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Impairment of glutathione metabolism and its impact on other biochemical constituents in patients of diabetes mellitus

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Abstract: The intention of this study was to evaluate the levels of antioxidant enzymes glutathione peroxidase and glutathione reductase with their effect on lipid peroxidation and other biochemical constituents in patients of type 2 diabetes mellitus and compare with normal subjects of the same population. Amplified oxidative stress is extensively accepted contributor in the development and progression of diabetes and its complications. Usually diabetes is accompanied by greater production of reactive oxygen species (free radical) or impaired antioxidant defenses. NADPH dependent reduction of oxidized glutathione is catalyzed by the Glutathione reductase (GR) enzyme that serves to keep up intracellular glutathione supplies and a favorable redox status. We evaluate the status of glutathione reductase activity and other biochemical constituents in human serum of diabetic patients as well as in control subjects. This study was conducted on 40 Type 2 diabetic patients and 40 age and sex matched healthy control subjects. Fasting blood glucose (FBG), glycosylated hemoglobin (HbA_{1c}), Total Cholesterol, HDL, LDL, TG, glutathione peroxidase (GPX), glutathione reductase (GR) activity and lipid peroxidation product (MDA) were measured by using UV-visible spectrophotometric technique and compared with normal healthy persons. The results were statistically evaluated. The study revealed that FBG, HbA1c, Total Cholesterol, LDL, TG and MDA were significantly higher whereas HDL, GPX and GR levels were significantly reduced in diabetics as compared to controls ($P<0.05$). Positive correlation of FBG with HbA1c ($r=0.529$, $P=0.0001$), Total cholesterol with LDL ($r=0.712$, $P=0.0001$), GR with MDA($r=0.562$, $P=0.0001$) whereas significant negative correlation was found between HbA1c with GR ($r=-.334$, $P=0.035$), HbA1c with MDA ($r=-.340$, $P=0.032$), HDL with TG ($r=-.313$, $P=0.049$), MDA with HbA1c ($r=-.340^{*}$, $P=0.032$). It can be concluded that decreased levels of glutathione peroxidase and glutathione reductase along with high level of lipid peroxidation may be a useful markers of oxidative stress in type 2 diabetics. The increase in free radical activity in type 2 diabetes mellitus together with insulin resistance can lead to activation of stress-sensitive pathways, which play an important role in the complication of diabetes.

Keywords: Diabetes mellitus Type 2, glutathione peroxidase, glutathione reductase, lipid peroxidation product.
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INTRODUCTION

ROS are constantly formed in the human body and are removed by an antioxidant defense system[1]. ROS are generally cytotoxic because they can cause damage to cellular components[2]. The mechanism of free radical production include glucose autoxidation, protein glycation, advanced glycation end products formation, and activation of polyol pathway, ultimately resulting in oxidative stress in a variety of tissues[3]. Some biological parameters involved in cell defense against oxygen radicals are vitamin C and E, erythrocyte glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase. Tissue glutathione plays a central role in antioxidant defense. Unusually high levels of free radicals and the concurrent decline of antioxidant defense mechanisms can cause to damage cellular organelles and enzymes, greater lipid peroxidation, and insulin resistance progression. The consequences of oxidative stress can advance the development of complications of diabetes mellitus. Hyperglycemia also impairs the endogenous antioxidant defense system in many ways during diabetes[4]. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells[5]. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols[6].

Glutathione system is one of the chief defense mechanism against oxidative stress. Glutathione in reduced state detoxifies reactive oxygen species such as H_2O_2 and lipid peroxides directly or by a glutathione peroxidase (GPX) catalyzed reaction. Glutathione reductase (GR) catalyzes the NADPH dependent reduction of oxidized glutathione (GSS), help to keep up intracellular glutathione stores and a favorable redox state[7]. Determination of GSH/GSSG is a good measure of oxidative stress of an individual[8]. The chief defensive roles of glutathione against oxidative stress are that, it can act as several detoxifying enzyme's cofactor, scavenge hydroxyl radical and singlet oxygen directly, participate in amino acid transport across plasma membrane and redevelop Vitamins C and E back to their active forms[9].

In this study, we want to evaluate the levels of an antioxidant enzyme glutathione peroxidase and reductase and their impact on other biochemical constituents including FBG, HbA_{1c}, HDL, LDL, TG,

and MDA in patients of type 2 diabetes and compared with normal subjects with good metabolic control, that whether the hyperglycemic induce variation in enzymatic levels affects other biochemical parameters or not in progression of diabetic complications.

MATERIALS AND METHODS

The study was conducted on 51 patients with type 2 diabetes both males and females (28 males and 23 females) between the age group 35-65 years who have registered at Baqai Institute of Diabetology and Endocrinology, Karachi, Pakistan. Fifty five (22 males and 33 females) age and sex matched control subjects were also selected from the general population at random for comparison. Ethical approval was obtained from the institutional review board (IRB) before the commencement of the study. Informed consent was taken from each individual at the time of recruitment in the study. All the patients who were diagnosed with type 2 diabetes using the ADA criteria i.e. FBG of ≥126mg/dl were included in the study. The patients who had any recent clinical evidence of cardiac, renal or liver dysfunctions and any hemoglobinopathy were excluded from the study. Blood samples were collected in tubes with EDTA as anticoagulant. Plasma was separated and analyzed for other biochemical parameters such as FBG, HbA_{1c}, HDL, LDL, TG, GPX, GR activity and MDA. Fasting blood glucose was estimated by following glucose oxidase method on UV-visible spectrophotometer[10].

The HbA_{1c} was analyzed by automatic D10 analyzer[11, 12].Total Cholesterol was estimated by enzymatic endpoint method (Randox Kit, Cat No: CH 200)[13], HDL and LDL was estimated by CHOD-PAP Assay method (Randox kit, Cat No: CH 203)[14,15], Triglycerides was estimated by GPO-PAP method[16]. Glutathione Peroxidase estimated by Randox kit method (estimation based on principle of Paglia and Valentine)[17]. Glutathione reductase was estimated by Randox kit method[18,19]. MDA was also estimated by Randox kit method on a UV-Visible Spectrophotometer[20, 21].

Data were statistically analyzed using Statistical Package for Social Sciences version 20 (SPSS Inc, Chicago, IL, USA). Independent samples were examined with student's t test. P-values and 95% confidence intervals (CI) were also calculated. P-value of < 0.05 was taken as significant for all comparisons.

RESULTS

The study was based 40 patients with diabetes type 2 (23 males and 17 females) and 40 healthy control subjects (17 males & 23 females) .The Mean age of control was 45.17±1.23 years and 50.87±1.59 years for patients. The results of biochemical parameters (FBG, HbA1c, Total Cholesterol, HDL, LDL, TG, GPX, GR and MDA) were compared in patients and control subjects. Significant increase in FBG, HbA1c, Total Cholesterol, LDL, TG and MDA were found in diabetic patients as compared to healthy control subjects (Table 1). Pearson correlation was used to evaluate the impact of impaired glutathione metabolism on different biochemical parameters and to find the significant correlation between biochemical parameters in diabetic patients (Table 2). Positive correlations was found in FBG with HbA1c, Total cholesterol with LDL, GR with MDA and negative correlation was found between HbA1c with GR ,HbA1c with MDA ,HDL with TG , (Table 2). The variation of biochemical constituents, antioxidant enzymes glutathione peroxidase & glutathione reductase and MDA level in control and diabetics on the basis of sex are shown in (Table 3).

Table 1: Comparison between control and diabetic subjects with respect to physical parameters and FBG, HbA1c, lipid profile, GPX, GR and MDA levels.

Parameters	Control (n=40)	Diabetic Patients (n=40)	P-Value
Age (Years)	45.17±1.23	50.87±1.59	0.0058*
Ht (Cm)	164.71±1.70	166.19±1.64	0.5328
Wt (Kg)	60.80±1.73	69.72±2.07	0.0014*
BMI(Kg /m2)	25.28±0.53	25.28±0.64	1.0000
FBG(mg /dl)	91.27±2.07	173.90±7.06	0.0001*
HbA1c (%)	4.70±0.05	8.01±0.31	0.0001*
TC(mg /dl)	162.84±3.47	227.94±4.60	0.0001*
HDL(mg /dl)	89.22±5.38	34.02±1.74	0.0001*
LDL (mg /dl)	58.01±4.52	142.63±4.04	0.0001*
TG (mg /dl)	145.84±10.09	173.89±10.70	0.0602
GPX (mg /gHb)	47.05±3.00	37.76±3.18	0.0368
GR (U /L)	39.57±2.70	19.38±1.40	0.0001*
MDA(µM)	8.81±0.27	13.07±0.62	0.0001*

Ht(Height),Wt (Weight),BMI(Body mass index), Fasting blood glucose (FBG), Glycosylated hemoglobin (HbA_{1c}), Total Cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL), Triglyceride (TG), Glutathione peroxidase (GPX), Glutathione Reductase (GR), Malondialdehyde(MDA), n = no of subjects, values are represented as mean ± SEM (Standard error of mean), * P < 0.05 is considered to be statistically Significant.

DISCUSSION

The mechanism of free radical production include glucose autoxidation, protein glycation, advanced glycation end products formation, and

activation of polyol pathway, ultimately resulting in oxidative stress in a variety of tissues[22].

Our study concluded that the levels of all the biochemical constituents such as Blood Glucose, Glycosylated hemoglobin, Total Cholesterol, LDL and Triglycerides were higher in diabetic patients as compare to control subjects which shows that the disturbance in carbohydrate metabolism affects all the other metabolic pathways. While the levels of antioxidant enzymes Glutathione peroxidase and reductase are lower in type 2 diabetic patients as compare to control subjects which shows that hyperglycemia induce oxidative stress which may occur through depletion of NADPH and consequently disturbance of glutathione metabolism[23].

Table 2: Correlations.

	Glucose	HbA1c	TC	HDL	LDL	TG	GPX	GR	MDA
Glucose	1	.529**	-.179	.141	-.050	.051	-.048	-.052	.496**
		.000	.268	.387	.759	.756	.769	.752	.001
	40	40	40	40	40	40	40	40	40
HbA1c	.529**	1	-.236	.289	-.066	-.082	-.109	-.334*	.060
	.000		.143	.070	.684	.613	.503	.035	.713
	40	40	40	40	40	40	40	40	40
TC	-.179	-.236	1	-.288	.712**	.170	.159	.186	.284
	.268	.143		.071	.000	.295	.327	.251	.076
	40	40	40	40	40	40	40	40	40
HDL	.141	.289	-.288	1	-.088	-.313*	-.233	-.051	-.015
	.387	.070	.071		.588	.049	.148	.756	.925
	40	40	40	40	40	40	40	40	40
LDL	-.050	-.066	.712**	-.088	1	-.115	.063	.252	.293
	.759	.684	.000	.588		.479	.700	.117	.067
	40	40	40	40	40	40	40	40	40
TG	.051	-.082	.170	-.313*	-.115	1	.033	.052	-.147
	.756	.613	.295	.049	.479		.838	.749	.367
	40	40	40	40	40	40	40	40	40
GPX	-.048	-.109	.159	-.233	.063	.033	1	.022	.037
	.769	.503	.327	.148	.700	.838		.893	.819
	40	40	40	40	40	40	40	40	40
GR	-.052	-.334*	.186	-.051	.252	.052	.022	1	.270
	.752	.035	.251	.756	.117	.749	.893		.092
	40	40	40	40	40	40	40	40	40
MDA	.496**	.060	.284	-.015	.293	-.147	.037	.270	1
	.001	.713	.076	.925	.067	.367	.819	.092	
	40	40	40	40	40	40	40	40	40

Depletion of NADPH occurs as a result of activation of polyol pathway in hyperglycemic condition which is also an NADPH dependent pathway[24]. Inconsistency was observed in biomarkers for oxidative stress such as glutathione peroxidase, glutathione reductase in diabetics. Decreased levels of glutathione and elevated concentrations of MDA are also observed in diabetes[25].

In present study significant decrease in the activities of antioxidant enzymes Glutathione Peroxidase and Glutathione Reductase are found between diabetic and non-diabetic individuals and this finding is supported by many studies [26, 27]. Blood GSH was significantly decreased in different phases of type2 DM such as: glucose intolerance and early hyperglycemia, within two years of diagnosis and before development of complications and in poor glycemic control. The pathophysiological significance of decreased glutathione levels in DM remains to be unclear. Some studies revealed no difference in whole blood GR activity in type1 and type2 DM patients compared to control subjects[28].

Current study shows positive correlation between Fasting Blood Glucose level with HbA1c & MDA and negative correlation with enzymatic levels (GPX & GR) (Figure 1). On the other hand negative correlation was found between HbA1c level and enzymatic levels (GPX & GR) and positive correlation between HbA1c and MDA(Figure 2). The variation of Biochemical constituents, antioxidant enzymes glutathione peroxidase and glutathione reductase and MDA level in control and diabetics on the basis of sex are shown in (Table 3).On the basis of these finding it is suggested that antioxidant enzymes levels should be utilized in clinical practice to improve vascular risk prediction in diabetics.

Table 3: Variations of FBG, HbA1c, lipid profile, GPX, GR and MDA in control and diabetic male and female subjects.

Parameters	Control (n=40)		Diabetic patients (n=40)	
	Male (n= 17)	Female (n=23)	Male (n= 23)	Female (n=17)
FBG	86.34 ± 2.79	94.91 ±2.77	178.30 ±9.83*	167.94 ±10.13*
HbA1c	4.57 ± 0.07	4.79 ±0.08	8.30 ±0.39*	7.62 ±0.52*
TC	166.72 ±4.11	159.98 ±5.21	228.85 ±5.81*	226.71 ±7.64*
HDL	92.89 ±9.26	86.50 ±6.51	33.87 ±2.06*	34.22 ±3.07*
LDL	49.80 ±6.22	64.08 ±6.19	141.00 ±5.50*	144.84 ±6.09*
TG	167.23 ±17.35	130.02 ±11.19	182.60 ±15.27	162.1 ±14.44
GPX	49.85 ±5.25	44.98 ±3.53	33.71 ±3.93*	43.22 ±5.11
GR	41.03 ±4.00	38.50 ±3.72	18.85 ±2.06*	20.10 ±1.83*
MDA	8.37± 0.29	9.13 ±0.42	13.70 ±0.90*	12.21 ±0.78

Fasting blood glucose (FBG), Glycosylated hemoglobin (HbA_1c), Total Cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL), Triglyceride (TG), Glutathione peroxidase (GPX), Glutathione Reductase(GR), Malondialdehyde (MDA), n = no: of subjects, values are represented as mean ± S.E.M (Standard error of mean).* Statistically significant as compared to control.

Decreased activity and efficiency of cellular antioxidant mechanisms with concurrent increased lipid peroxidation represent the pathogenic

connection between hyperglycemia and expansion of endothelial dysfunction. Furthermore, the extent of oxidative stress and lack of defensive antioxidant mechanisms in Type 2 Diabetic patients is reliant on the metabolic control of diabetes and the incidence of complications. Intensity of oxidative stress in Type 2 diabetic patients is greater when compared with normal healthy individuals as controls. Antioxidant enzymes (Glutathione Peroxidase and Glutathione Reductase) levels as surrogate marker in early detection of diabetic complications. Suggesting it that, they may contribute in finding micro and macrovascular complications in diabetics.

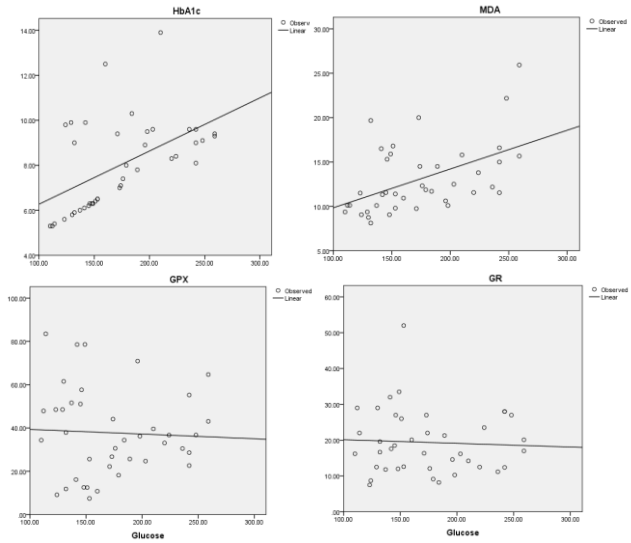

Figure 1: Correlation between FBG level with HbA1c and MDA and negative correlation with enzymatic levels (GPX and GR).

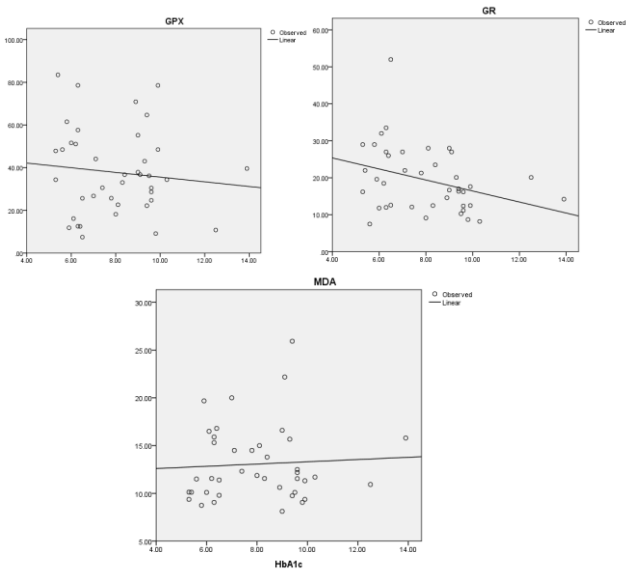

Figure 2: Correlation between HbA1c level with enzymatic levels (GPX and GR) and positive correlation between HbA1c and MDA.

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