

Evaluation of HbA1c and Oxidation Status in Diabetic Patients (Type 2) in Erbil Governorate

Sarwar Nawzad Jahfar
Department of Biology, College of
Education, University of Salahaddin
sarwarnawzad@gmail.com

Nadir Mustafa Qadir Nanakali
Department of Biology, College of
Education, University of Salahaddin
Cihan University-Erbil
nadirnanakali@gmail.com

Abstract

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in either insulin secretion or insulin action, or both. The chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. This study was carried out from the period of December 2010 to June 2011. Fasting blood samples were obtained from one hundred fifty-two diabetic patients (59 males and 93 females) of type 2 and 50 apparently clinically healthy individuals were selected as a normal group (29 males and 21 females).

The results showed that the mean HbA1c percentage were significantly higher in diabetic patients (9.18%) than normal group percentage (5.57%). Results showed that mean serum Superoxide dismutase activity in diabetic (0.202) IU/L was significantly higher than normal (0.123) IU/L groups. While mean serum glutathione levels (37.726) $\mu\text{mol/L}$ was significantly lower in diabetic patients, than in normal group (42.931) $\mu\text{mol/L}$. beyond that mean vitamin E (9.9) $\mu\text{mol/L}$ was significantly lower in diabetic patients, than normal group (15.6) $\mu\text{mol/L}$. The results revealed high level were of serum MDA (1.60) $\mu\text{mol/L}$ in diabetic patients compared to in normal group (1.27) $\mu\text{mol/L}$. we concluded that diabetes mellitus affects negatively the level of lipid profile and antioxidants vitamin E and Glutathione while lead to increased Malondialdehyde, patients of diabetes mellitus have high HbA1c Values.

Keywords: HbA1c, Antioxidants, Diabetes.

Introduction:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in either insulin secretion or insulin action, or both. The chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Nathan, 1993). Latest report by International Diabetes Federation demonstrated that 366 million people had diabetes till 2011 by 2030 this will have risen to 552 million, The number of people with type 2 diabetes is increasing in every country and 80% of people with diabetes live in low-and middle-income countries. The greatest numbers of people with diabetes are between 40 to 59 years of age. 183 million people (50%) with diabetes are undiagnosed, as for mortality diabetes caused 4.6 million deaths in 2011 (IDF, 2011).

Prevalence estimates of *Diabetes Mellitus* in 2007 in Iraq was 14,699 at the age range of 25-79 which participate in the global prevalence by 6% according to International Diabetes Federation (IDF) atlas 2007, and this number will rise to 25,477 at the same age range by 2025 (IDF, 2007). At the last nine months 406 new diabetic cases have been registered in Layla Qasim Clinic for diabetes-Erbil, which added to the total number of diabetic patients exceeded 10.000 including both types 1 and 2, but the majority is type 2 diabetes (Qadir, 2010).

In diabetes, impaired lipid metabolism involves both quantitative and qualitative changes often referred to as a diabetic dyslipidemia. The major lipid disorders detected in type 2 diabetes are triglycerides, total cholesterol, LDL, and HDL concentrations (Erkelens, 1998).

Metabolic control of hyperglycemia will prevent in alteration in peroxidation and the lipid metabolism, which may help in good prognosis and preventing manifestation of vascular and secondary complication in diabetes mellitus (Suryawanshi *et al*, 2006). During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation, Free radicals are generated as by-products of normal cellular metabolism; however, several conditions are known to disturb the balance between ROS production and cellular defense mechanisms in diabetic patients. This imbalance can result in cell dysfunction and destruction resulting in tissue injury, the increase in the level of ROS in diabetes could be due to their increased production and/ or decreased destruction by non-enzymic and enzymic catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) antioxidants (Bonnefont rousset *et al*, 2000).

The levels of the antioxidant enzymes critically influence the susceptibility of various tissues to oxidative stress and are associated with the development of complications in diabetes. Also this is particularly relevant and dangerous for the beta islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses (West, 2000). Antioxidant treatments have been proposed to be prospective in the treatment and prevention the progression of diabetes and the occurrence of complications resulted from diabetes. Which is include endogenous (enzymatic antioxidants), mainly, superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase, and glutathione reductase and exogenous (non-enzymic antioxidants) such as vitamins A, C, and E, glutathione (Cadenas and Packer, 2002).

The aims of this study were to evaluate the oxidative stress status through measuring malondialdehyde and antioxidants. In order to determine the differences in physiological and biochemical parameters among diabetic patients according to gender.

Material and Method

In this study two groups (patients and controls) were studied. Fifty clinically healthy individuals were selected randomly (29) males and (21) females as control group ages were ranged from (34-62) and one hundred fifty-two patients with type 2 diabetes were selected randomly in Layla Qasm center for diabetic patients in Erbil city (152) which included (59) males and (93) females, their age was ranged from (25-65).

Venous blood samples about (8-10 ml) were taken from all patients and control individuals after overnight fasting. About 3 ml of the samples was collected in K3-EDTA tubes and kept cool to determine HbA1c at the same day. The remaining volume were 6-8 ml which collected in regular plastic tubes and centrifuged minimum of 10 minutes at (2000) rpm serum was used for serum antioxidant and oxidant tests.

Colorimetric Determination of Glycosylated Hemoglobin

Glycosylated hemoglobin determined using HbA1c determination kit (Stanbio), in this procedure a preparation of hemolyzed whole blood is mixed with a weakly binding cation-exchange resin. The non-glycosylated hemoglobin (HbA1) binds to the resin, leaving (HbA₂) free to be removed by means of a resin separator in the supernate. The percent of HbA₂ is determined by measuring the absorbance values at 415 nm of the HbA₂ fraction and of the total Hb fraction, calculating the ratio of absorbances R, and comparing this ratio to that of a glycohemoglobin standard carried through the same procedure (Abraham *et al.*, 1978). Results arc express as HbA1, but can be converted or derived as HbA1c by using a conversion factor or when using a HbA1c value for the standard. Values of HbA1c less than 3-6.5 % consider as normal range.

Determination of Serum Superoxide Dismutase Activity (SOD)

The superoxide dismutase activity was estimated by using modified biochemical Nitroblue tetrazolum (NBT) method. This method includes the using of sodium cyanide as peroxidase enzyme inhibitor and it depends on indirectly determination of SOD activity according to change of phormazin absorbance intensity, which was formed by reaction of superoxide with Nitroblue tetrazolum dye after serum radiation (Brown and Goldstein, 1983). The decreasing in phormazin absorbance intensity was used as indicator for SOD activity.

Determination of Reduced Glutathione (GSH)

The level of serum GSH was determined by using Ellman's reagent which modified by (Al-Zamely *et al*, 2001). The principle of determination includes the reaction between the glutathione in serum and Ellman's reagent which gives a color and read at 412 nm using spectrophotometer method.

Determination of Serum Vitamin E (A-Tocopherol)

Tocopherol can be estimated using Emmeric- Engle reaction which based on the reduction of ferric to ferrous ions by tocopherols, which then forms a red colour with 2, 2'- dipyridyl-tocopherol and carotenes are first extracted with xylene and the extraction read at 460 nm to measure carotenes. A correlation is made for these after adding ferric chloride and reading at 520 nm (Shakila, 2010).

Determination of Serum Malondialdehyde (MDA)

The assessment of the lipid peroxidation process is achieved via determination the end product, malondialdehyde. The level of serum MDA was determined spectrophotometrically with a thiobarbituric acid (TBA) solution (Reddy *et al*, 2011).

Data Analysis

Computerized statistical analysis were performed using Statistical package for science service (SPSS) version 19.0 computer software. The data were expressed as Mean and standard error of mean (Mean $\pm$ S.E.M.). The differences in mean values between two groups were analyzed by 2-tailed t-test. Probability level of P value (P<0.01) and (P<0.05) level of significance was considered to be statistically significant.

Results and Discussion

We had two groups studied for different parameters, case group which were included diabetic patients of type 2 and normal group which apparently healthy

individuals were selected. Table (1) shows the mean of glycosylated hemoglobin percentage in diabetic and normal groups. The results obtained revealed that the mean of Glycosylated hemoglobin levels were 9.189 ± 0.255 % and 5.570 ± 0.186 % in diabetic and normal groups respectively. The values obtained in diabetic group showed that there was a significant difference in HbA1c means of diabetic patients. When compared to normal non-diabetic group this result supported by (Manjunatha *et al.*, 2011). In results of current study the HbA1c level of diabetic patients were found to be above 7% , this result is supported by the guidelines of American diabetes association which demonstrate that an HbA1c of 6.5 percent or higher indicates a diagnosis of diabetes.

Results of our study revealed that SOD activity in type 2 diabetic patients 0.202 ± 0.010 IU/L was higher than the activity of SOD in non-diabetic patients 0.123 ± 0.006 IU/L (normal) group as it is clear in table (2), that mean SOD activity of diabetic patients increase these results was consistent with results of (Aydin *et al.*, 2001; Chug *et al.*, 2001 and Martínez-sánchez *et al.*, 2005). Increase of SOD activity is sign of oxidative stress in the diabetic patients by increased level of free radical which may resulted from hyperglycemia. Hyperglycemia can directly cause increased ROS generation. Glucose can undergo autoxidation and generate $\text{OH}^\bullet$ radicals. In addition, glucose reacts with proteins in a non-enzymatic manner leading to the development of amadori products followed by formation of AGEs. ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of superoxide radicals (Johansen *et al.*, 2005). Reactive species can be eliminated by a number of enzymatic and non-enzymatic antioxidant mechanisms. SOD immediately converts superoxide to H_2O_2 , which is then detoxified to water either by catalase in the lysosomes or by glutathione peroxidase in the mitochondria.

Table (1): Mean $\pm$ S.E. of glycosylated hemoglobin in diabetic patients (type 2) and normal group.

Groups	HbA1c %	T. value	P. value	normal range
Diabetic	$9.189 \pm .255$	11.458**	.000	4-6.5 %
Normal	$5.570 \pm .186$			

Table (2) Mean $\pm$ S.E. of serum superoxide dismutase activity in both diabetic patients (type 2) and normal group

Groups	SOD IU/L	T. value	P. value
Diabetic	0.202 ± 0.010	6.814**	.000
Normal	0.123 ± 0.006		

The values serum glutathione obtained in diabetic group exceeded values of non-diabetic group (Table 3) but it there were no significance difference between Them $37.726 \pm 1.217 \mu\text{mol/L}$ $42.931 \pm 1.977 \mu\text{mol/L}$ respectively. Glutathione is the most important endogenous antioxidant in most organisms, including humans (Meister 1991). GSH is often used as a biological marker for the occurrence of oxidative stress (Meister, 1991) Glutathione is the major cellular antioxidant that protects against environmental toxicants as well as reactive oxygen species (ROS) mediated cell injury. Although there is no significance difference between diabetic and non-diabetic but we observe that diabetic patients had lower glutathione levels, which matches with previous studies that suggested that individual with DM may have lower GSH levels than age-matched non-diabetic subject controls. (Kharb *et al*, 2000; Beard, 2001 and Chugh *et al*, 2001). The reason behind the decrease of glutathione it is suggested that GSH metabolism is altered in type 2 diabetic. Several studies support the hypothesis that in DM, chronic hyperglycemia increases the polyol pathway as well as AGE formation and free radical generation rate, leading to increase GSH oxidation. (Martínez-sánchez *et al.*, 2005).

Table (3) Mean $\pm$ S.E. of serum glutathione in both diabetic patients (type 2) and normal group

Groups	Glutathione $\mu\text{mol/L}$	T. value	P. value
Diabetic	37.726 ± 1.217	6.814	.026
Normal	42.931 ± 1.977		

Table (4) shows the mean of fasting serum malondialdehyde concentration in diabetic and normal groups. The results obtained revealed that the mean serum MDA levels were 1.603 ± 0.048 and $1.272 \pm 0.067 \mu\text{mol/L}$ in diabetic and normal groups, respectively. In the present study, lipid peroxidation product level MDA was significantly ($P < 0.01$) increased in type 2 diabetes mellitus compared to control subjects. There are several studies reported an increase level of MDA in serum type2 diabetes (Sundaram *et al.*, 1996; Chugh *et al*, 2001; and Maritim *et al*, 2003). Measurement of malondialdehyde is by far the indicator of oxidative damage to cells and tissue. MDA is produced from the breakdown of polyunsaturated fatty acids. MDA has been recognized as an important indicator of lipid peroxidation and oxidative stress-related disease states.

This finding suggests that, type 2 diabetic patients differ in their susceptibility to oxidative stress. Increase levels of lipid peroxidation may cause oxidative injury to blood cells, cross-linking in membrane proteins and lipids .On the other hand, the current study disagreement with the results of Jorge who found that there is no

significant difference between serum MDA in type 2 Diabetes and control group (Jorge et al, 2005). The difference in MDA level may be due to many factors like, racial variation, seasonal variation and geographical localities, social, the amount of antioxidants such as (vitamin A, C, and E,) daily intake with the diet and eating habit of the people.

Table (4) Mean $\pm$ S.E. of serum malondialdehyde in diabetic patients (type 2) and Normal

Groups	MDA $\mu\text{mol/L}$	T. value	P. value
Diabetic	1.603 $\pm$ 0.048	3.981**	.000
Normal	1.272 $\pm$ 0.067		

In the current study, an elevation in MDA levels, the lipid peroxidation product as a marker of oxidative stress, were observed to be significant in diabetic patients. This clearly shows that diabetic patients were exposed to an increased oxidative stress via lipid peroxidation.

There are several factors believed to be effective in increasing lipid peroxidation as a result MDA these factors are abnormal increase in levels of lipid (cholesterol and TG), lipoprotein (HDL-C and LDL-C) and lipid peroxides (MDA) in serum may be due to the abnormal lipid metabolism (Suryawanshi et al, 2006) -. Increase of lipid peroxide may be due to the increased glycation of protein in diabetes mellitus. The glycated protein might themselves act as a source of free radicals (Suryawanshi et al, 2006).

A deficiency of the antioxidant activity of vitamins (C and E) has been related to higher concentration of peroxide. There may be imbalance between production and scavenging of free radicals produced due to the lack of antioxidant system. (Suryawanshi et al, 2006) In addition, there is a clear association between lipid peroxide and glucose concentration, which may be also thought to play a role in increased lipid peroxidation in diabetes mellitus.

Results in table (5) revealed that diabetic patient group had less serum vitamin E $9.900 \pm 0.522 \mu\text{mol/L}$ than the non-diabetic group (normal) $15.689 \pm 0.694 \mu\text{mol/L}$ and the difference was highly significant. Our result is constituent with results of other (Yanagawa et al, 2001). Vitamin E is one of the most effective antioxidants in animals. It is composed of various subfamilies of which tocopherols and tocotrienols are the most studied. The structural difference between the two subfamilies is that tocotrienols possess three double bonds in their isoprenoid side chain, and this structural difference results in differences in their efficacy and potency as antioxidants.

Table (5) Mean $\pm$ S.E. of serum vitamin E in diabetic patients (type 2) and Normal

Groups	Vitamin E $\mu\text{mol/L}$	T. value	P. value
Diabetic	9.900 $\pm$ 0.522	6.658**	.000
Normal	15.689 $\pm$ 0.694		

Our results showed significance decrease of vitamin E in diabetic patients compared to control group, this decrease of vitamin E in diabetic patients is due consumption of vitamin E in prevention of Lipid peroxidation. Vitamin E prevents lipid peroxidation in plasma membrane by maintaining lipids present in plasma membrane specially unsaturated fatty acids and decrease the oxidation in diabetic patients and leads to decrease vitamin levels in blood. And it is not replaced by more because of limited diet of diabetic patients it may not contain enough vitamin E, and they didn't take vitamin supplementation.

Conclusion

The results indicated that there is imbalance between serum lipid peroxidation and plasma antioxidant systems in patients with type2 diabetes mellitus. Decreased levels of Glutathione, and vitamin E antioxidants with increase of superoxide dismutase activity in diabetic patients.

Increased level of MDA in serum of type 2 diabetes was detected which is the product of lipid peroxidation. Gender differences among diabetic patients some parameters were significant and other were not.

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