The Decrement of Glutathione Peroxidase Activity Associated with the Elevated Diene Hydroperoxide Levels in Serum of Patients with Diabetes Mellitus Type I

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Abstract:-

The glutathione peroxidase, vitamin E and dienhydroperoxide in serum were investigated in 30 subject with Type 1 (insulin-dependent) diabetes mellitus and in 30 healthy others. The mean conjugated hydroperoxide(CDH) value in the serum of diabetic patients were significantly higher than those in the control subjects (conjugated dienhydroperoxide $p<0.01$). The Glutathione peroxidase activity and the levels of Vitamin E significantly decreased(GPx $p<0.05$ while Vitamin E $p<0.01$). These data suggest that hyperglycemia induces oxidative stress in sera of patients compared with healthy controls and that antioxidants which used in the study are effective against oxidative injury.

Conjugated Dienes Hydroperoxides (CDH), Reactive Oxygen Species (ROS), Glutathione peroxidase (GPx), Poly Unsaturated Faty Acids (PUFAs), Catalase (CAT),.Reduced Glutathione(GSH) .Oxidized Glutathione(GSSG)

نقصان فعالية أنزيم الكلوتاثيون بيروكسيديز يرتبط بزيادة مستوى الدايين هيدروبيروكسيد في مصل مرضى السكري المعتمد على الأنسولين

ألخلاصة:-

تم قياس فعالية أنزيم الكلوتاثيون بيروكسيديز,و مستويات فيتامين E والدايين هيدروبيروكسيد في مصل الأشخاص المصابين بمرض السكري المعتمد على الأنسولين وكذلك الأشخاص الأصحاء,أظهرت النتائج انخفاضا معنويا في فعالية إنزيم الكلوتاثيون بيروكسيديز وكذلك مستويات فيتامين E بينما ازدادت مستويات الدايين هيدروبيروكسيد في مصل مرضى السكري مقارنة بمستوياتها لدى الأشخاص الأصحاء.هذه النتائج دليل لمقاومة الإجهاد

ألتأكسدي لدى الأشخاص المصابين بمرض السكري المعتمد على الأنسولين من قبل مضادات الأكسدة VitE & GPx.

Introduction:-

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels[1].Type 1 diabetes is one of the most frequent chronic diseases[2].This type is characterized by the autoimmune destruction of the pancreatic beta cells[3]. Consequent chronic exposure of tissues to supraphysiologic levels of blood glucose can lead to adverse intracellular outcomes, a process known as glucose toxicity [4,5]. Possible mechanisms of action for glucose toxicity include the formation of advanced glycosylation end products and glucosamine, increased protein kinase activity, autooxidation of glucose, and increased levels of reactive glycolytic intermediates such as glyceraldehyde-3-phosphate or dihydroxyacetone phosphate [6,7,8]. All these processes usually are accompanied by the formation of reactive oxygen species (ROS), setting up the potential for oxidative stress[9]. Glutathione peroxidase (GPx, EC 1.11.1.9) has a mechanism for detoxification of peroxides in cells[10]. This reaction plays a crucial role in protecting cells from damage by the action of free radicals, which are formed by peroxide decomposition. Lipid components of the cell are especially susceptible to reactions with free radicals, resulting in lipid peroxidation. GPx enzymes reduce hydrogen peroxide (H_2O_2) and a wide variety of organic peroxides (R-OOH) to the corresponding stable alcohols (R-OH) and water using cellular glutathione as the reducing reagent[11,12]. Most cellular glutathione peroxidases are tetrameric enzymes consisting of four 22 kDa monomers, each of which contains a selenocysteine moiety in the active site[10] .The selenocysteine participates directly in electron donation to the peroxide substrate and becomes oxidized in the process. The enzyme then uses reduced glutathione as a hydrogen donor to regenerate the selenocysteine. GPx enzymes also exist as non-selenium (non-Se) containing enzymes[13].

Elevated levels of lipid peroxides are accompanied by an increase in peroxyl radicals,

which can inactivate NO through the formation of lipid peroxynitrites [14,15]. Thus a deficiency of GPx would theoretically lead to an increase in ROS and a decrease in bioavailability NO. Because GSH represents one of the most important intracellular antioxidants, primarily as a co-substrate for GPx, we hypothesized that this antioxidant system plays a central role in protecting the vasculature in states of increased oxidant stress.

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defense system and is exclusively obtained from the diet. The term "vitamin E" refers to a family of eight naturally occurring homologues that are synthesized by plants from homogentisic acid[16]. The major biologic role of vitamin E is to protect PUFAs, the other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals[17]. Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation, a series of chemical reactions involving the oxidative deterioration of PUFAs[18].

The initial products of lipid peroxidation are conjugated dienic hydroperoxides [19]. These active substances decompose either into various aldehydes or, if the original fatty acid is arachidonic acid, into isoprostanes, (shown fig 1).

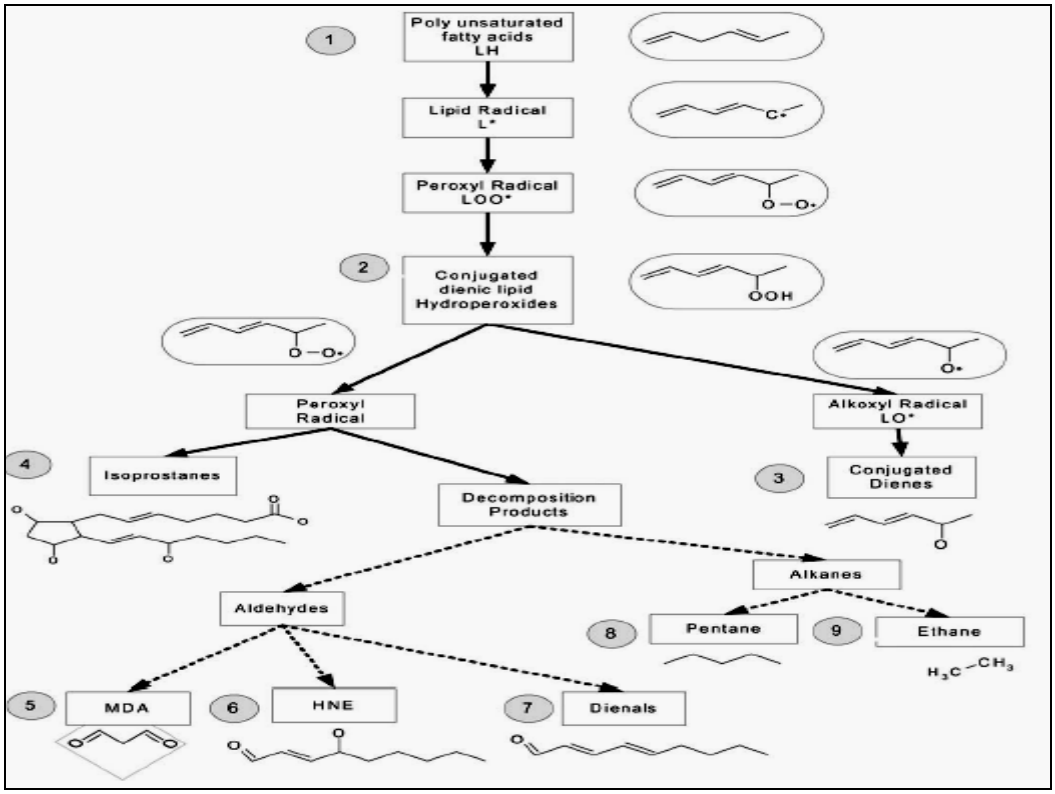


Fig 1:-The Products and Pathways Relating to Lipid Peroxidation. This Figure Describes the Various Products Polyunsaturated Fatty Acids (1) Lipid Hydroperoxides (2) Conjugated dienes (3) Isoprostances (4) MDA (5) HNE (6) Dienals (7) Alkanes(8,9).

All these products of degradation and decomposition are used in assessing oxidative stress, including hydro- peroxides (LOOH), commonly expressed as conjugated dienes hydroperoxides (CDH), as well as the widely used end products malondialdehyde (MDA) [20] and isoprostanes .

The aims of the present study were determine whether oxidative damage occurs, and to what degree, at diabetes mellitus type I disease evolution in patients with clinical manifestations and to assess the oxidant/antioxidant balance in the whole diabetic group in relation to other healthy control group. The indicative parameter of conjugated dienehydroperoxide with one of the enzymatic antioxidant system activity (glutathione peroxidase (GPx)), and one of the endogenous radical scavengers (α-tocopherol (vit E)) were evaluated. This is a new study to describe diene hydroperoxide inthe diabetes mellitus.

Patients

We studied 30 type I diabetic patients (15 males,15 females; ages 26-36 years). All patients were diagnosed at the Diabetes Unit of AL-Qadisyia general Hospital. The study also included 30 healthy individuals, aged 26-36years who were not taken any medication.

Blood Sample Collection

Blood samples were drawn in the fasting state and processed within 20 min of collection. After clotting, serum was separated by centrifugation and divided in three aliquots.

Materials and Methods

Reagents

All reagents, unless otherwise indicated, were obtained from Sigma Chemical Co. (St. Louis, USA).

Determination of the Total Glutathione Peroxidase Activity (Se&non Se Enzyme):-GPx activity was measured by the method described by Rotruck *et al*. [21]. Briefly, reaction mixture contained 0.2 ml of 0.4 M Tris-HCl(BDH) buffer pH 7.0, 0.1 ml of 10 mM sodium azide(Fluka), 0.2 ml of serum, 0.2 ml glutathione,0.1 ml of 0.2 mM Cumene hydroperoxid(Fluka)e. The contents were incubated at 37°C for 10 min. The reaction was arrested by 0.4 ml of 10% TCA, and centrifuged. Supernatant was assayed for glutathione content by using Ellman's reagent (19.8 mg of 5,5'-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate).

Conjugated Diene Hydroperoxide (CDH) levels was measured by the method described by Pryor *et al.* [22]. α-Tocopherol (Vit E) level was measured by the method described by Toro *et.al*[23]. Briefly, the reduction of ferric ions to ferrous ions produce by α-tocopherol and the formation of a red coloured complex with α-ά-dipyridyl at 520 nm.
(UV-Visible spectrophotometer double beam Shemadzu-1601(Japan 2005) used in this research).

Statistical:-

The results are expressed as number, range, convedance interval C.I 95% and whenever possible as mean ±SD(SE) of number of observation .The data were performed using Microsoft Excel version 6. The hypothesis testing was performed using student's "t" and correlation test taking $p \leq 0.05$ as the lowest limit of significance .

Results:-

Table 1 show GPx activity in serum of diabetic and control groups. Which were significantly lower than (11%) in serum of type I diabetic patients versus healthy control.

Table(1):-Glutathione Peroxidase Activity (U/L) in Sera of Patients and Healthy Controls.

	Sex	Mean	SD	SE	95 % C.I		P	Sign.
					Upper	Lower		
Control	M	169	38.59	10.0	189.2	148.8	--------	-------
	F	165	35.7	9.21	185.8	144.16	--------	-------
Type I	M	149.16	37.73	9.73	171.16	127.15	<0.05	Sign.
	F	147.6	38.9	10	177.22	125	<0.05	Sign.

α-Tocopherol (Vit E), an effective lipophilic antioxidant and free radical scavenger, was determined in serum of diabetic and control subjects. Significant decreases of α-tocopherol levels in serum of type 1 diabetic patients were observed when compared with their respective control subjects (Table 2).

Table(2):- Vitamin E Levels (mg/l) in Sera of Patients and Healthy Controls.

	Sex	Mean	SD	SE	95 % C.I		P	Sign.
					Upper	Lower		
Control	M	8.883	1.368	0.353	9.68	8.0	--------	-------
	F	9.05	1.437	0.37	9.84	8.25	--------	-------
Type I	M	7.8	1.405	0.36	8.61	7.0	<0.01	Sign.
	F	8.05	1.38	0.356	8.85	7.7	<0.01	Sign.

Finally, the assess the overall conjugated diene hydroperoxide level in serum. Compared with healthy control subjects, a marked increase of CDH level (38%) was found in type 1 diabetic patients compared with healthy control subjects (Table 3).

Table (3):- CDH Levels (µ mole/L) in Sera of Patients and Healthy Controls.

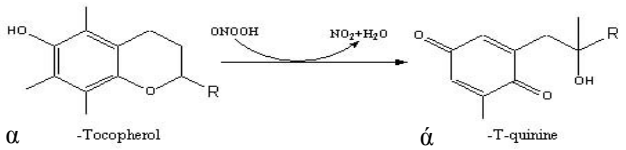
	Sex	Mean	SD	SE	95 % C.I		P	Sign.
					Upper	Lower		
Control	M	9.25	1.54	0.39	10.13	8.36	--------	-------
	F	9.4	1.5	0.26	10.0	8.8	--------	-------
Type I	M	14.41	3.67	0.94	16.5	12.3	<0.01	Sign.
	F	14.6	3.63	0.86	16.5	12.65	<0.01	Sign.

Discussion:

Oxidative stress plays a role in the development of diabetic complications. In the diabetic state, protein glycation and glucose auto-oxidation can lead to the formation of free radicals [24]. The main free radicals that occur in this diseased state are superoxide ($O_2^{\bullet -}$), hydroxyl ($^{\bullet}OH$) and peroxyl ($LOO^{\bullet}$) radicals. These free radicals all might play a role in DNA damage, glycation and protein modification reactions, and in lipid oxidative modification in diabetes. Certain enzymes play an important role in antioxidant defense, such as cellular GPx which present in all tissues; however, various diseases may influence its level. A decrease in the level of the enzyme has been observed in patients suffering from diseases such as Favism [25](a disease associated with extreme hemolytic crisis) or hairy cell leukemia[26].Table (1) show the GPx activity were found to be lower in the present study. The decrement of GPx activity may be beyond to that patients with Diabetes mellitus have low levels of selenium, (Hamanishi *et al.* demonstrated that GPx is a selenocysteine-containing enzyme and a low serum selenium level induces a decrease in GPx enzyme activity [27]).In addition, hyperhomocysteinemia which increased in serum of diabetic patients, is one of the risk factors for atherosclerotic vascular disease. It has been reported that homocysteine inhibited the expression of GPx and lead to an increase in reactive oxygen species [28]. Also, the elevated of $O_2^{\bullet -}$ levels in serum of patients with Diabetes Mellitus due to the depression of GPx activity.($O_2^{\bullet -}$ anions have been shown to inactivate GPx and activated CAT [29]). The reduction of the GPx might be a result of high glucose concentration, previous study [30] has been reported that enzymatic inactivation might occur through glycation governed by hyperglycemia; thus increased glycation in diabetic patients and the subsequent reactions of proteins might affect amino acids close to the active sites of the molecule or disturb the stereo chemical configuration, there by provoking structural and functional changes in proteins. Also, the low GPx activity could be directly illustrate by the low GSH content found in serum of diabetic patients, since GSH is a cofactor of this enzyme. There fore, low GSH content necessitate low GPx activity, which may produce increased

oxidative stress inclination. *In vitro* studies have shown that although GPx is a relatively stable enzyme, it may be inactivated under conditions of severe oxidative stress[31]. Several animal models suggest that increasing intakes of antioxidant substances increased the activity of GPx in sera and tissue of diabetic animals[32,33,34,35].

Vitamin E is an example of a phenolic antioxidant. Such molecules readily donate the hydrogen from the hydroxyl (-OH) group on the ring structure to free radicals, which then become unreactive. On donating the hydrogen, the phenolic compound itself becomes a relatively unreactive free radical because the unpaired electron on the oxygen atom is usually delocalized into the aromatic ring structure thereby increasing its stability[36].Table (2) show Vitamin E levels were found to be lower in the present study. The low Vitamin E could be beyond to its antioxidant property, this property ensures the protection of PUFAs and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals[37]. It is particularly effective in preventing lipid peroxidation, a series of chemical reactions involving the oxidative deterioration of PUFAs. Elevated levels of lipid peroxidation products are associated with numerous diseases and clinical conditions[18].The elevated of peroxynitrate level in serum of Diabetic patients[38] could be the essential cause to decrement Vitamin E because α-tocopherol was regarded as a defense substance against peroxynitrate attack, the protective action of the α-tocopherol was showed by equation below[39].



Serum CDH levels has been found to be increased in this study as show in table (3). The elevated CDH level could be beyond to a higher production of reactive oxygen species, which ascribed to protein glycation and/or autoxidation caused by an hyperglycemic environment, and peroxidation of cellular structures (a consequence of free radical activity) is thought to play an important role in diabetic complications [40]. The second cause to increase CDH levels was the lowering of GPx activity, which can lead to a relative accumulation of 12-HpETE(12-hydroperoxy-eicosatetraenoic acid), the main CDH formed from arachidonic acid, and such an increase could activate signal transduction pathways leading to arachidonic acid release [41],also Pang *et al*. were demonstrated that a tendency to decreased GPx activity could increase both the intracellular peroxide level and oxidative damage [42] .The other cause to high CDH levels may be the elevated free fatty acids (FFAs) in plasma, which can increase products of peroxidation [39].

In summary, we report significant differences between the diabetic and control groups. However, the defense mechanisms were fairly efficacious against oxidative stress under diabetic conditions. This was demonstrated by the variable levels of antioxidative enzyme(GPx), the relatively low concentration of Vitamin E ,and high levels of CDH. Figure 2

show the relationship between these parameters.

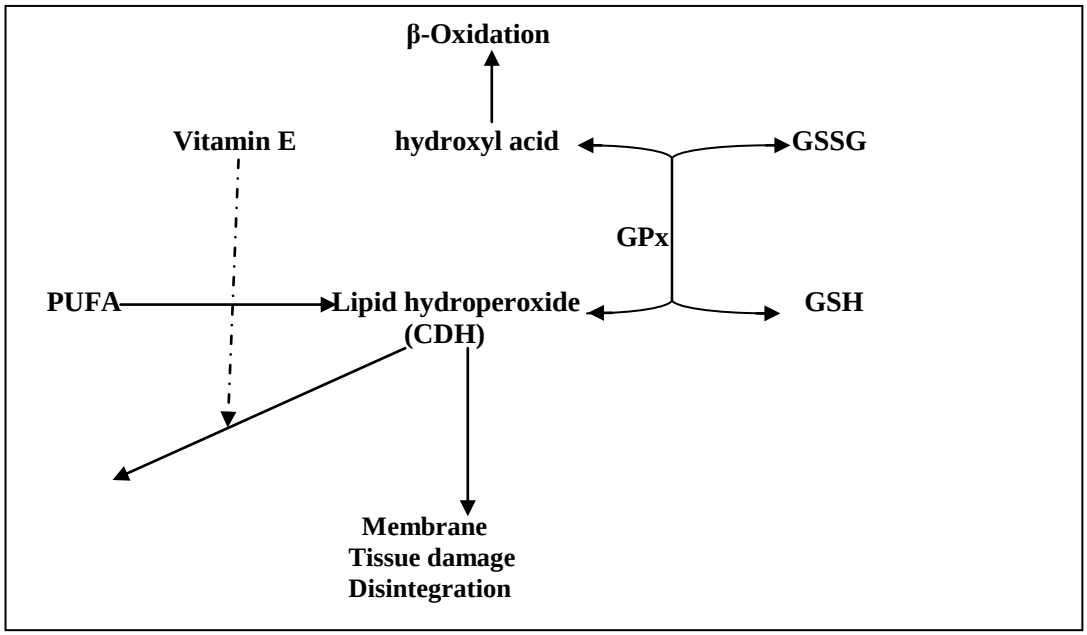


Fig 4:- The Relation between GPx, CDH, and Vitamin E [43] .

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