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Investigation of the relationship between butyrylcholinesterase, lipoic acid synthetase and glutathione peroxidase activities in sera of diabetes mellitus type 2 patients

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Abstract---Diabetes mellitus type 2 is the world's most common chronic metabolic disease it's characterized by an increased level of blood glucose without changes in insulin liberation in the first stage. The goal of this study was to see the relationship between glutathione peroxide (GPX) activity, butyrylcholinesterase (BuChE) concentration, fasting blood glucose (FBG), and Lipoic acid synthetase (LIAS) concentration in sera of type 2 diabetes patients, and as biomarkers for illness progression monitoring and prediction. ELISA kits were used to assess all biomarkers in this study. In this study, FBG and HbA1c levels in patients are significantly higher ($P > 0.05$) than in the control group. The activity levels of Gpx were lower in diabetic patients ($46.5 \pm 11.81 \text{ U/ml}$) than in healthy controls ($59.72 \pm 17.2 \text{ U/ml}$) ($p < 0.001$). The Concentration of Butyrylcholinesterase (BuChE) in control was $13.127(14.158-12.219) \text{ pg/ml}$ whereas, its Concentration in patients group significantly increased ($p < 0.001$) to $15.787(14.278-17.657) \text{ pg/ml}$. whereas lipoic acid synthetase (LIAS) concentration in the control was insignificantly increased to $11.66(13.38-10.19) \text{ pg/ml}$ than in patients $8.77(6.57-10.04) \text{ pg/ml}$ ($P = 0.824$). We conclude there is a negative relationship between type 2 diabetes, gender, age, and BMI. HbA1c, FBG, LIAS, BuChE, and GPX are useful biomarkers for disease monitoring and should be used in standard clinical diagnostic processes to assess patient clinical conditions.

Keywords---Butyrylcholinesterase, Lipoic acid, glutathione peroxidase, diabetes mellitus.

1 Introduction

Diabetes mellitus type 2 (T2DM) is an endocrine disorder characterized by impaired insulin action and production. T2DM is becoming more common over the world, to the point where it is now considered a worldwide issue. Hyperglycemia is the most common sign of T2DM [1–2]. Hyperglycemia promotes an increase in oxidative stress [such as reactive oxygen species (ROS) and reactive nitrogen species (RNS)] generation as well as inflammation [3]. The best and most often used test for identifying diabetes is fasting blood glucose (FBS), which has a cutoff point of >126 mg/dl. However, there are some disadvantages to using FBS, such as the fact that clients must fast for around 8 hours and it is not applicable in the afternoon. Furthermore, in centralized screening, the HbA1c test, which assesses the fraction of glycated haemoglobin, is recommended. In addition, the HbA1c test determines the fraction of glycated haemoglobin, in diabetes. [4]. In addition to its utility in diagnosing diabetes, HbA1c is an important marker for assessing microvascular issues and plasma glucose [5]. Cholinesterase is a high-activity enzyme that catalyzes the hydrolysis of acetylcholine and other choline esters. Cholinesterase is divided into two categories: The first is acetylcholinesterase (AChE), which is found mostly in the nervous system, muscles, and erythrocytes and has a high affinity for acetylcholine. Second, butyrylcholinesterase (BuChE), also known as pseudocholinesterase, plasma cholinesterase, or serum cholinesterase, is a -glycoprotein that has a low affinity for acetylcholine and is found in the nervous system, liver, and other organs [6]. The glycoprotein butyrylcholinesterase (BuChE; EC 3.1.1.8) is found in the central and peripheral nervous systems, as well as the majority of tissues. The most frequent type is a homotetramer, which consists of 574 amino acids and nine glycosidic chains connected to the asparagine residue. Several investigations have connected BuChE activity to obesity, coronary artery disease, adiposity, type 2 diabetes, and hepatic fat storage. The plasmatic form of BuChE is produced by the liver. Choline and aliphatic esters such as butyrylthiocholine, butyrylcholine, propionylthiocholine, and Propionylcholine may be broken down [7]. ROS are produced by normal cell metabolism and play a crucial role in biology. While ROS are necessary for life, they can harm macromolecules such as lipids, proteins, and nucleic acids due to their high chemical reactivity. As a result, defence mechanisms in cells are triggered to control the generation of ROS and prevent oxidative harm. The majority of anti-ROS defence mechanisms are scavenging enzymes such as superoxide dismutases (SODs), catalase, peroxiredoxins, thioredoxins, and glutathione peroxidases [8]. The largest family of thiol antioxidant enzymes, glutathione peroxidases (GPx) (EC1.11.1.9), was the first family of selenoenzymes found in mammals.

These proteins with one or more selenocysteine residues are known as selenocysteine proteins [9]. It has been revealed that there are around five distinct mammalian GPx in the biological system. They use reduced glutathione (GSH) to catalyze the conversion of hydrogen peroxide to water or alcohol, depending on whether the hydrogen peroxide is organic or not. After giving electrons, reduced GSH is oxidized to glutathione disulfide (GSSG). Though they carry out comparable processes, each type of GPx has a different substrate. The truth is that GPx can reduce a variety of hydroperoxides, making them an important intracellular antioxidant in protecting the system from ROS-mediated cell death

[10]. The type II mitochondrial fatty acid synthase is involved in the production of lipoic acid (LA), also known as thioctic acid, in mitochondria (FAS II). FAS II produces octanoic acid in an acyl carrier protein (ACP)-bound way. This process is catalyzed by lipoic acid synthetase (LIAS), a highly conserved enzyme found in both prokaryotes and eukaryotes [11]. LA synthetase has two [4Fe-4S]-type iron-sulfur (FeS) clusters that act as sulfur donors and are essential for enzyme activity [12]. The potential role of LIAS expression as a marker for the diagnosis of DN was assessed. Also, LIAS expression can discriminate micro albumin uric(microalbuminuria) patients from those who had normal levels. So, it seems to be a useful marker for early diabetic detection [13]. The research aims to investigate the relationship between butyrylcholinesterase with GPX, and lipoic acid synthetase.

2 Materials and Methods

2.1 Ethical statement

Every volunteer has given written informed permission. This research has received ethical approval for scientific research from the MOH and MOHSER ethics committees in Iraq.

2.2 Study Population

A total of (90) people, ranging in age from 30 to 60, were diagnosed with type 2 diabetes mellitus. All of them have more than a one-year history of sickness. The samples were acquired at Hilla's Al-Marjan hospital. The diabetic patients were diagnosed using WHO guidelines. 30 seemingly healthy control volunteers (of both sexes) were chosen as healthy controls; they were non-smokers, drank no alcohol, and had no history of chronic disorders. Before participating in this study, all persons have signed a written consent form.

2.3 Serum collections

After obtaining the patient's written consent, 4 (ml) Venous blood samples were obtained in gel tubes after a 12-hour fast. at 2400 xg for 15 minutes Centrifugation was used to isolate serum. Biochemical markers such as FBS, HbA1c, glutathione peroxidase (GPx), lipoic acid synthetase (LIAS), and butyrylcholinesterase (BChE) were measured in serum. HbA1c levels were determined using Cobas c 111 analyzers (Roche, Switzerland) in Al-Marjan hospital in Hilla city.

2.4 Butyrylcholinesterase (BChE) Concentration

The levels of Butyrylcholinesterase (BChE) Concentration were determined using an ELISA kit following the manufacturer's instructions (Human Butyrylcholinesterase ELISA Kit, SUN LONG, China). The sandwich ELISA method is based on the binding of Butyrylcholinesterase to antibodies coated on the wells.

2.5 Glutathione peroxidase assay

The activity of GPX in serum was measured by using an Enzyme-Linked Immunosorbent assay (ELISA) according to the manufacturer's instructions (Human Glutathione Peroxidase ELISA Kit, Bioassay Technology Laboratory (BT Lab, China). The sandwich ELISA method is based on the binding of glutathione peroxidase to antibodies coated on the wells.

2.6 lipoic acid synthetase

The levels of lipoic acid synthetase were determined using an ELISA kit following the manufacturer's instructions (Human lipoic acid synthetase (LIAS) ELISA Kit, SUN LONG, China). The sandwich ELISA method is based on the binding of lipoic acid synthetase (LIAS) to antibodies coated on the wells.

2.7 FBS

The levels of FBS were determined using a kit following the manufacturer's instructions (GLUCOSE GOD-PAP – BIOLABO, France). The Trinder Method is a technique that Trinder created. When GOD and POD are coupled, glucose is converted to gluconic acid and hydrogen peroxide, which combines with chloro-4-phenol and PAP to produce scarlet quinone imine. The coloured complexes' absorbance, which is proportional to the glucose concentration in the material, is measured at 500nm.

3 Results and Discussion

In this study some of the demographic characteristics were assessed by analyzing questioners answered by direct interviews with control and case groups as indicated presented in table (1), Height, weight, BMI, age, and gender did not differ significantly between the two groups studied ($P > 0.05$).

Table 1
displays the study population's anthropometric indices

Parameters	Healthy control (n = 30)	patients (n = 60)	P-value
Age (years)	44.47 ± 8.760	47.55 ± 8.033	0.099
Height (cm)	170.63 ± 10.749	171.87 ± 9.146	0.571
weight (kg)	75.53 ± 8.228	77.83 ± 7.293	0.180
BMI (kg/m ²)	25.960 ± 1.880	26.347 ± 1.441	0.281
Gender (M/F)	20/10	38/22	0.755

Other studies support our findings, indicating that there are no significant variations in age, height, or weight between the two groups ($P > 0.05$). [14,15]. which shows that there are insignificant differences in age, height, or weight between the study groups tested ($P > 0.05$). [15,16]. T2DM patients had a significantly greater BMI than healthy controls ($p = 0.016$). [14]. Another study demonstrated significant weight and BMI variations between the two groups ($P = 0.003, 0.007$, respectively) [16]. Ruderman et al. discovered a link between type 2

patients and various symptoms, such as FBG, obesity, dyslipidemia, and hypertension [17].

3.1 FBG and HbA1c Levels

There is a significant increase ($p < 0.001$) of FBG and HbA1c in patients compared with the control group as shown in Table (2). The concentration of FBG in control was (5.593 ± 1.145) mM while its concentration in the case was significantly increased to (9.485 ± 2.201) mM. The level of HbA1c in control was (5.135 ± 0.718) % while its levels in the case were significantly increased to (7.639 ± 1.495) %.

Table 2
Serum glucose and HbA1c levels of Diabetes subjects and healthy controls

Biomarker	Control	Patients	df	F	P-value
FBG mM	5.593± 1.145	9.485± 2.201	1/88	82.256	<0.001
HbA1c %	5.135±0.718	7.639± 1.495	1/88	75.102	<0.001

Several studies back up our findings. In diabetic type 2 patients, FBS (fasting blood sugar), and HbA1C were all significantly higher than in healthy people. Fasting insulin blood concentrations, on the other hand, there was no statistically significant difference between the two groups, according to the findings. [15]. In comparison to the control group, the patient group had statistically substantially greater weight, BMI, insulin, FBG, and HbA1c levels ($P > 0.05$) [16]. Glycated HbA1c is often utilized as a glycemic control clinical indication. It is a simple examination of mean glucose levels over the previous 2-3 months that is used to identify patients who have undiagnosed type-2 Diabetes or who are at risk of developing it [18]. The average blood glucose level over the previous three months is represented by glycated haemoglobin (HbA1c). As a result, HbA1c is a critical biochemical marker for determining long-term blood glucose levels and as a monitoring tool for glycemic control in patients [19]. HbA1c is formed when haemoglobin binds to glucose in the bloodstream and becomes glycated [20]. FBS of patients was 175.5 (mg/dL) according to the results, which is substantially higher than the control group's mean of 74.17 (mg/dL) ($p < 0.01$). In terms of glycated haemoglobin HbA1c, type-2 diabetes patients had a substantially higher mean value of 8.852 compared to 5.16 in the control group ($p < 0.01$) [19]. FBG and HbA1c levels are higher in patients than in healthy people, fasting blood glucose and HbA1C were found to have a substantial connection [21].

3.2 Glutathione Peroxidase (Gpx) Activity

The present study shows that the activity of Glutathione Peroxidase in control was 59.723 ± 17.2 U/ml whereas, its activity in the patient's group significantly decreased ($p < 0.001$) to 46.5 ± 11.8 U/ml as shown in Table (3).

Table 3
Glutathione Peroxidase (Gpx) Activity in patients and control groups.

Biomarker	Control 30	Patients 60	df	F	P-value
GPX (U/ml)	59.72 ±17.2	46.5 ±11.81	1/88	18.095	<0.001

Another study [14] found that GPx activity was reduced in those diabetic patients. In contrast, we found no statistically significant changes ($P > 0.05$) between the two groups in plasma GPx activities [16]. In addition, compared to healthy controls, diabetes groups had increased GPX activity. It has been claimed that the system of antioxidants counteracts the effects of increasing free radicals in the early stages of type 2 diabetes, but that by the late stages, the balance between free radical creation and antioxidant defence has shifted [22]. It has been proposed that excessive levels of oxidative stress markers in the blood hinder the production of antioxidant enzymes by oxidizing transcription factors in the early stages of type 2 diabetes [23].

3.3 Butyrylcholinesterase (BuChE) Concentration

The present study shows that the Concentration of Butyrylcholinesterase (BuChE) in the control was 13.127 (14.158-12.219) pg/ml whereas, its concentration in the patient's group significantly increased ($p < 0.001$) to 15.787(14.278-17.657) pg/ml as shown in Table (4).

Table 4
Butyrylcholinesterase (BuChE) concentration in patients and control groups

Biomarker	Control 30	Patients 60	P-value
BuChE pg/ml	13.127(14.158-12.219)	15.787(14.278-17.657)	<0.001

Diabetes-induced metabolic alterations have been shown to influence blood Butyrylcholinesterase (BuChE) in numerous studies. Increases in plasma, serum, and BuChE in patients' serum were found in a recent study [24]. BuChE may play a role in the development of type 2 diabetes via interacting with amyloid fibrils. [25]. Furthermore, type 2 diabetes mellitus has been related to a locus on chromosome 3q27, which is near the chromosomal locus of BuChE. BuChE inhibits the production of amyloid fibrils in vitro, according to a recent study [26]. Butyrylcholinesterase and type 2 diabetic Mellitus the relationship between serum BuChE activity and type 2 diabetes mellitus and metabolic syndrome components have been studied in people in several research. A total of 107 people with type 1 and type 2 diabetes were studied [27]. In type 2 diabetes, while BuChE activity was correlated with insulin sensitivity, it was not related to others such as gender, age, BMI or type of treatment of diabetes. However other studies have shown that BuChE activity was related to serum lipid profile and parameters of obesity in addition to type 2 patients. Among 259 Japanese subjects with type 2 diabetes, BuChE activity correlated with waist circumference, visceral fat area, and subcutaneous [28].

3.4 Lipoic Acid Synthetase Concentration

The lipoic acid synthetase (LIAS) concentration in the present study has appeared insignificant differences ($p= 0.824$) between control and patients with T2DM, the results showed that the concentration of LIAS in patients was 8.77(6.57-10.04) pg/ml while its concentration in the control was insignificantly increased to 11.66(13.38-10.19) pg/ml as shown in figure (1).

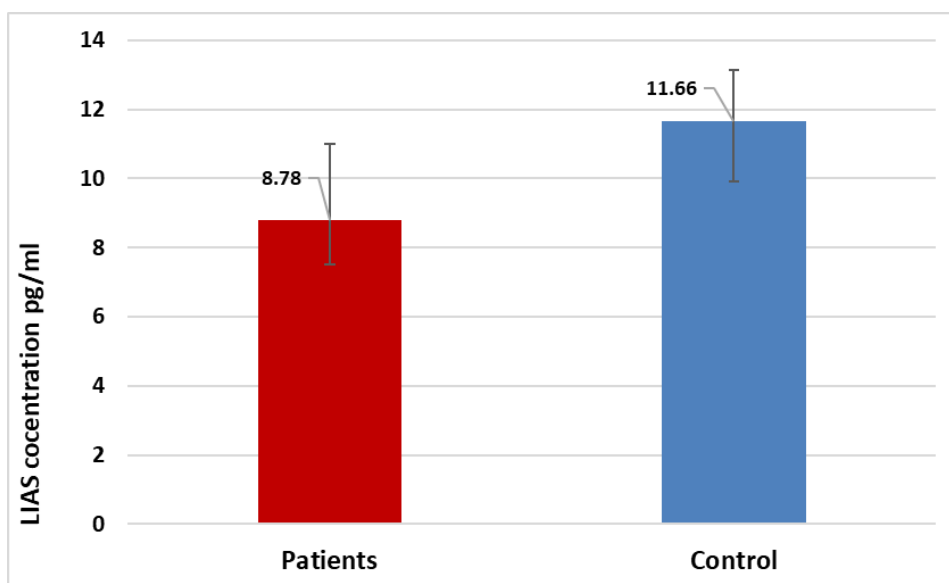


Figure 1. lipoic acid synthetase concentration in serum of T2DM patients and control groups

Increased oxidative stress is a feature of diabetes mellitus, and it has a deleterious impact on mitochondrial integrity and function. As a result of its participation in lipoic acid production, LIAS has physiologic significance in diabetes [29]. Mutations in the structural gene LIAS or a lack of synthesis of the FeS cluster cofactors can induce LA synthetase deficiency. Defects in LA synthetase affect all lipoyl-containing enzymes, including the 2-oxoacid dehydrogenases and the Glycine cleavage system. LIAS mutations were discovered in three index individuals with atypical nonketotic hyperglycemia and altered mitochondrial energy metabolism [11]. Lipoic acid synthetase (LIAS) and GPX showed a significant positive Correlation ($P < 0.05$). BuchE with lipoic acid synthetase (LIAS) and GPX had a significant negative correlation ($p < 0.05$). HbA1c showed a significant positive correlation ($P < 0.05$) with BuchE and lipoic acid synthetase (LIAS), a negative correlation with GPX (Table 5). (Figure 2).

Table 5
Correlation among the biomarker's levels in type 2 diabetic patients

Variable	GPX	LIAS	BuchE	HbA1c
GPX	1	0.304**	-0.037	-0.259*
LIAS	0.304**	1	-0.230*	-0.419**

BuchE	-0.037	-0.230*	1	0.366**
HbA1c	-0.259*	-0.419**	0.366**	1

* Significant correlation ($p < 0.05$). ** Significant correlation ($p < 0.01$).

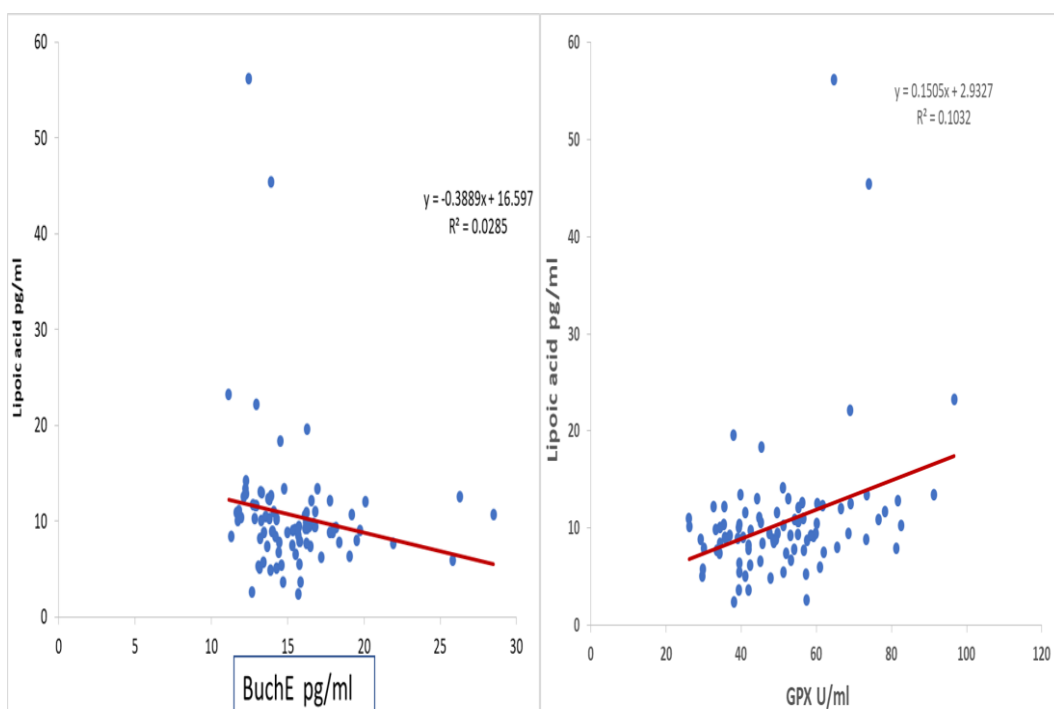


Figure 2. The correlation between LIAS and Gpx and BuchE.

The activity of GPx declines, due to decreased H₂O₂ generation, Furthermore, an increase in superoxide radicals could render GPx inactive, when compared to age- and sex-matched controls, LIAS expression is dramatically reduced in tissues from animal models of diabetes and obesity [30]. Serum and placental butyrylcholinesterase activity in type 2 diabetes mellitus showed a strong positive correlation with total antioxidant activity in serum, Butyrylcholinesterase may, therefore, be involved in reducing oxidative stress in diabetic pregnancy [31]. The glycosylated haemoglobin (HbA1c levels were significantly increased ($p < 0.001$), and antioxidant enzyme activities such as glutathione peroxidase (GPx), was significantly decreased ($p < 0.001$) in T2DM, there were good negative correlations of HbA1c and GPx ($r = -0.79$) [32]. Other results showed a significant increase in HbA1c in the diabetic group; in addition to a positive correlation between HbA1c and GPx, and FBS [33].

Conclusion

We conclude there is a negative relationship between type 2 diabetes, gender, age, and BMI. HbA1c, FBG, LIAS, BuchE, and GPx are useful biomarkers for disease monitoring and should be used in standard clinical diagnostic processes to assess patient clinical conditions.

Conflict of Interest

The authors have no conflict of interest to be declared.

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