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Study Of Malondialdehyde, Reduced Glutathione, And Peroxy Nitrite Levels In Type 2 Diabetics Patients

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ABSTRACT

The study was conducted to investigate the difference in the serum malondialdehyde (MDA), glutathione (GSH), and peroxy nitrate (PN) levels between type 2 (T2DM) diabetes patients and normal subjects. MDA, GSH, and PN levels in sera of 100 patients and 80 participants in the control group were evaluated. A statistically significant difference was found between patients and the control group in terms of MDA, GSH, and PN levels. A decrease in GSH activity was detected (P<0.0001), while MDA and PN levels increased significantly (P<0.0001). The high levels of patients versus control ratio of MDA and PN levels probably suggests the occurrence as a mechanism of tissue damage in cases of T2DM. Moreover, it is recommended that the patient levels of MDA, GSH, and PN should be evaluated in insulin resistance patients.

Keywords: Type2 diabetes mellitus, Oxidative stress, Malondialdehyde, Reduced glutathione, Peroxy nitrite.

INTRODUCTION

Diabetes mellitus is a major worldwide health problem characterized by chronic hyperglycemia [1], predisposing to markedly increased coronary artery, cerebrovascular and peripheral vascular diseases, with up to 80% of deaths in people with diabetes caused by cardiovascular disease [2], and serious morbidity and mortality related to development of nephropathy, neuropathy, and retinopathy [3,4]. Excessive urine production with a compensatory thirst is main signs of diabetes mellitus. There are two major forms of diabetes mellitus, characterized by an absolute and a relative insulin deficiency type 1, and type 2 respectively. Type 2 is the most common form, accounts for 90-95% of all diabetes cases adults aged 20-79 years, and results from a combination of insulin resistance and impaired insulin secretion[5]. Diabetes mellitus is characterized by absolute or relative deficiencies in insulin secretion and/or action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism [6]. Changes in human behavior and lifestyle over the last century have resulted in a dramatic increase in the incidence of diabetes worldwide [7, 8]**.** Type 2 diabetes is rapidly becoming pandemic, and although the origin of this disease is not entirely clear, it is accepted that insulin resistance is important in its pathogenesis and that defects in insulin secretion by pancreatic β-cells lead to hyperglycemia and the onset of diabetes [9, 10]. It is interesting that the proportion of diabetes is higher in women than in men [10]. Type 2 diabetes is

a heterogeneous condition due to reduced tissue sensitivity to the action of insulin (insulin resistance) and impaired insulin secretion [11-13].

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. A currently favored hypothesis is that disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling. Oxidative stress is the common pathogenic factor leading to insulin resistance, β-cell dysfunction, impaired glucose tolerance (IGT) and ultimately to type 2 DM (T2DM) [14]. Furthermore, this mechanism has been implicated as the underlying cause of both the macrovascular and microvascular complications associated with T2DM [15].

MATERIALS AND METHODS

Patients and control subjects: A total of 120 Iraqi type 2 diabetes mellitus patients (T2DM) consist both males and females recruited in the Al-Najaf center for diabetes and Endocrine center in Al-Sadder medical teaching city/ Najaf / Iraq. Normal eighty eligible matched ages was recruited from subjects who attended for a routine medical check; they were defined as healthy by physical examination (weight, height, and blood pressure measurements, chest X ray, respiratory, and eye examination). The diagnosis of diabetes mellitus type 2 was assigned by physician. Both diabetic type 2 patients and healthy control had not any of other diseases, and those which have are excluded.

Sample Collection: Disposable syringes and needles were used for blood collection .Venous blood samples, 5 ml were collected from patients and healthy volunteers in plane tubes. The blood samples were collected at fasting. After allowing the blood to clot at room temperature for 15 min, blood samples were centrifuged at 3000 xg for 15 min. Sera were separated, and stored at -20 C˚ for estimation of the concentrations of glucose and markers of oxidative stress.

Methods: Validated laboratory spectrophotometric procedures were used as described by Koubaa et al. [16]. Type 2 diabetic patients and healthy subjects were undergo to assessment of biochemical parameters such as fasting plasma glucose (FPG) [17], malondialdehyde (MDA) assayed by using thiobarbituric acid reactive substance method described by Guidet B, Shah (18), reduced glutathione (GSH) measured based on 5,5-Dithiobis (2-nitrobenzoic acid) DTNB according to Sedlak, J. and Lindsay [19,20], and peroxy nitrite (PN) [21].

Statistical analysis: Descriptive statistics were expressed as mean ± SD. The SPSS for windows program was used for statistical analysis. Since the data obtained were normally distributed, the independent student's t-test (unpaired t-test) was performed to compare type 2 diabetic patients with their healthy normal groups. The Pearson product moment correlation coefficient was used to assess the relationship among these oxidative stress values with age and body mass index of type 2 diabetic patients and healthy normal subjects. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

The study encompassed 120 T2DM patients who showed increase blood glucose levels more than 13mM, whose average age was 37±11.06 years. The control group included 80 healthy individuals, whose average age was 36.65 ± 11.04years. Since we compare type 2 diabetic patients with healthy individual for the difference of the malondialdehyde (MDA), glutathione (GSH), peroxy nitrite (PN), and fasting plasma glucose (FPG). The data outcome demonstrates that FPG in type 2 DM patients was significantly higher

than that in the normal group (P<0.0001). Both levels of serum MDA and PN showed increase significantly different from the normal healthy group (P<0.0001). In the other hand GSH level was significantly lower than in normal individual (P<0.0001) (table1).

Table 1. Database of age, gender, FPG, and oxidative stress markers in type 2 diabetic patients (mean ± S.D)

Variables	Control (n=80)	Type 2 diabetes (n=120)	P Value
Age	36.65 ± 11.04	37.0 ± 12.06	
Gender (M/F)	55/25	65/35	
FPG mmol/L	5.45 ± 0.45	13.48 ± 3.52	< 0.000
MDA µmole/L	8.75 ± 1.99	16. 84 ± 4.2	< 0.000
GSH µmole/L	256.4 ± 60.91	196.68 ± 39.76	< 0.000
PN µmole/L	9.88 ± 3.45	20.16 ± 13.36	< 0.000

The 100 type 2 diabetic patients and 80 healthy subjects were categorized into two groups according to gender, so there were (65 males, 35 females), and (55 males, 25 females) for diabetics and healthy group respectively. Each group compared with corresponding group to appraise the influence of sex on the malondialdehyde (MDA), glutathione (GSH), and peroxy nitrite (PN). Both MDA and PN levels were found to be significantly (p<0.0001) raised in type 2 diabetic males and females in comparison with those corresponding control groups. Significant decreases were observed for the levels of GSH (p<0.001) in in both diabetic males and females when compared with those of healthy corresponding groups. (table 2).

Table 2. Malondialdehyde (MDA), glutathione (GSH), and peroxy nitrite (PN) in type 2 diabetic males and females

Variables (µM)	Male Mean±SD		Female Mean±SD	
MDA	Control (55) Patients (65)	8.54 ± 1.7 17.27 ± 3.99 *	Control (25) Patients (35)	9.1 ± 2.3 15.95 ± 4.5 *
GSH	Control (55) Patients (65)	259.4 ± 64.1 200.1 ± 29.7 *	Control (25) Patients (35)	251.7 ± 54.9 192.5 ± 53.05 *
PN	Control (55) Patients (65)	9.54 ± 3.35 21.3 ± 15.5 *	Control (25) Patients (35)	10.65 ± 3.67 18.01 ± 7.76 *
FPG mmole/L	Control (55) Patients (65)	5.4 ± 0.47 13.4 ± 3.51 *	Control (25) Patients (35)	5.57 ± 0.42 13.52 ± 3.62 *

* p Value < 0.0001

To inspect the linkage of oxidative stress markers with the ages of diabetics patients , the linear regression analysis was used to esteem the data. A significant MDA (r = 0.18, p<0.05), PN (r = 0.167, p<0.05), positive correlation was appeared with ages of type 2 diabetics. furthermore, significant negative correlations were obvious for GSH (r = - 0.07, p>0.05), with ages of the diabetics (table 3).

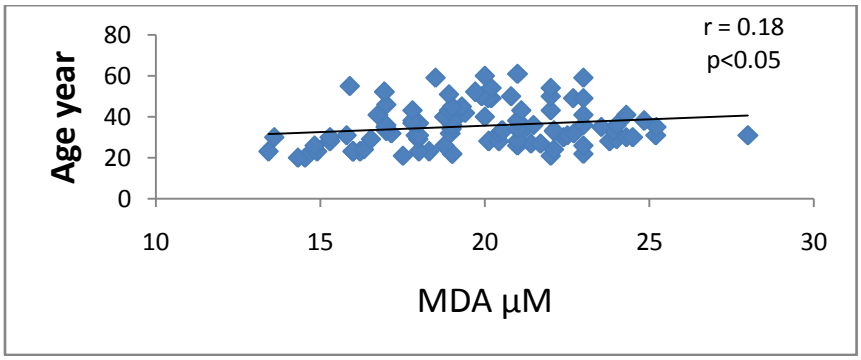
Table 3. Linear regression analysis of malondialdehyde (MDA), glutathione (GSH), and peroxy nitrite (PN) levels with ages of type 2 diabetic patients.

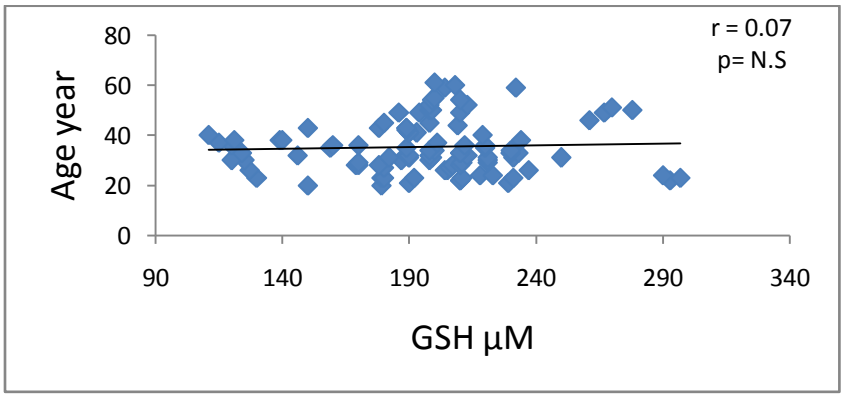
Variables	r	P value
MDA	0.18	0.05
GSH	0.07	N.S
PN	0.167	0.05

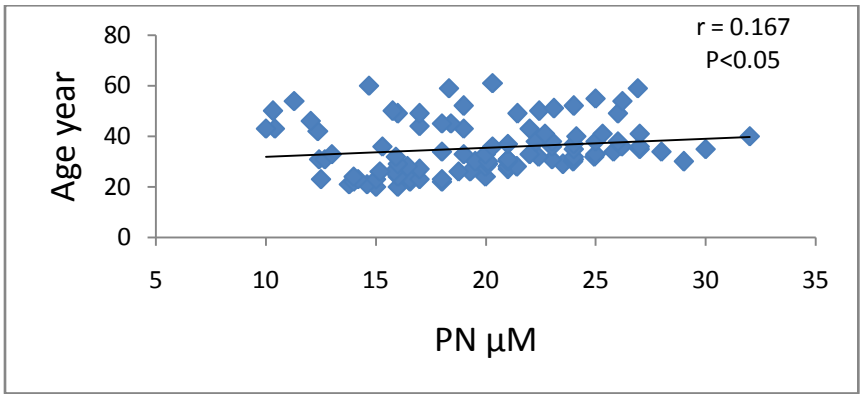
Type 2 diabetics patients and healthy subjects were classified according to the body mass index (BMI) into 2 subgroups as over weight (BMI 25 - 30Kg/m^2) and normal weight (BMI < 25 Kg/m^2), and the values of oxidative stress markers among those subgroups were compared. All oxidative stress markers (MDA, GSH. and PN) failed to give significant differences when compared overweight patients with those of normal weight group (table 4).

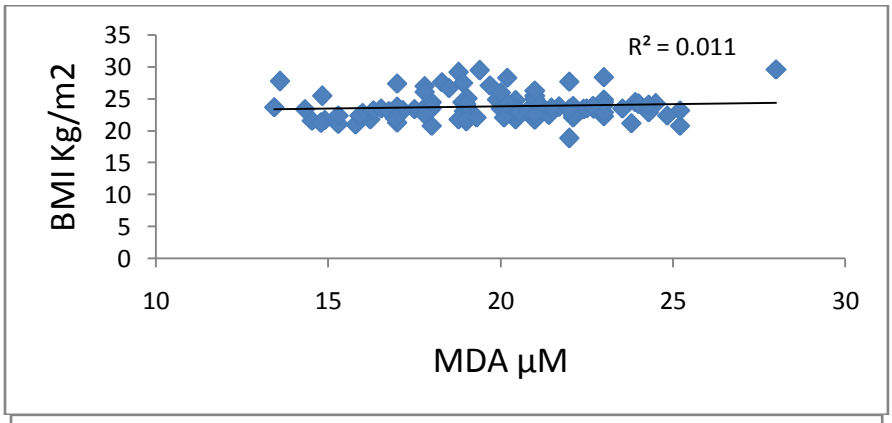
Table 4. Malondialdehyde (MDA), glutathione (GSH), and peroxy nitrite (PN), in overweight and normal weight type 2 diabetic patients

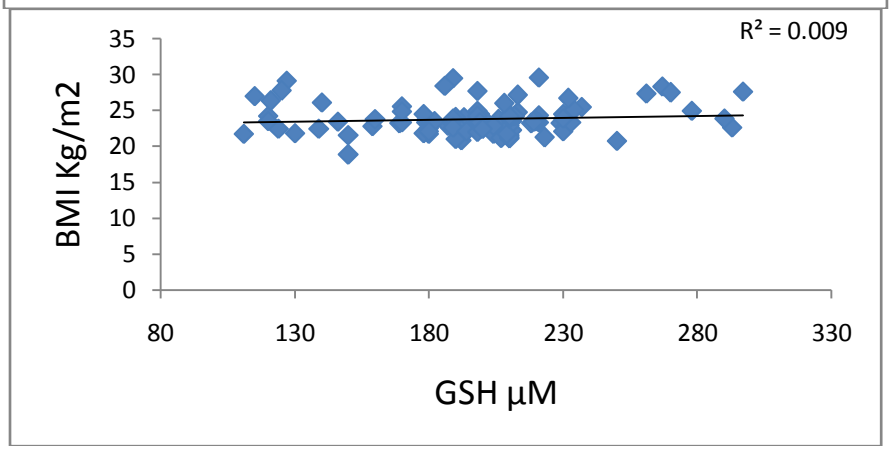
Variable	Group (n)	Mean ± SD	P value
MDA µM	Normal weight (74)	16.86 ± 3.9	
	Over weight (26)	16. 7 ± 4.89	N.S
GSH µM	Normal weight (74)	196.89 ± 35.7	
	Over weight (26)	199.12 ± 49.0	N.S
PN µM	Normal weight (74)	19.56 ± 7.6	
	Over weight (26)	21.89 ± 23.62	N.S

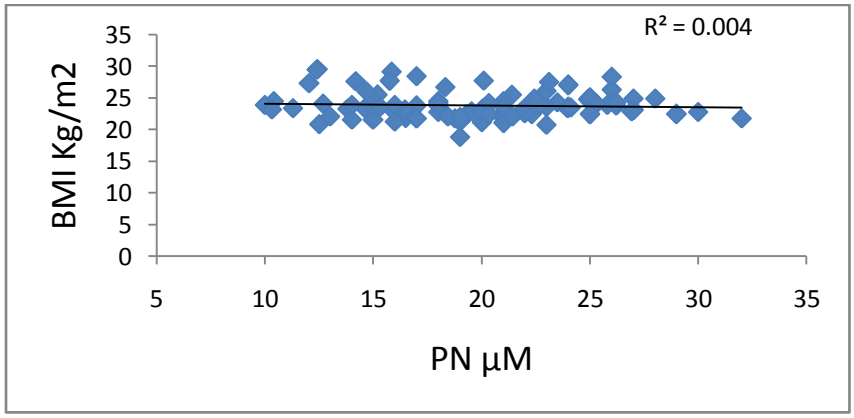





r = 0.167
P<0.05
Age year
PN µM


R² = 0.011
BMI Kg/m2
MDA µM


R² = 0.009
BMI Kg/m2
GSH µM


R² = 0.004
BMI Kg/m2
PN µM

Diabetes is associated with a number of metabolic alterations and principal among these is hyperglycemia. Known squeal of hyperglycemia such as cellular damage, increased extra cellular matrix production and vascular dysfunction have all been implicated in the pathogenesis of vascular disease type 2 diabetes. Free radicals and oxidative stress may act as a common pathway to diabetes itself, as well as to its complications [22]. The higher oxidative stress as expressed by plasma MDA concentration was present type 2 diabetic patients as compared with normal subjects. This is in agreement with the idea that higher oxidative stress and lipid peroxidation are present in type 2 diabetes mellitus as part of the metabolic syndrome [23]. Insulin resistance is closely related to hypertension, obesity [24].

In present study serum MDA and PN is significantly increased in cases of type 2 diabetes with complications as compared with controls. It is in accordance with previous findings of that hyperglycemia induces overproduction of oxygen free radicals in diabetes [25]. Increased levels of the products of oxidative damage to lipids have been detected in serum of diabetic patients and their presence correlates with the development of vascular complications [26]. Increased PN levels is because of increased nitric oxide synthase expression due to high glucose level [27]. This could probably attribute to increased oxidative stress which may further cause complications of type 2 DM.

Enzymatic scavengers like glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione molecules (GSH) etc. protect the system from deleterious effects of reactive oxygen species. The antioxidant activity of glutathione peroxidase (GPx) decrease when the insulin level is elevated with hyperglycemia as consequence of long term increased oxidative stress and exhaustion of antioxidant defense mechanisms. Impaired glucose metabolism leads to oxidative stress [28], and protein glycation produces free radicals[29]. furthermore, the decreases in GPx activity, could at least in part result from inactivation of the enzymes by H_2O_2 or by glycation, which are known to occur during diabetes [30-32]. The alterations of oxidative stress markers in the studied type 2 diabetic patients were found to be higher than those of type 1. The reason was unclear, but we believe that the glycemic status, the duration of the disease and increased insulin resistance in type 2 may be implicated in such variation[33]. Increased levels of the products of oxidative damage to lipids have been detected in serum of diabetic patients and their presence correlates with the development of vascular complications [26]. This could probably attribute to increased oxidative stress which may further cause complications of type 2 DM. In healthy individuals, oxidative damage to tissue is prevented by a system of defense which includes antioxidant enzymes and small molecules with scavenging ability such as antioxidant vitamins. Mechanisms involved in the increased oxidative stress in diabetes include not only oxygen free radical generation due to nonenzymatic glycation, auto oxidation of glycation products, but also changes in the tissue content and activity of antioxidant defense systems [34]. Reduced glutathione functions as a direct free radical scavenger as a cosubstrate for glutathione peroxide (GPx) which explained decreased GSH concentration with increased oxidative stress.

The high MDA elevation in both gender of type 2 diabetic patients when compared with corresponding control groups. while failed to exhibit any difference variation between males and females when compared together. this is disagree with the idea which says that females have higher lipid building than males who have higher muscle tissue composition than that of females. So in such environment of hyperinsulinemia, it is possible to increase MDA level and lipid peroxidation in both sex. The decrease in GSH levels in females more than that in males confirms the suggestion that females are more fatty than males and so that their antioxidant system could be severely exhausted due to hyperinsulinemia and increase of ROS generation. Hyperinsulinemia is also a major cause of obesity, because insulin causes the body to store more fat. It also disrupts sodium metabolism, so it increases water retention[35].
The elevation in MDA levels pointed out gradual increase with increase age. The negative correlations of GSH as markers of oxidant defense system, show that their body levels decrease as diabetic progress with age. The cause of lower level of GSH is the loss enhanced oxidation in insulin resistant elder diabetics. Another possible cause is that the lower content may be a result of either decreased synthesis or their

increased degradation. Decreased GSH concentrations could have a marked effect on the detoxification capacity of a senescent organism, since a major function of GSH is in the detoxification of peroxides produced by normal metabolism and of xenobiotics. Thus , this lower capacity may provide toxicological bases for aging[36]. The results demonstrated that the contents of MDA increased as a function of advancing human age[37]. Lipid profile of an individual depends on many factors like life style, type of food, stress, disease, exercise and age. In diabetic patients, the adipose tissues shows a high rate of turnover, possibly due to increased catecholamine-mediated β-adrenoceptor activity, with high activities of hormone-sensitive lipase as well as lipoprotein lipase, these will increase with age causing raising of MDA levels in older patients[38]. So we found that there was an environment of progress of oxidative stress in insulin resistant type 2 diabetics with aging, so high insulin levels could be considered as a direct or indirect stimuli to initiate oxidation and related diabetic complications[39].

The data denote that tissues in obesity and diabetes mellitus exhibit insulin resistance and abundant data that relate insulin resistance to alter patterns of enzyme activity, as determined within muscle homogenates [40]. These results correspond to the insulin resistance syndrome[41]. MDA values in the two subgroups which suggest that there is a maximum level of lipid peroxidation reached in insulin resistant type 2 diabetic patients. So from this finding we can suggest a relationship between insulin resistance and oxidative stress, rather than between oxidative stress and body mass index in type 2 diabetic patients.

APPLICATIONS

The high levels of patients versus control ratio of MDA and PN levels probably suggests the occurrence as a mechanism of tissue damage in cases of T2DM. Moreover, it is recommended that the patient levels of MDA, GSH, and PN should be evaluated in insulin resistance patients.

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