The Correlation between Glutathione Peroxidase Activity and Diene Hydroperoxide Level in Serum of Patients with Diabetes Mellitus Type II

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Abstract :-

The present study was designed to evaluate the oxidative stress-related parameters in sera of diabetic patients and healthy control . Some of the enzymatic and non-enzymatic factors were measured in sera of diabetic comparing with control groups. The results show that glutathione peroxidase activity and the levels of Vitamin E significantly decreased, while CDH levels increased in sera of patients (male and female).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.

Key word:- ROS:Reactive Oxygen Species ,CDH: Conjugated Diene Hydroperoxide, GSH; reduced glutathione, GSSG; oxidized glutathione, GRD; glutathione reducates, GPX; glutathione peroxidase,AR; aldose reductase.

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. GPx VitE

(CDH) (ROS)-:

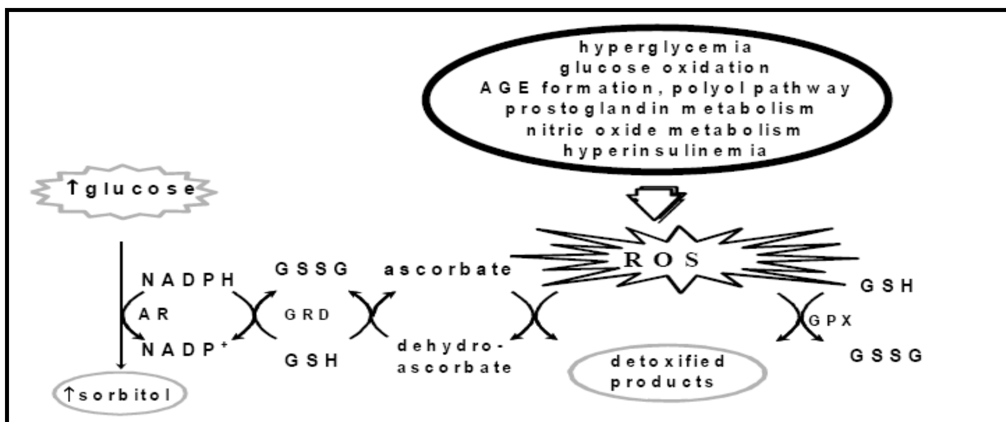
(GRD) (GSSG) (GSH)

. (AR) (GPx)

Introduction:-

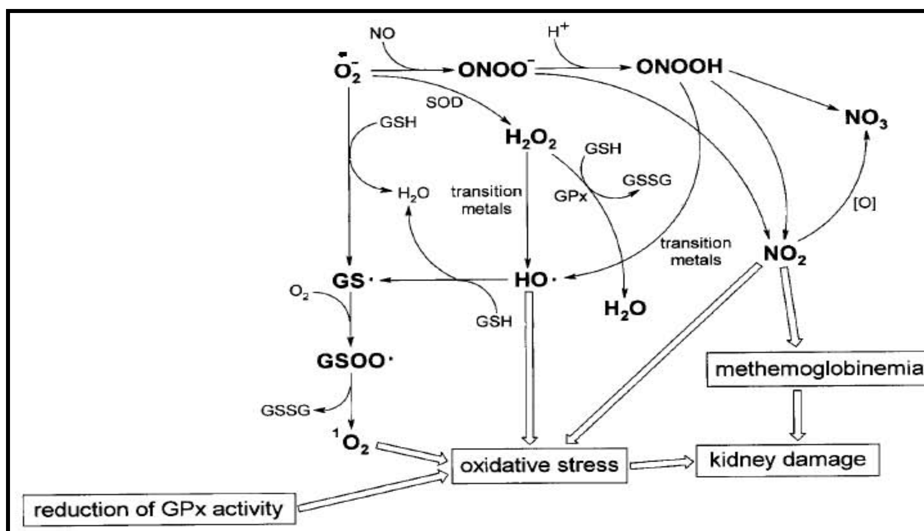
Diabetes mellitus (DM) is a syndrome characterized by abnormal insulin secretion, derangement in carbohydrate and lipid metabolism, and is diagnosed by the presence of chronic hyperglycemia. Diabetes is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality related to the development of nephropathy, neuropathy and retinopathy. The prevalence of type 2 DM among adults varies from less than 5% to over 40% depending on the population in question [1]. Its due to increasing obesity, sedentariness and dietary habits in both Western and developing countries, the prevalence of type 2 DM is growing at an exponential rate while type 1 DM is less common[2].

Type 2 diabetes mellitus is polygenic in origin and usually begins in adulthood, although specific genes for subtypes of this disease that occur earlier in life, referred to collectively as maturity-onset diabetes of the young, have been identified [3]. The onset of type 2 diabetes is insidious, thus hyperglycemia develops gradually and often goes untreated for years until symptoms become clinically obvious. 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 C 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 are usually accompanied by the formation of reactive oxygen species (ROS), setting up the potential for oxidative stress.(show fig.1)



Figure(1):- Mechanisms for Increased Oxidative Stress in Diabetes Mellitus. (Taken from 9).

The enzyme glutathi.one peroxidase (GPx) is a selenocysteine- containing protein that has an important role in the cellular defense against oxidant stress[10] by utilizing reduced glutathione (GSH) to reduce hydrogen peroxide (H_2O_2) and lipid peroxides to their corresponding alcohols[11]. GPx are known as antioxidative enzymes in various tissues which physiologically suppress the oxidative stress by catalyzing the removal of ROS.(As show in fig2)



Fig(2):- Possible Pathways for the Generation of Reactive Species which Cause Oxidative Stress(Taken from[24]).

GPx exists in several isoforms, and the most abundant intracellular isoform is cellular GPx, or GPx-1[10]. Hydrogen peroxide forms the toxic oxygen species hydroxyl radical ($OH^{•}$), which is highly reactive and causes lipid peroxidation, and hydroxide anion (OH^{-}), which promotes alkaline tissue

damage, a process that is counterbalance in part by catalase and GPx-1- dependent reduction to H_2O. Elevated levels of lipid peroxides are accompanied by an increase in peroxyl radicals, which can inactivate $NO^{\bullet}$ through the formation of lipid peroxynitrites [12,13], although the precise molecular mechanism(s) by which these peroxyl radicals form remains speculative [13]. Thus a deficiency of GPx-1 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 cosubstrate for GPx-1.We hypothesized that this antioxidant system plays a central role in protecting the vasculature in states of increased oxidant stress.

Vitamin E is thought to be the major nonenzymatic antioxidant present in the lipid structures of cells [14]. It is a donor antioxidant (reductant), which appears to react with peroxyl radicals to inhibit the propagation cycle of lipid peroxidation. Its characteristics as an antioxidant in organic solutions of fatty acids are well studied. In lipid solutions and dispersions, it inhibits radical formation linearly with time until it is depleted, and then oxidation accelerates, taking place at the same rate as if vitamin E had not been present [15].Its inhibition of oxidation of liposomal membranes and lipoproteins [16] has the same general features.

The aims of the present study were to determine whether oxidative damage occurs, and to what degree, at diabetes mellitus type II 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 diene hydroperoxide with one of the enzymatic antioxidant system activity (GPx), and one of the endogenous radical scavengers (α-tocopherol(vit E)) were evaluated.

Materials and methods Patients

We studied 30 type II diabetic patients (15 males, 15 females; ages 36-44 years). All patients were diagnosed at the Diabetes Unit of the Marjan educational Hospital. The study also included 30 healthy individuals, aged 36-44 years who did not take 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.

Reagents

All reagents, unless otherwise indicated, were obtained from Sigma Chemical Co. (St. Louis, USA) and used without any further purification.

Determination of the total glutathione peroxidase activity(Se& non Se dependent enzyme)

GPx activity was measured by the method described by Rotruck *et al*. [17]. 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(BDH), 0.2 ml of serum, 0.2 ml glutathione,0.1 ml of 0.2 mM Cumene hydroperoxide(Fluka). The contents were incubated at 37°C for 10 min. The reaction was arrested by 0.4 ml of 10% TCA(BDH), 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(CD) levels were measured by the method described by Pryor &Castle [18].

α-Tocopherol (Vit E) level was measured by the method described by Toro *et.al*[19]. Briefly, α-Tocopherol reacts with α-ά dipyridyl to produce a complex ,which has λmax in 520nm.

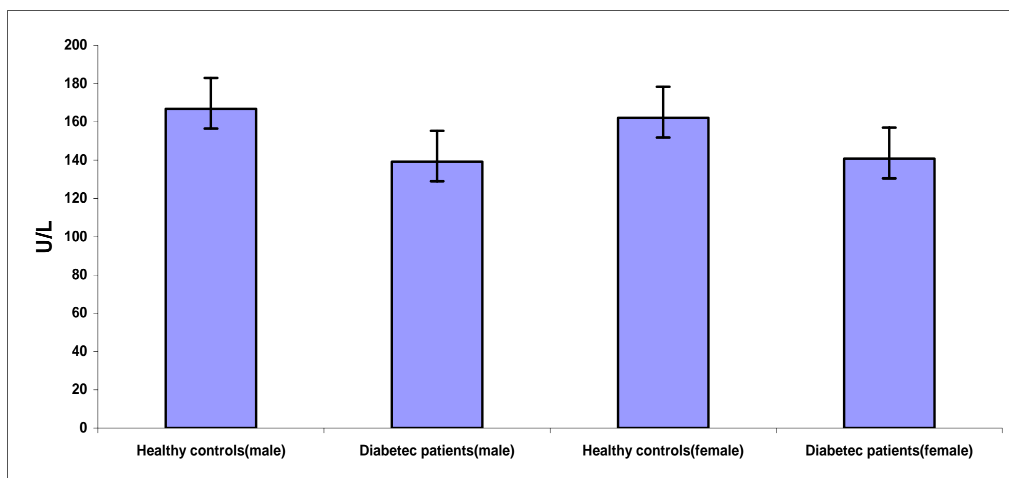
(UV-Visible spectrophotometer double beam Shemadzu-1601(Japan 2005) used in this research).

Statistical analysis:-

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.

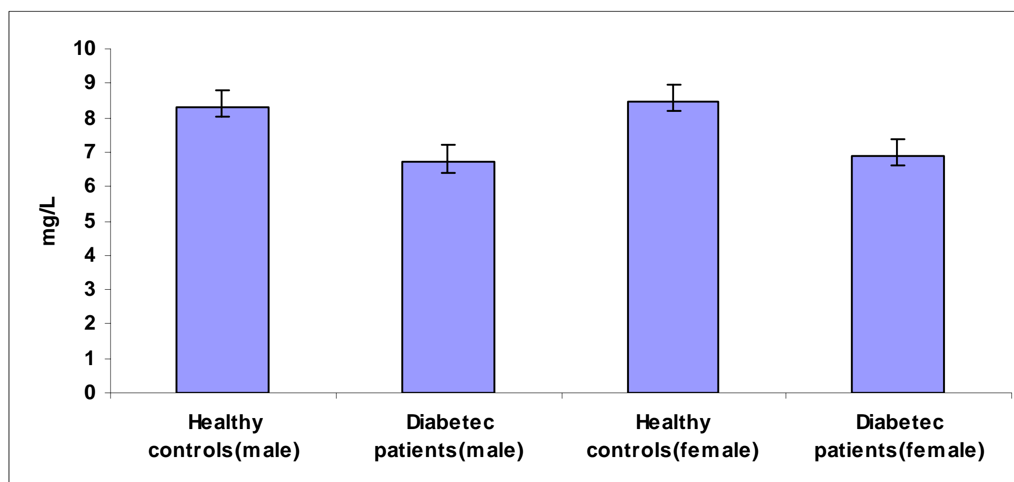
Results:-

The glutathione peroxidase GPx was investigated. Figure 3 show GPx activity in serum of diabetic and control groups. GPx activity was significantly lower than (11%) in serum of type II diabetic patients versus healthy control.



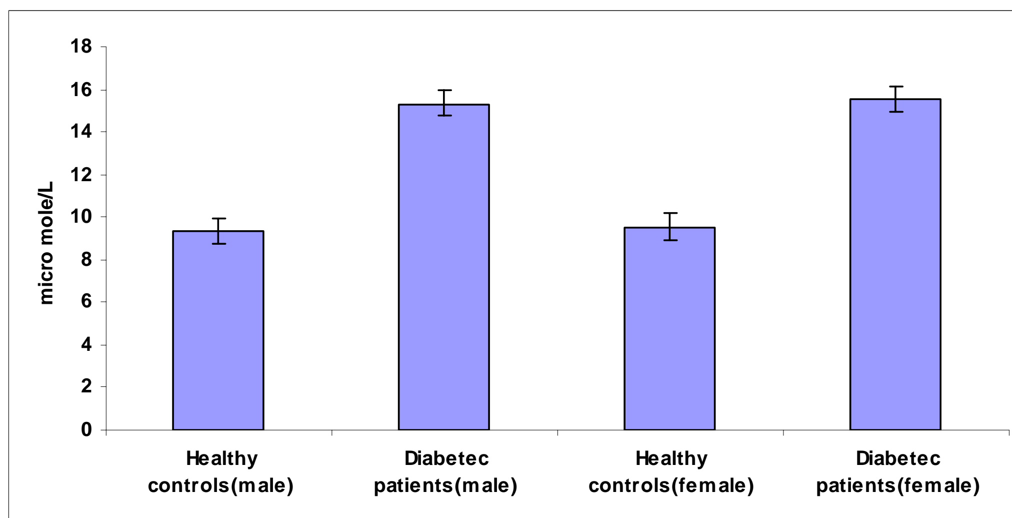
Figure(3):- (GPx) Activity from Type 2 Diabetic Patients and from Respective Control Subjects.

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 2 diabetic patients were observed when compared with their respective control subject.



Figure(4):- α-Tocopherol (Vit E) Level in Serum of Diabetic Patients (Type 2) and from Respective Control Subjects.

Finally, the assessment the overall CDH level in serum were done . Compared with healthy control subjects, a marked increase of CDH level (40%) was found in type 2 diabetic patients compared with healthy control subjects (Fig.5).



Figure(5):- Conjugated Diene Hydroperoxide Level in Serum of Diabetic Patients (Type 2) and from Respective Control Subjects.

Discussion:-

Glutathione peroxidase (GPx) is an antioxidant enzyme that reduces hydrogen peroxide and lipid peroxides [20]. Hyperglycemia is associated with increased reactive oxygen species in both animal and human studies [21].As shown in table (1), GPx activities were found to be lower in the present study.

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

	Sex	Mean	SD	SE	95 % C.I		P	Sign.
					Upper	Lower		
Control	M	166.8	39.8	10.3	190.09	143.5	--------	-------
	F	162.1667	44.808	11.56	188.3	136.3	--------	-------
Type II	M	139.2	62.7	16.2	175.6	102.4	0.01	Sign.
	F	140.75	61.51	15.7	176.2	105.25	0.01	Sign.

The depletion of GPx activity may be beyond to its broader protective spectrum than catalase in catalyzing the reduction of H_2O_2 and other hydroperoxide, including lipid hydroperoxide[22]. However,the elevated levels of $O_2^{\bullet -}$ in serum of patients with Diabetes Mellitus produce the depression GPx activity.($O_2^{\bullet -}$ anions have been shown to inactivate GPx and activated CAT [23]).The elevation of homocysteine concentrations in serum of patients with type II DM could be directly elucidate the decrement of

GPx activity(Upchurch[25] shown that elevated homocysteine concentrations suppress GPx expression in endothelial cells *in vitro* and in mildly hyper homocysteinemic mice *in vivo* and suggested that this effect may account, in part, for the vascular oxidant stress of hyper homocysteinemic states).The low GPx activity could be illustrated directly 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[26].

Previous study [27] showed 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 by provoking structural and functional changes in proteins.

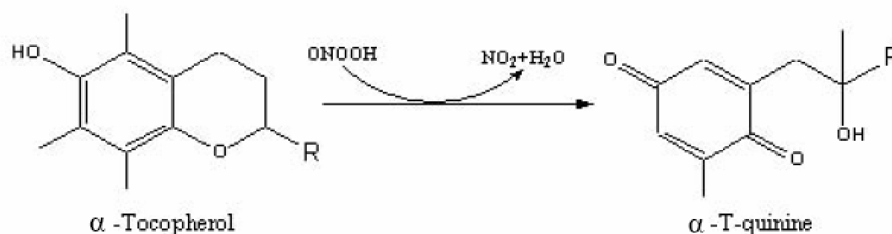
Vitamin E is a one of four fat-soluble vitamins that are necessary for the body to function normally. Vitamin E exists in eight different forms of which the most important is α-tocopherol because it is the most active[28]. As show in table (2) Vitamin E levels were found to be lower in the present study.

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

	Sex	**Mean**	**SD**	**SE**	**95 % C.I**		**P**	**Sign.**
					Upper	Lower		
Control	M	8.291	1.9	0.49	9.4	7.2	--------	-------
	F	8.41	1.92	0.5	9.54	7.28	--------	-------
Type II	M	6.7	1.1	0.28	7.33	6.06	0.01	Sign.
	F	6.88	1.14	0.29	7.35	6.22	0.01	Sign.

The low Vitamin E could be beyond its antioxidant property. This property ensures the stability of the membranes of blood components such as erythrocytes, leukocytes and platelets and provides protection from oxidative damage by scavenging excess free radicals produced by oxidative stress[29].

However, Wagner *et al.* found that vitamin E slows the rate of free radical-mediated lipid peroxidation in cells[16].The elevated level of peroxynitrate in serum of Diabetic patients[30] 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[31].



A higher production of reactive oxygen species has been attributed 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[32]. CDH is considered as a marker of oxidative stress and it was found that serum CDH to be increased in this study as show in table(3).

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

	Sex	Mean	SD	SE	95 % C.I		P	Sign.
					Upper	Lower		
Control	M	9.35	2.27	0.58	10.66	8.03	--------	-------
	F	9.5	2.5	0.64	10.94	8.05	--------	-------
Type II	M	15.36	2.2	0.56	16.6	14.09	0.00	Sign.
	F	15.5	2.2	0.56	16.7	14.2	0.00	Sign.

The elevated CDH level could be changed to lowering GPx activity, which can lead to a relative accumulation of 12-HpETE(12-hydroperoxy-eicosatetraenoic acid), the main hydroperoxide formed from arachidonic acid, and such an increase could activate signal transduction pathways leading to arachidonic acid release[33], Pang *et al.* were also

demonstrated that a tendency to decreased GPx activity could increase both the intracellular peroxide level and oxidative damage[34].The other cause to increase CDH may be the Elevated free fatty acids (FFAs) in plasma, which can increase products of peroxidation[35].

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.

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