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Evaluation of Glutathione S Transferase and Glutathione Levels in Serum of Normal Individuals and Patients with Diabetes Mellitus

Entedhar Riffat Sarhat
Department of Biochemistry / College of Dentistry / Tikrit University

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ABSTRACT:-

Diabetes Mellitus (DM) is considered as a member of oxidative stress syndrome. It is associated with an imbalance between types of free radicals and scavenging system. Sixty patients with DM were studied for the changes in their Glutathione s- Transferase (GSTs) and Glutathione (GSH) in the blood. Who attended out patient, and Primary health care In Tikrit Teaching Hospital in Tikrit City. This Study concluded that GST activities are significantly higher in serum of patients with type I and II diabetes mellitus disease than that of controls, $P \leq 0.05$. But there was a significant decrease in levels of GSH activity in both types of diabetic patients.

Key words: Glutathione s- Transferase (GSTs), Glutathione (GSH), & Diabetes Mellitus (DM).

تقييم فعالية أنزيم الكلوتاثايون- اس- ترانسفريز والكلوتاثايون المختزل في مصل الدم للأشخاص الطبيعيين و المصابين بمرض السكري

انتظار رفعت سرحت

المستخلص:-

يعد مرض السكري من العناصر المتلازمة لفرط الأكسدة ذات الصلة بالتوازن بين أنواع الجذور الحرة ونظام الكاسحات. تمت دراسة التغيرات في أنزيم الكلوتاثايون- اس- ترانسفريز والكلوتاثايون المختزل في دم المرضى المصابين بمرض السكري البالغ عددهم ستين مريضا . أجريت على المرضى المراجعين لمراكز الصحية لمستشفى تكريت التعليمي في تكريت. أشارت هذه الدراسة إلى أن الأنزيم GST يرتفع مستواه بشكل ملحوظ في مصول المرضى المصابين بالنوع الأول والثاني من المرض السكري عن مستوياتها في المجموعة الضابطة ولكن الدراسة اظهر انخفاضا ملحوظا في مستوى أنزيم GSH في المرضى لكلا نوعي مرض السكري.

INTRODUCTION:-

Diabetes mellitus is a very complex chronic disease with syndrome of hyperglycemia. Its result from absolute or relative decrease in insulin secretion from β-cell of the islet of langerhans. The cause of diabetes mellitus is not fully understood. Free radicals formation is involved in the pathogenesis of diabetes and the development of diabetic complications. In type1; β-cells are prone to be destroyed by free radicals because of the low antioxidant enzyme nature [1]. Type 2 DM is a heterogeneous disorder, that is, very different pathologic events result in the same clinical symptom. Genetic abnormalities, or environmental factors, or obesity, which may induce β -cells malfunction and/or insulin resistance can cause mild hyperglycemia which further develops to type 2 DM. Some studies indicate that there are alterations in free radicals generation and antioxidant enzymes. There free radicals activity in type 2 DM patients was increased as measured by the markers of free radicals activity [2].

Glutathione is γ-glutamyl cysteinyl glycine, the most abundant non proteinthiol compound, found in almost every cell; it can not enter most cells directly and therefore must be made inside the cell, from its three constituent amino acids: glycine, glutamic acid and cysteine molecule that give the glutathione molecule its biochemical activity [3, 4]. Glutathione is the major antioxidant produced by the cells, protecting it from the harmful effect of free radicals (oxygen radical or oxy radical); these highly reactive substances if left unchecked, will damage or destroy key cell components i.e. (membranes, DNA) in microsecond. Also, it is a very

important detoxifying agent enabling the body to get rid of toxins and pollutants. It forms a soluble compound with toxins that can be excreted through the urine or the gut. Liver and kidney which contain high levels of glutathione as they have the greatest exposure to toxins. The lung is also rich in glutathione partly for the same reason [4]. In diabetic patient GSH deficiency resulting from NADPH is used in the polyol pathway where glucose is reduced to sorbitol by aldose reductase. An increased activity in this pathway causes a depletion of reduced GSH, which may weaken the antioxidant defense [9, 10].

Glutathione –S- Transferase (Ec2.5.1.18) is ubiquitous multifunctional enzymes. GSTs are thought to play a physiological role in initiating the detoxification of potential alkylating agents, including pharmacologically active compounds [11]. The transferases increase the nucleophilic properties of reduced glutathione (GSH) and they catalyze the formation of thioether bond by conjugated hydrophobic compounds with GSH to form products consist very stable thioether bond; these products excreted by the bile as GSH conjugates and then cleavage glycine or glutamine and acetylation the free amino group of cysteinyl residue to give the end product (Mercapturic acid) [9]. Base on their sequence homology, substrate specificity and immunological cross reactivity, GSH have been grouped into five species-independent classes of isoenzymes [12]. Four of these classes (alpha. Pi, mu and theta) comprise cystosolic enzymes, a fifth rather distinct form is micrisomal. All cystosolic glutathione-S- transferases are found to be homo- or hetero-dimmer enzymes (from within the same class) with a relative molecular weight of 50 000 Dalton. They are active over a wide variety of substrates with considerable overlap [13].

MATERIALS AND METHODS:-

This research was conducted in Tikrit Governorate, Tikrit Teaching Hospital. The samples included (16) patients suffering from typeII of diabetes aged between (34) and (60) years; and (15) patients with type I diabetes aged between (13) and (60) years, controlled with (30) healthy individuals aged between (13) and (60) years. The blood was drawn from venous of fasting patients recently diagnosed with diabetes mellitus (type I and TypeII) and healthy subjects were used as control. And then centrifuged to be used serum samples for detection of variable in this study.

Determination of serum Glutathione S-Transferase [15]. The activity was determined by using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. Were; the difference between the first absorbance at 1st minute and 10th.

Determination of serum glutathione [16]. 5, 5 –Dithiobis (2-nitrobenzoic acid) (DTNB) is a disulfide chromagen that is readily reduced by sulfhhydryl group of GSH to an intensely yellow compound. The absorbance of the reduced chromagen is measured at 412 nm.

RESULTS:-

Sixty patients with DM (15 with type IDM), and (15 with type II DM), and 30 healthy individuals served as control were enrolled in the present study.

Age and Sex: The mean age ± SD of patients with type I DM was (31.97±6.9 years VS 29 ±6.1 years of control), while in type 2 DM was (52±8.8 years VS 48.04±8.4years of control).

Biochemical Parameters: Table 1, 2, and 3: illustrated the differences between the mean of levels of Glutathione –S-Transferase in serum of healthy control and patients with type I and II diabetes mellitus (DM) disease respectively.

DISCUSSIONS:-

Glutathione –S-Transferase Serum GSTs activity in diabetic patients (with type I and Type II DM) was significantly higher than control activity, $P<0.05$. The mean± SD or serum GSTs levels of patients with type I DM was 4.47+ 1.9 µ/L VS 2.81± 1.73 µ/L of control), while in patients with TypeII DM was (3.99± 0.43 µ/L VS 2.96 ± 1.65 µ/L of control as in table (2). Glutathione –S-Transferase is an antioxidant enzyme that catalyzes the reaction between reduced glutathione (GSH) and drugs, xenobiotics and other toxic compounds, rendering them more water soluble and finally exerted from the body [13]. Some chemical compounds, which augment oxygen products, have generated

toxic effect such as many drugs and xenobiotics, which contain quinine groups, cause free radicals. This radicals reduces the molecule oxygen immediately, the super oxide anion (O20-) comes into existence and, following that other reactive oxygen species (ROS) are generated(17,18,19).

Glutathione levels In this work, serum GSH activity in diabetic patients (with type I and type II DM) was significantly lower than control activity, $P<0.05$. The mean± SD of serum GSTs levels of patients with type I DM was 0.2 ± 1.9 µmol/L VS 1.52± 0.65 µmol/L of control), while in patients with TypeII DM was (0.83± 0.17 µmol/L VS 2.96 ± 0.38 µmol/Lof control). Serum GSH plays a central role in antioxidant defense. GSH detoxifies ROS such as H2O2 and lipid peroxides directly or in a glutathione peroxidase (GPX) catalyzed mechanism. GSH also generates the major aqueous and lipid phase antioxidant, ascorbate and ά-tocopherol. Glutathione reductase (GRD) catalyzes the NADPH dependent reduction of oxidized glutathione (GSSG) serving to maintain intracellular GSH stores and a favorable redox status GSH catalyzes the reaction between the –SH group and potential alkylating agents (3, 22, 23). The result of this study show significant negative correlation ($r = -0.309$, $P<0.05$) between levels of GSH and levels of GST activity in serum of patients with type2 DM, but this correlations was not significant in patients with type 1 DM($r= -0.2045$, $P<0.05$). These results probably were due to the; depletion serum GSH levels lead to slightly accumulation of intracellular homcysteine, which induces endothelial cells damage and slightly increases risk of cardiovascular disease in diabetic patients. Presence of high level of toxic compounds in diabetic patients, especially with type 2 DM (treatment with hypoglycemic such as tablets f danil, glucphage……) induce accumulation of GST enzyme and altered GSH metabolism, therefore toxic compounds increase ROS production that decreases levels of GSH, while increases synthesis of GST enzyme to play a protective role(14,19).

Table (1) Levels of Glucose Serum of Healthy Control and Patients with Type I and II Diabetes Mellitus (DM) Disease

	Type I DM	Control	Type II DM	Control
Glucose	280.2±75.6 mg/dl	95.7±12.6 mg/dl	188.7±48.6 mg/dl	105.03±11.28 mg/dl

P<0.05

Table (2) Levels of Glutathione –S-Transferase in Serum of Healthy Control and Patients with Type I and II Diabetes Mellitus (DM) Disease

	Type I DM	Control	Type II DM	Control
GSTs	4.47± 1.9 µ/L	2.81± 1.73 µ/L	3.99± 0.43 µ/L	2.96 ± 1.65 µ/L

P<0.05

Table (3) Levels of Glutathione in Serum of Healthy Control and Patients with type I and II Diabetes Mellitus (DM) Disease

	Type I DM	Control	TypeII DM	Control
GSH	0.68± 0.2 µmol/L	1.52± 0.65 µmol/L	0.83± 0.17 µmol/L	2.96 ± 0.38 µmol/L

P<0.05

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