy patients because the patient was subjected to a precise medical examination by the neurologist, according to the Toronto system, which diagnosed the patient has peripheral neuropathy if the score after the examination ≥6.

5. Conclusion

The present study has reported the improvement in insulin resistance in treated diabetic and neuropathy patients with metformin and sulfonylureas or together. These drugs are a promising treatment for patients with type 2 diabetes and also for those with peripheral neuropathy. The present study has also shown that oxidative stress is a risk factor for diabetes and peripheral neuropathy.

Ethical Approval: This study was approved by the scientific committee in the College of Science for Women, and a verbal consent form was obtained from each participant enrolled in the study.

Conflict of Interest: None.

Source of Funding: None.

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