A Comparative study on oxidative stress and antioxidant status in type II diabetes mellitus with and without altered lipid pattern

Apoajela Aposallah Ahmed

Chemistry department, Faculty of Education, Obary, Sebha university

Abstract:

Much research has been done regarding the role of free radicals in various diseases and the role of antioxidants in prevention of diseases. Diabetes mellitus is known to cause imbalance between free radicals and antioxidants and as a consequence leading to oxidative stress. The study is to make a comparative study on oxidative stress and antioxidant status in type II diabetic patients with and without alteration in lipids. Degree of lipid peroxidation was measured in terms of serum malondialdehyde (MDA) along with lipid profile. Glucose, hemosylated hemoglobin (HbA1c) and antioxidant superoxide dismutase (SOD) were also measured in the present study. Total 80 known diabetic patients were studied. These patients were grouped in accordance with the changes in lipid pattern that one group (group I) having diabetes mellitus with alteration in lipids and the other group (group II) having diabetes mellitus without alteration in lipids. Results showed through the present study that MDA level was significantly higher in group I compared with group II. The same was also true for the level of HbA1c. There was also significant increase in total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglyceride in group I compared with group II. There were significant decrease in HDL-cholesterol and superoxide dismutase (SOD) in group I compared with group II. The present study has showed that the alteration in lipids leads to an increase in plasma lipid peroxidation (MDA) which is in parallel to the decrease in the antioxidant (SOD) and plasma HDL, eventually resulting in increase in free radicals and imbalance occurs between free radicals and antioxidants causing oxidative stress, which all indicate type II diabetic patients are at risk of cardiovascular complications due to oxidative stress.

Keywords: free radical, antioxidant and oxidative stress.

Introduction:

Diabetes mellitus:

Diabetes mellitus is a common metabolic disorder resulting from defects in insulin secretion or action or both is characterized by hyperglycemia often accompanied by glycosuria, polydipsia, and polyuria. 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. 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 nonenzymic and enzymic antioxidants. The level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes [27].

Free radicals:

A free radical can be defined as any molecular species capable of independent existence that contains one or more unpaired electron in an atomic orbital. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants. They are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism. Free radicals can be formed continuously in the cells either from normal essential metabolic processes in the human body or from external sources. The formation in the cells occurs as a consequence of both enzymatic and nonenzymatic reactions. Enzymatic reactions, which serve as source of free radicals, include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis, and in the cytochrome P-450 system. Free radicals can also be formed in nonenzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing reactions. The external sources which contribute to producing free radicals include ionizing reactions, cigarette smoking, air pollutants, and industrial chemicals. At low or moderate concentration some of the free radicals play beneficial physiological role *in vivo* this include defense against infectious agents by phagocytosis, energy production and cell growth. . Free radicals are highly reactive species, capable of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids. Free radicals attack important macromolecules leading to cell damage and homeostatic disruption [33].

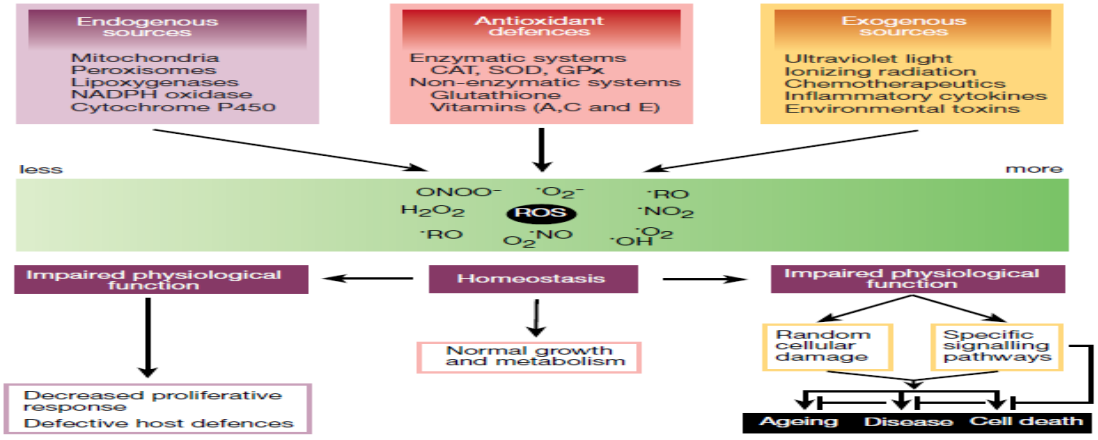


Figure 1 The sources and cellular responses to reactive oxygen species (ROS) [34].

Free radicals in biology:

Free radical reactions are expected to produce progressive adverse changes that accumulate with age throughout the body. Such "normal" changes with age are relatively common to all. Cancer and atherosclerosis, two major causes of death, are "free radical" diseases. Cancer initiation and promotion is associated with chromosomal defects and oncogene activation. It is possible that endogenous free radical reactions, like those initiated by ionizing radiation, may result in tumor formation. Studies on atherosclerosis reveal the probability that the disease may be due to free radical reactions involving diet-derived lipids in the arterial wall and serum to yield peroxides and other substances [33].

Antioxidants:

Antioxidants are any substance that delay or inhibits oxidative damage to a target molecule by reacting and donating an electron to a free radical to neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property. The body produces different endogenous antioxidants to neutralize free radicals and protect the body from different disease leads by the tissue injury. Exogenous antioxidants which are externally supply to the body through food also play important role to protect the body. The body has developed several endogenous antioxidant defense systems classified into two groups enzymatic and non-enzymatic. The enzymatic defense system includes different endogenous enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and non-enzymatic defense system included vitamin E (α-tocopherol), vitamin C (ascorbic acid) , vitamin A (B-carotene) and reduced glutathione (GSH) [31,33].

Oxidative Stress:

The term is used to describe the condition of oxidative damage resulting when there is an imbalance between free radical production and antioxidant defenses .In other words, there is an excess of ROS and decrease in antioxidants levels, this may cause tissue damage by physical, chemical, psychological factors that lead to tissue injury in human and causes different diseases. Oxidative stress is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids. Short-term oxidative stress may occur in tissues injured by trauma, infection, heat injury, hypertoxia, toxins, and excessive exercise. These injured tissues produce increased radical generating enzymes (e.g., xanthine oxidase, lipogenase, cyclooxygenase) activation of phagocytes, release of free iron, copper ions, or a disruption of the electron oxidative transport chains of phosphorylation, producing excess ROS. The initiation, promotion, and progression of cancer, as well as the side-effects of radiation and chemotherapy, have been linked to the imbalance between ROS and the antioxidant defense system. ROS have been implicated in the induction and complications of diabetes mellitus, age-related eye disease, and neurodegenerative diseases such as Parkinson's disease [31,33].

Oxidative stress and human diseases:

A role of oxidative stress has been postulated in many conditions, including atherosclerosis, inflammatory condition, certain cancers, and the process of aging. Oxidative stress is now thought to make a significant contribution to all inflammatory diseases (arthritis, vacuities, glomerulonephritis, lupus erythematous, adult respiratory diseases syndrome), ischemic diseases (heart diseases, stroke, intestinal ischema), hemochromatosis, acquired immunodeficiency syndrome, emphysema, organ transplantation, gastric ulcers, hypertension and preeclampsia, neurological disorder (Alzheimer's disease, Parkinson's disease, muscular dystrophy), alcoholism, smoking-related diseases, and many others. An excess of oxidative stress can lead to the oxidation of lipids and proteins, which is associated with changes in their structure and functions [33].

Materials And Methods:

Materials:

Sample size:

The study group consists of 80 diabetes mellitus patients between the age 35-65 years of both sexes (male & female). The samples of these patients were collected from Alshorok lab at Obary region. The patients were divided into two groups (40 patients for each group). Group I for patients having diabetes mellitus with alteration in lipids and the other group for patients having diabetes mellitus without alteration in lipids.

Collection of samples and site of experiment:

Fasting and random venous blood sample (5ml) of diabetic patient was collected in sodium fluoride to detect glucose and EDTA to detect HbA1c. Serum was made to detect lipids, MDA and SOD. The samples were analyzed in clinical biochemistry section of Alshorok lab.

Methods and Biochemical analysis:

Routine analysis:

• Serum or plasma glucose: The method used is GOD-PAP method.
• Plasma HbA1c: The method used is Cation-Extchange Resin method.
• Serum Total cholesterol: The method used is CHOD-POD method.
• Serum HDL-Cholesterol: The method used is CHOD-POD method.
• Serum LDL-Cholesterol: It is calculated value by Friedwald's equation.
• Serum Triglyceride: The method used is GPO-PAP method.

b- Oxidative stress marker:

• MDA (malondialdehyde): The method used is Beuge method.

Antioxidant enzyme:

• SOD: The method used Misra and Fridovich method.

- Instrumentation:
The following instruments were used during the course of study:
• Centrifuge
• Incubator
• Colorimeter

Quantitative determination of glucose in plasma:
Method Name: CHOD-PAP method
Procedure:
Preparation of working reagent:
Transfer 1 vital Enzyme powder to one bottle of (100ml) reagent No.3 (working Buffer). Mix gently to dissolve which is ready to use.

Pipette into test tube marked	Blank	Standard	Test
Serum	-	-	0.02 ml
Standard	-	0.02 ml	-
Working Reagent	3 ml	3 ml	3 ml

Mix well and incubate for 15 minutes at 37℃ or 20 minutes at 30℃ . Mix well and measure the absorbance of standard and test against the reagent blank at 500 nm (480-520 nm).

Quantitative determination of Glycosylated Hemoglobin
Method used: Ion Exchange Resin method.
Preparation of working reagent:
The Ion Exchange Resin tubes & the Lysing Reagent are ready to use.
Procedure:
Hemolysate preparation:
• Dispense 0.5 ml Lysing Reagent into tubes labeled as control(c) & Test(T).
• Add 0.1ml of the reconstituted control & well mixed blood sample into the appropriately labeled tubes. Mix until complete lysis is evident.
• Allow to stand for 5 minutes.

Glycosylated hemoglobin separation:
• Remove cap from Ion-Exchange Resin tubes and label as control & Test.
• Add 0.1ml of the hemolysate from step A into the appropriately labeled Ion Exchange Resin tubes.
• Insert a resin separator into each tube so that the rubber sleeve is approximately 1cm above the liquid level of the resin suspension.
• Mix the tubes on rocker, rotator or a vortex mixer continuously for 5 minutes.
• Allow the resin to settle, then push the resin separator into the tubes until the resin is firmly packed.

• Pour or aspirate each supernatant directly into a cuvette and measure each absorbance against distilled water.

c- Total hemoglobin fraction:

• Dispense 5 ml of distilled water into tubes labeled as control & test.
• Add to it 0.02 ml of hemolysate from step A into the appropriately labeled tube. Mix well.
• Read absorbance against distilled water.

Quantitative determination of cholesterol in serum:

Method used: CHOD/POD method.

Procedure:

Preparation of working reagent: All reagents are ready to use.

Pipette into test tube marked	Blank	Standard	Test
Serum	-	-	10µl
Cholesterol Standard	-	10µl	-
Cholesterol Reagent	1000 µl	1000 µl	1000 µl

Mix well and incubate for 5 minutes at 37℃. Mix well and measure the absorbance of standard and test against the reagent blank at 505 nm(490-530).

Quantitative determination of HDL cholesterol in serum

Method used: CHOD/POD method

Procedure

Preparation of working solution:

• Take 0.5 ml of serum/plasma into a glass tube.
• Add 50 µl precipitating reagent.
• Mix well, leave it for 10 minutes at room temperature.
• Centrifuge at 3000 rpm for 10 minutes.
• Take the clear supernatant for HDL-cholesterol.

Mix well and incubate for 5 minutes at 37℃. Mix well and measure the absorbance of standard and test against the reagent blank at 510 nm.

Pipette into test tube marked	**Blank**	**Standard**	**Test**
Supernatant Sample	-	-	10µl
Standard	-	10µl	-
Enzyme Reagent	1000 µl	1000 µl	1000 µl

Quantitative determination of triglycerides in serum
Method used: GPO-PAP, End point Assay.
Procedure:
Preparation of working solution: All reagents are ready to use.

Pipette into test tube marked	**Blank**	**Standard**	**Test**
Serum/*Plasma*	-	-	10µl
Standard	-	10µl	-
Reagent	1000 µl	1000 µl	1000 µl

Mix well and incubate for 10 minutes at 37℃. Mix well and measure the absorbance of standard and test against the reagent blank at 505 (490-550 nm.

Quantitative of MDA in Serum or Plasma

Reagents required:

- 375mg TBA.
- 0.25N HCl.
- 15g TCA.

Preparation of working solution:

- Dissolve 375 mg of TBA in 2ml of 0.25N HCl
- Add 15g of TCA and made up to 100ml.

Procedure:

- Heat the solution at 50℃ for 5-10 min.
- Take 1ml of the solution.
- Add 1ml of serum to the solution.

- Incubate it in boiling water for 15 min.
- Then take it out and leave it till it becomes cool.
- Centrifuge it at 3000 Rpm for 10 min.
- Measure it against blank at 535 nm.

Quantitative determination of SOD activity in Serum

Reagents required:

- Carbonate Buffer (0.2M).
- KCl (0.015M).
- Epinephrine (0.025M).

Preparation of the Sample:

- Collect blood without using an anticoagulant such as heparin, citrate, or EDTA.
- Allow blood to clot for 30 minutes at 25℃.
- Centrifuge the blood at 2000 Rpm for 15 minutes at 4℃. Pipette off the top yellow serum layer without disturbing the white buffy layer.

Preparation of working solution:

- **Preparation of Carbonate buffer (0.2M, pH-10.2):**

Carbonate buffer consists of Na2CO3 and NaHCO3 and these two chemicals have to be prepared in 100 ml of distilled water. To do so the following formula which can be used to obtain the value which is then dissolved in 100 ml of distilled water.

The formula is : $w = \frac{M \times M.W \times \text{desired volum}}{1000}$

To prepare the carbonate solution

A) 2.12 g of Na2CO3 1n 100 ml gives 0.2 M solution

B) 1.65 g of NaHCO3 in 100 ml gives 0.2 M solution

33 ml of A + 17 ml of B gives 50 ml of buffer solution of 0.2 M.

- **Preparation of potassium Chloride (KCl): 0.0115M**

The formula is : $w = \frac{M \times M.W \times \text{desired volum}}{1000}$

$$w = \frac{0.015 \times 74.6 \times 100}{1000} = 0.111\text{gm}$$

So, about 0.111 gm has to be balanced and dissolve it 100ml of distilled water in order to get 0.015M of KCl.

- **Preparation of epinephrine: 0.025M**

The formula which is used to prepare 0.025M of epinephrine as follows:

$$V = \frac{\text{desired M} \times \text{desiredv} \times \text{Eq.W}}{\%\text{purity} \times \text{specific gravity} \times 1000}$$

$$V = \frac{0.025 \times 10 \times 100 \times 14.09}{98.8\% \times 1 \times 1000} = 3.5\text{ml}$$

So, 3.5 ml of epinephrine was taken and the volume was made to 10 ml by adding distilled water to get 0.025 M of epinephrine and add to it 10ml of distilled water so that we get 0.025 of epinephrine.

Procedure:

- Take 1.0 ml of carbonate buffer (0.2M, pH 10.2).
- Add 0.8 ml KCl (0.015).
- Add 0.1 ml of serum and water to make the final volume 3 ml.

- The reaction is started by adding 0.2 ml of epinephrine (0.025M).
- Absorbance is measured at 480 nm at 15 second intervals for one minute.

Results:

The experimental results of the present study have been presented and summarized in the form of tables and figures along with appendix. The various data which have been obtained during the study. All the results are subjected to statistical analysis in order to find out the mean, standard deviation, P value and t test of each group. All the results obtained are shown in this chapter and whether there is statistical significant or not between the two groups for each parameter.

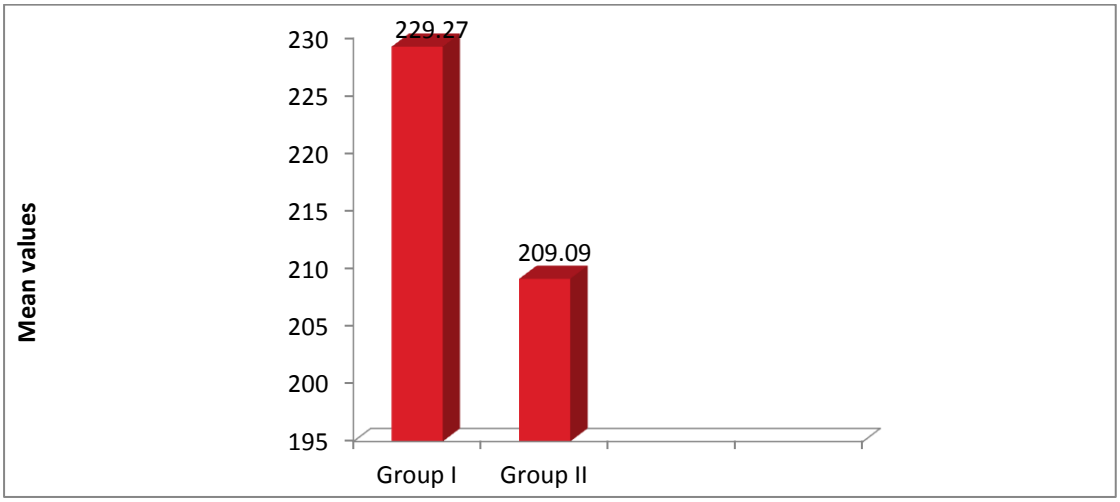
Table 1: The mean, standard deviation and SEM of Blood sugar (α. Dglucose) levels in diabetic patients with and without alteration in lipids pattern.

Groups	Mean±SD	SEM
Group I	229.27±62.21	12.44
Group II	209.09±52.74	10.54

Table 2: The P value significance and t-test values of glucose in plasma.

Groups	P values Significance	t-test
Group I	Not statistically significant	1.23
Group II		

Figure 1: Blood sugar (α. D glucose) level in plasma of diabetic patients with and without alteration in lipids pattern.



The mean, standard deviation, P value and t-test value for glucose in the two groups are shown in table 1 and 2 respectively. In the present study it was found that there is no significant difference in the glucose level between the two groups. There is no significant increase in glucose level in group I compared to the group II.

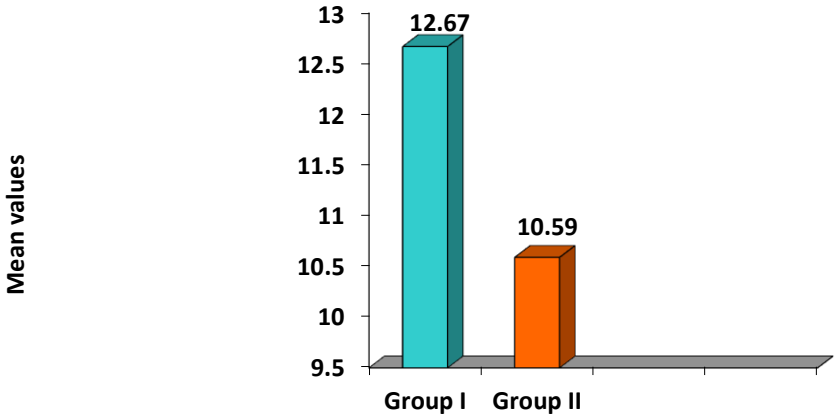
Table 3: The mean, standard deviation and SEM Hemosylated Hemoglobin (HbA1c) level in diabetic patients with and without alteration in lipid profile.

Groups	Mean±SD	SEM
Group I	12.67±3.59	0.71
Group II	10.59±2.96	0.59

Table 4: P values significance and t-test values of Hemosylated hemoglobin (HbA1c) in plasma.

Groups	P values Significance	t-test
Group I	Statistically significant	2.23
Group II		

Figure 2: Hemosylated hemoglobin (HbA1c) level in plasma of diabetic patients with and without alteration in lipids.



The mean, standard deviation, P value and t-test value for HbA1c in the two groups are shown in table 2.1 and 2.2 respectively. In the present study it was found that there is significant difference in the HbA1c level between the two groups. There is significant increase in HbA1c level in group I compared to the group II.

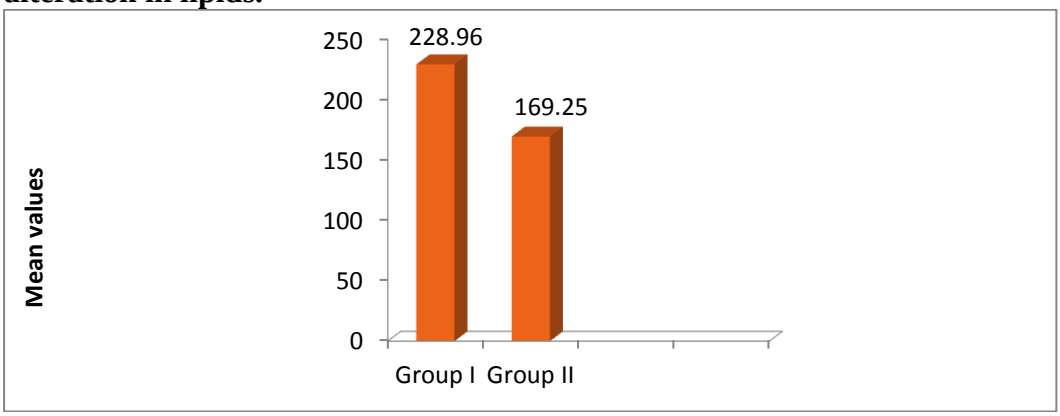
Table 5: The mean, standard deviation and SEM of Cholesterol levels in serum of diabetic patients with and without alteration in lipids.

Groups	Mean±SD	SEM
Group I	288.96±71.17	14.23
Group II	169.25±20.32	4.06

Table 6: P values significance and t-test value of cholesterol in plasma.

Groups	P values Significance	t-test
Group I	Statistically significant	8.087
Group II		

Figure 3: Cholesterol level in plasma of diabetic patients with and without alteration in lipids.



The mean, standard deviation, P value and t-test value for cholesterol in the two groups are shown in 3.1 and 3.2 respectively. In the present study it was found that there is significant difference in the cholesterol levels between the two groups (group I & group II). There is significant increase in cholesterol level in group I compared to the group II.

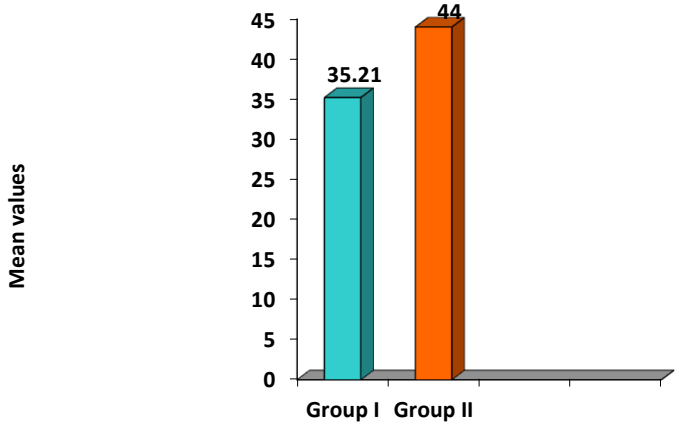
Table 7: The mean, standard deviation and SEM of HDL-cholesterol levels in plasma of diabetic patients with and without alteration in lipids.

Groups	Mean±SD	SEM
Group I	35.21±6.51	1.30
Group II	44.00±6.15	1.23

Table 8 : P value significance and t-test values of HDL-cholesterol in plasma.

Groups	P values Significance	t-test
Group I	Statistically significant	4.90
Group II		

Figure 4: The mean of HDL-cholesterol levels in plasma of diabetic patients with and without alteration in lipids.



The mean, standard deviation, P value and t-test value for HDL-cholesterol in the two groups are shown in the table 4.1 and 4.2 respectively. In the present study it was found that there is significant difference in the HDL-cholesterol levels between the two groups (group I & group II). There is significant decrease in HDL-cholesterol levels in group I compared to the group II.

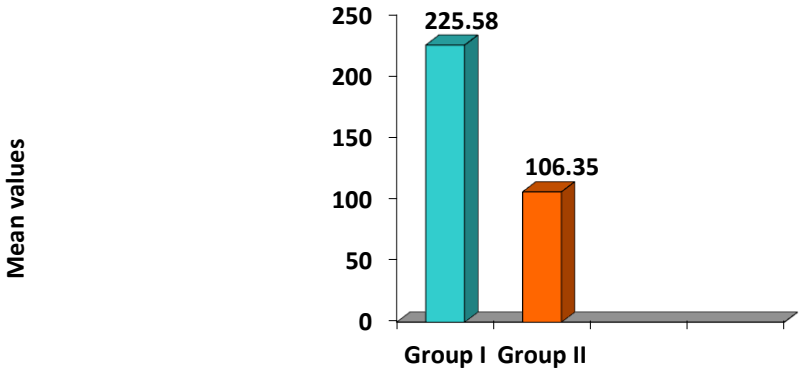
Table 9 : The mean, standard deviation and SEM of LDL- cholesterol in plasma of diabetic patients with and without alteration in lipids.

Groups	Mean±SD	SEM
Group I	225.58±69.58	13.91
Group II	106.35±14.74	2.94

Table 10 : P value significance and t-test value of LDL-cholesterol in plasma.

Groups	P values Significance	t-test
Group I	Statistically significant	8.38
Group II		

Figure 5: The mean values of LDL-cholesterol in serum of diabetic patients with and without alteration in lipids.



The mean, standard deviation, P value and t-test value for LDL-cholesterol in the two groups are shown in the table 5.1 and 5.2 respectively. In the present study it was found that there is significant difference in the LDL-cholesterol levels between the two groups (group I & group II). There is significant increase in HDL-cholesterol levels in group I compared to the group II.

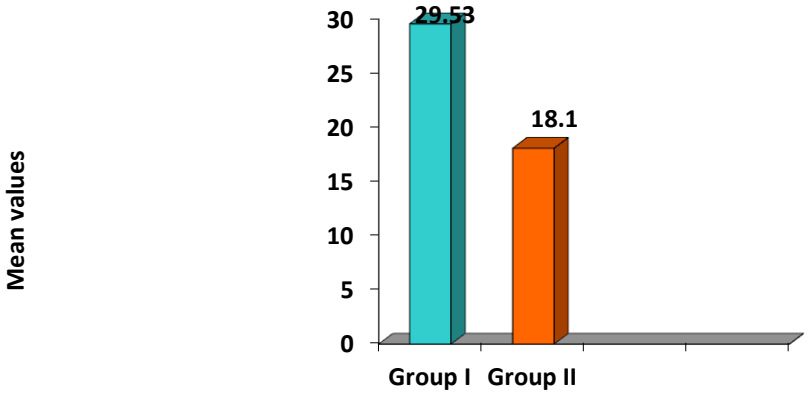
Table 11 : The mean, standard deviation and SEM of VLDL-cholesterol in serum of diabetic patients with and without alteration in lipids.

Groups	Mean±SD	SEM
Group I	29.53±10.76	2.15
Group II	18.10±4.52	0.90

Table 12 : P value significance and t-test of VLDL-cholesterol in serum.

Groups	P values Significance	t-test
Group I	Statistically significant	4.89
Group II		

Figure 6 : The mean of VLDL levels in serum of diabetic patients with and without alteration in lipids.



The mean, standard deviation, P value and t-test value for VLDL-cholesterol in the two are shown in table 6.1 and 6.2 respectively. In the present study it was found that there is significant difference in the VLDL-cholesterol levels between the two groups (group I & group II). There is significant increase in VLDL-cholesterol levels in group I compared to the group II.

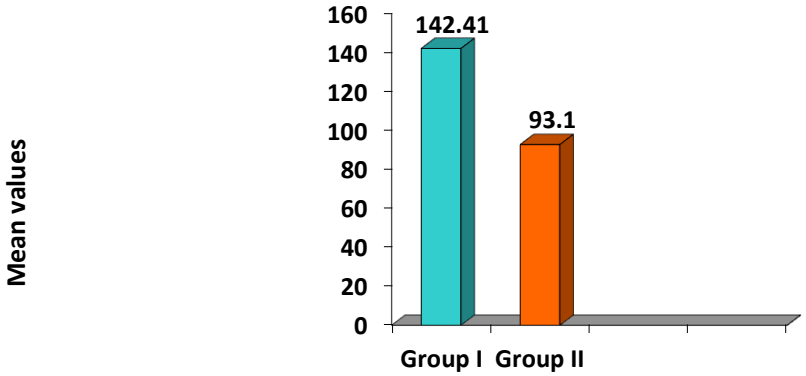
Table 13 : The mean, standard deviation and SEM of Triglyceride in serum of diabetic patients with and without alteration in lipids.

Groups	Mean±SD	SEM
Group I	142.41±52.78	10.55
Group II	93.10±28.74	5.75

Table 14 : P value significance and t-test value of Triglyceride levels in serum of diabetic patients with and without alteration in lipids.

Groups	P values Significance	t-test
Group I	Statistically significant	4.10
Group II		

Figure 7 : The mean of Triglyceride levels in serum of diabetic patients with and without alteration in lipids.



The mean, standard deviation, P value and t-test value for Triglyceride in the two groups are shown in table 7.1 and 7.2 respectively. In the present study it was found that there is significant difference in the Triglyceride levels between the two groups (group I & group II). There is significant increase in Triglyceride levels in group I compared to the group II.

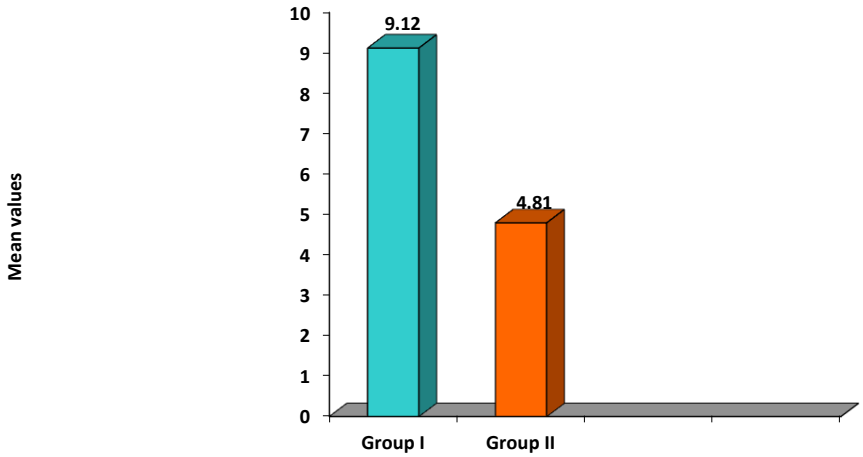
Table 15 : The mean, standard deviation and SEM of MDA in plasma of diabetic patients with and without alteration in lipids.

Groups	Mean±SD	SEM
Group I	9.12±2.87	0.57
Group II	4.81±2.78	0.55

Table 16: P value significance and t-test value of MDA levels in serum of diabetic patients with and without alteration in lipids.

Groups	P values Significance	t-test
Group I	Statistically significant	5.39
Group II		

Figure 8: The mean of MDA levels in serum of diabetic patients with and without alteration in lipids.



The mean, standard deviation, P value and t-test value for MDA in the two groups are shown in table 8.1 and 8.2 respectively. In the present study it was found that there is significant difference in the MDA levels between the two groups (group I & group II). There is significant increase in MDA levels in group I compared with the group II.

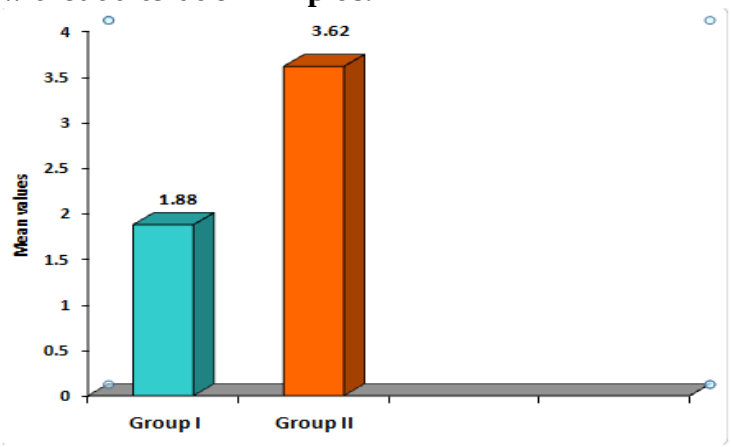
Table 17: The mean, standard deviation and SEM of SOD activity in plasma of diabetic patients with and without alteration in lipids.

Groups	Mean±SD	SEM
Group I	1.88±0.95	0.19
Group II	3.62±1.39	0.27

Table 18: P value significance and t-test value of SOD activity in serum of diabetic patients with and without alteration in lipids.

Groups	P values Significance	t-test
Group I	Statistically significant	5.16
Group II		

Figure 9: The mean of SOD activity levels in serum of diabetic patients with and without alteration in lipids.



The mean, standard deviation, P value and t-test value for SOD activity in the two groups are shown in table 9.1 and 9.2 respectively. In the present study it was found that there is significant difference in the SOD activity levels between the two groups (group I & group II). There is significant decrease in SOD activity levels in group I compared to the group II.

Discussion:

Diabetes mellitus has been known to be a state of excess generation of free radicals contributed by several mechanisms, including hyperglycemia and reducing in antioxidant levels causing oxidative stress which leads to the development and complications of diabetes mellitus. Oxidative stress is the existence of products called free radicals and reactive oxygen species (ROS) which are formed under normal physiological conditions but become deleterious when not being quenched by the antioxidant systems [27].

Excessive production of free radicals has been observed in type II (non-insulin dependent) diabetes by several studies. Study has shown increase in lipid peroxidation due to elevated free radicals in type II diabetes mellitus [2], the study was also supported by [31]. Lipid peroxidation products (MDA levels) were higher in patients with uncontrolled type 2 diabetes compared to patients with controlled type 2 diabetes [12]. It has been investigated that patients with diabetics have higher MDA and hemosylated hemoglobin levels in their blood serum [10]. It was observed that the decrease in the activity of the SOD was in parallel to the increase of lipid peroxidation. This decrease in SOD activity may be due to the effect of increased oxygen-derived free radicals on SOD [1]. It was also found that decrease in SOD activity of patients with changes in lipid profile [15]. Study which has been made by (McMurray et al 1993) has shown an increase in MDA, to be indirectly proportional to levels of enzymatic antioxidant activity. The decrease in SOD activity results from the effect of increased oxygen-derived free radicals [4].

In the present study HbA1c, cholesterol, LDL-cholesterol, VLDL-cholesterol, triglyceride and MDA were statistically significant between the two groups, they were higher in the group I compared with the group II. Furthermore, the concentrations of HDL-cholesterol and SOD activity were significantly lower in group I as compared to

group II. The same findings were observed by [10]. High levels of total cholesterol appear due to increased cholesterol synthesis or may be attributed to decreased muscular exercise or inhibition of cholesterol catabolism [23].

Triglyceride levels changes according to the glycemic control or may be due to insulin deficiency which results in faulty glucose utilization causing hyperglycemia and mobilization of fatty acids from adipose tissue [23].

The increased plasma lipid peroxidation and decreased HDL-cholesterol that have been observed during the study in patients with type II diabetes mellitus indicate that they may predispose to the development of cardiovascular complications. The present study also showed the direct correlation between lipid peroxidation and serum lipids in diabetes mellitus [18].

Lipid peroxidation (MDA) which is markedly increased in the present study in group I compared with the group II may be due to increased level of lipids, lipoprotein and lipid peroxides in plasma [23, 9]. Elevated levels of lipid peroxidation (MDA) in diabetes mellitus may be also due to the alteration of function of erythrocytes membrane leading to inhibition the activity of superoxide dismutase enzyme(SOD) which leads to accumulation of superoxide radicals causing the maximum peroxidation and tissue damage [7]. Study showed that oxidative stress may be increased in diabetes due to the increase in Lipid Profiles and Lipid Peroxidation in Patients with Type 2 Diabetes [14].

Estimation of lipid peroxidation along with other lipid profile in diabetes mellitus is very useful as it may serve as a useful monitor to judge the progress of the patient.

.

Conclusion:

The present study 'A comparative study on oxidative stress in diabetic patients with and without altered lipid pattern' was carried out at Alshorok lab in Obary region. The study group consists of 80 diabetes mellitus patients between the age 35-65 years of both sexes (male & female). The study was carried out by the collection of Fasting and random venous blood sample of diabetic patient. The patients were divided into two groups.

• Group I: 40 blood samples of diabetic patients with alteration in lipids.
• Group II:40 blood samples of diabetic patients without alteration in lipids.

All the samples were analyzed for glucose, HbA1c, cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, triglyceride, MDA and SOD activity.

It is concluded from the present study that with progression of diabetes, changes in lipids lead to increase in MDA levels and as a consequence lipid peroxidation takes place which leads to an increase in free radicals activity in type 2 diabetes and then an imbalance occurs in the antioxidant status to compact the oxidant injury. Therefore, good metabolic control of hyperglycemia will prevent in alteration in lipid metabolism and peroxidation, which may help in good prognosis and preventing manifestation of vascular and secondary complication in diabetes mellitus and also monitoring of antioxidant parameters in diabetic patients could be of vital importance in prevention of development of complications in diabetic patients.

Refrences:

1- Azza Dandana, Imen Gammoudi, Salima Ferchichi, Hinda Chahed, Hlima Ben Limam, Faouzi Addad, Abdelhedi Miled (2011). Correlation of oxidative stress parameters and inflammatory markers in tunisian coronary artery disease patients. Int J Biomed Sci ; 7 (1): 6-13).
2- Benedicta D'Souza, Vivian D'Souza, S Sowmya, G Seema, C N Hemalatha, M Anu and G Sajana(2008). A Comparative study on oxidative stress and antioxidant status in ischemic stroke patients with and without diabetes. Indian Journal of Clinical Biochemistry, 23 (3) 218-222.
3- Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Keaney JF, Jr., Creager MA (2003). Oral antioxidant therapy improves endothelial function in Type 1 but not Type 2 diabetes mellitus. Am J Physiol Heart Circ Physiol 285:H2392-2398.
4- Celik S, Baydas G, Yilmaz O (2002). Influence of vitamin E on the levels of fatty acids and MDA in some tissues of diabetic rats. Cell Biochem Funct 20:67-71.
5- Chugh SN, Dhawan R, Kishore K, Sharma A, Chugh K (2001). Glibenclamide vs gliclazide in reducing oxidative stress in patients of noninsulin dependent diabetes mellitus--a double blind randomized study. J Assoc Physicians India 49:803-807.
6- Ceriello A, Bortolotti N, Motz E, Crescentini A, Lizzio S, Russo A, Tonutti L, Taboga C (1998). Meal-generated oxidative stress in type 2 diabetic patients. Diabetes Care21:1529-1533.
7- Freitas, J.P., Filipe, P.M. and Radrigo, F.G. (1997). Lipid peroxidation in Type-II normolipidemic diabeticpatients Diabetes. Res. Practy. 36 (2), 71-75.
8- Haber CA, Lam TK, Yu Z, Gupta N, Goh T, Bogdanovic E, Giacca A, Fantus IG (2003). N-acetylcysteine and taurine prevent hyperglycemia-induced insulin
9- H. Surekha Rani, G. Madhavi1, V. Ramachandra Rao, B.K. Sahay and A. Jyothy (2005). Risk Factors For Coronary Heart Disease In Type Ii Diabetes Mellitus Indian Journal of Clinical Biochemistry, 20 (2) 75-80
10- Hanachi. P, Rashid Haydari Moghadam and Latiffah A.L (2009). Investigation of Lipid Profiles and Lipid Peroxidation in Patients with Type 2 Diabetes. European Journal of Scientific Research ISSN 1450-216X Vol.28 No.1, pp.6-13.
11- Je HD, Shin CY, Park HS, Huh IH, Sohn UD (2001). The comparison of vitamin C and vitamin E on the protein oxidation of diabetic rats. J Auton Pharmacol 21:231-236.
12- Jain SK, McVie R, Jackson R, Levine S N & Lim G (1999).Effect of Hyperketonemia on Plasma Lipid Peroxidation Levels in Diabetic Patients. Diabetes Care; 22:1171-75.
13- Kaul N, Siveski-Iliskovic N, Hill M, Khaper N, Seneviratne C, Singal PK (1996). Probucol treatment reverses antioxidant and functional deficit in diabetic cardiomyopathy. Mol Cell Biochem 160-161:283-288.
14- Maxwell S R J, Thomason H, Sandler D, Leguen C, Baxter M A, Thorpe, G H G, Jones A F. and Barnett A.H (1997). Antioxidant Status in Patients with Uncomplicated Insulin-Dependent and Non-Insulin-Dependent Diabetes Mellitus. Eur J Clin. Invest; 27: 484-90.
15- M.A. IBRAHIM, M.H. IBRAHIM, H.A. AYOUB(2008). Diabetes melllitus as an oxidative stress. Romanian J. Biophys., Vol. 18, No. 3, P. 195–208.

16- M. Salem, S. Kholoussi, N. Kholoussi, R. Fawzy (2010). Malondialdehyde and trace element levels in patients with type 2 diabetes mellitus. Archives Of Hellenic Medicine: Issn 11-05-3992.
17- 17) Madhur Gupta and Suresh Chari (2006). Proxidant And Antioxidant Status In Patients Of Type Ii Diabetes Mellitus With IHD. Indian Journal of Clinical Biochemistry, 21 (2) 118-122.
18- Mohammd Abbas Khulaibus Al-Nashi (2008).Oxidative stress and hyperlipedemia in diabetes mellitus. Volume 3, Issue 2, March – August
19- M Mahboob, M F Rahman, P Grover (2005). Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. Singapore Med J; 46(7) : 322.
20- Nur Kebapci, Ý Belgin Efe, Fahrettin Aky.z, Emine Sunal, Canan Demir.st.(1999).Oxidative stress and anti-oxidant therapy in type-2 diabetes mellitus. Turkish Journal of Endocrinology and Metabolism, 4 : 153-162.
21- Nakhjavani M, Esteghamati A, Nowroozi S, Asgarani F, Rashidi A, Khalilzadeh O (2010). Type 2 diabetes mellitus duration: an independent predictor of serum malondialdehyde levels. Singapore Med J; 51(7) : 582.
22- Nur Kebap, Belgin Efe, Fahrettin Akyuz.Emine Sunal and Canan Demir.st (1999). Oxidative Stress and Anti-Oxidant Therapy in Type-2 Diabetes Mellitus. Turkish Journal of Endocrinology and Metabolism, 4 : 153-162.
23- N.P. Suryawanshi, A.K. Bhutey, A.N. Nagdeote, A.A. Jadhav and G.S. Manoorkar (2006). Study Of Lipid Peroxide And Lipid Profile In Diabetes Mellitus. Indian Journal of Clinical Biochemistry, 21 (1) 126-130.s
24- Paolisso G, D'Amore A, Balbi V, Volpe C, Galzerano D, Giugliano D, Sgambato S, Varricchio M, D'Onofrio F (1994). Plasma vitamin C affects glucose homeostasis in healthy subjects and in non-insulin-dependent diabetics. Am J Physiol266:E261-268.
25- R. G. AHMED (2005). The Physiological And Biochemical Effects Of Diabetes On The Balance Between Oxidative Stress And Antioxidant Defense System. Medical Journal of Islamic World Academy of Sciences 15:1, 31-42.
26- Rama Srivatsan , Sujata Das, Ranjita Gadde, Krishna Manoj-Kumar , Snigdha Taduri, Nageshwara Rao, Ramesh B., Akanksha Baharani, Kaajal Shah, Srilakshmi C. Kamireddy, Priyatham G., Balakumaran T.A. , Shubha Seshadri, Asha Kamath MPhil, Anjali Rao (2009). Antioxidants and Lipid Peroxidation Status in Diabetic Patients with and without Complications. Archives of Iranian Medicine, Volume 12, Number 2: 121 – 127.
27- S.A. Moussa(2008). Oxidative stress in diabetes mellitus Vol. 18, No. 3, P. 225–236, Bucharest.
28- Shadia A. Fathy, Safinaz A. El-Habashy, Wafaa I. Rasheed, Azza S. Helmy, Gamila S.M. El- Saeed, Omima M. Said and Safaa M. Youssef (2009). Diagnostic Potential of oxidative stress indicators in egyptian type 1 diabetic Children and Adolescents Journal of Applied Sciences Research, 5(12): 2524-2534.
29- Sarita N. Shinde , Vithal N. Dhadke , Adinath N. Suryakar (2011). Evaluation of oxidative stress in type 2 diabetes mellitus and follow-up along with vitamin E supplementation. Ind J Clin Biochem 26(1):74–77.
30- Santini SA, Marra G, Giardina B, Cotroneo P, Mordente A, Martorana GE, Manto A, Ghirlanda G (1997). Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. Diabetes 46:1853-1858.

31- Soliman G Z A (2008). Blood lipid peroxidation (superoxide dismutase, malondialdehyde, glutathione) levels in Egyptian type 2 diabetic patients. Singapore Med O R I G I N A L A R T I C L E J; 49(2) : 129.
32- Saikat Sen1, Raja Chakraborty1, C. Sridhar, Y. S. R. Reddy1, Biplab De (2010). Free Radicals, Antioxidants, Diseases And Phytomedicines: Current Status And Future Prospect. Volume 3, Issue 1, July – August ; Article 021
33- T Adachi, M Inoue1, H Hara, E Maehata, and S Suzuki (2004). Relationship of plasma extracellular-superoxide dismutase level with insulin resistance in type 2 diabetic patients. Journal of Endocrinology (2004) 181, 413–417.
34- V Lobo, A Patil, A Phatak, N Chandra (2010). Free radicals, antioxidants and functional foods: Impact on human health. July-December Volume 4 | Issue 8.
35- V. A. Kangralkar, Shivraj D. Patil, R. M. Bandivadekar (2010). Oxidative Stress And Diabetes. International Journal of Pharmaceutical Applications. Vol 1, Issue 1, June, pp 38-45.

الملخص

العديد من الدراسات تم القيام بها حول دور الجذور الحرة في العديد من الأمراض وكذالك دور مضادات الأكسدة في منع حدوث الأمراض. من المعروف أن داء السكري يعتبر أحد الأمراض التي تؤدي إلى حدوث عدم التوازن بين الجذور الحرة ومضادات الأكسدة وهذا يؤدي إلى ما يعرف بالإجهاد التأكسدي. هذه الدراسة تهدف إلي القيام بدراسة مقارنة على الإجهاد التأكسدي ومضادات الأكسدة لمرضي النوع الثاني (الغير معتمد على الانسولين) للداء السكري مع أو بدون حدوث تغير في مستويات الدهون. درجة تأكسد الدهون تم قياسها من خلال قياس MDA بالترافق مع قياس مستويات الدهون. في هذه الدراسة أيضا تم قياس كل من الجلوكوز و HBA1c ومضاد الأكسدة Superoxide dismutase (SOD). استهدفت الدراسة حوالي 80 من مرضي الداء السكري الغير معتمد علي الانسولين حيث تم تقسيمهم اعتمادا علي التغيرات في مستويات الدهون إلي مجموعتين كل مجموعة تتكون من 40 من مرضى الداء السكري. المجموعة الأولى مرضى مصابين بداء السكري مع وجود تغير في مستويات الدهون أما المجموعة الثانية أيضا مرضي مصابين بالداء السكري مع عدم حدوث تغير في مستويات الدهون. الدراسة اظهرت أن مستويات كل من الكولسترول الكلي والجلسريدات الثلاثية والـ LDL و HBA1c وVLDLوMDA كانت مرتفعة في المجموعة الاولي بالمقارنة مع المجموعة الثانية أي ان هناك فروق معنوية بين المجموعتين. أيضا أظهرت النتائج أن هناك انخفاض في مستويات الـ HDL و الـ SOD في المجموعة الأولى بالمقارنة مع المجموعة الثانية أي أن هناك فروق معنوية بين المجموعتين. الدراسة أظهرت أن التغير في مستويات الدهون يؤدي إلى زيادة في أكسدة الدهون (ارتفاع MDA) والذي يكون بالتوازي مع انخفاض مضاد الاكسدة (SOD) والـ HDL وبالتالي زيادة في مستويات الجذور الحرة محدثة عدم التوازن بينها وبين مضادات الاكسدة وبالتالي حدوث الاجهاد التأكسدي وهذا يشير إلى أن مرضي النوع الثاني لداء السكري عرضة للإصابة بمضاعفات أمراض القلب نتيجة لحدوث الإجهاد التأكسدي.