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ASSESSMENT OF THE LEVEL OF OXIDATIVE STRESS AND SOME BIOCHEMICAL VARIABLES AMONG PEOPLE WITH T2DM IN KIRKUK GOVERNORATE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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ASSESSMENT OF THE LEVEL OF OXIDATIVE STRESS AND SOME BIOCHEMICAL VARIABLES AMONG PEOPLE WITH T2DM IN KIRKUK GOVERNORATE

By Abdulhameed Mote Abdullah ABDULLAH

May 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

ASSESSMENT OF THE LEVEL OF OXIDATIVE STRESS AND SOME BIOCHEMICAL VARIABLES AMONG PEOPLE WITH T2DM IN KIRKUK GOVERNORATE

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Master of Science in Chemistry

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May 2022

The current study aimed to estimate the levels of llipid profile and oxidative stress in T2DMM patients compared to healthy controls. 120 subjects (males and females) patients suffering from T2DMM and 30 non-diabetics subjects (control) were used in this study. All subjects are adults aged between 30-70 years. The study was done in different hospitals in Kirkuk city, in the period from October 2021 to January 2022. A glucose level in serum of T2DMM patients shows a significant ($P < 0.05$) increase than healthy subjects. A uric acid level in serum of diabetes mellitus patients shows a significant ($P < 0.05$) increase compared to control group. Lipid profile showed significant ($P<0.05$) increased and decreased in between studied groups. Malondialdehyde levels showed a significant ($P < 0.05$) increase in patients of diabetes type II compared with control group and significant ($P < 0.05$) decreased in antioxidant enzymes. It's concluded that the both types of diabetes mellitus type II lead to induces oxidative stress and significant ($P < 0.05$) differences in Lipid profile.

2022, 42 pages

Keywords: Oxidative stress, Antioxidants lipid profile, Uric acid

ÖZET

KIRKÜK VALİLİKİNDE T2DM'Lİ KİŞİLERDE OKSİDATİF STRES VE BAZI BİYOKİMYASAL DEĞİŞKENLERİN DÜZEYİ DEĞERLENDİRİLMESİ

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Mayıs 2022

Mevcut çalışma, sağlıklı kontrollere kıyasla T2DMM hastalarında lipid profili ve oksidatif stres düzeylerini tahmin etmeyi amaçladı. Bu çalışmada T2DMM'den muzdarip 120 denek (erkek ve kadın) ve 30 diyabetik olmayan denek (kontrol) kullanıldı. Tüm denekler 30-70 yaş arası yetişkinlerdir. Çalışma Kerkük şehrinde farklı hastanelerde Ekim 2021 ile Ocak 2022 tarihleri arasında yapıldı. T2DMM hastalarının serumundaki bir glikoz seviyesi, sağlıklı deneklere göre önemLi ($P < 0.05$) bir artış gösterir. Diabetes mellitus hastalarının serumundaki ürik asit seviyesi, kontrol grubuna kıyasla önemLi ($P < 0.05$) bir artış göstermektedir. Lipid profili, çalışılan gruplar arasında anlamLı ($P<0.05$) artış ve azalma gösterdi. Malondialdehit seviyeleri, tip II diyabetli hastalarda kontrol grubuna göre önemLi ($P < 0.05$) bir artış ve antioksidan enzimLerde önemLi ($P < 0.05$) düşüş gösterdi. Diabetes mellitus tip II'nin her iki tipinin de oksidatif stresi indüklediği ve Lipid profilinde önemLi ($P < 0.05$) farklılıklara yol açtığı sonucuna varılmıştır.

2022, 42 sayfa

Anahtar Kelimeler: Oksidatif stres, Antioksidanlar lipid profili, Ürik asit

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I would like to thank my thesis advisor, Assoc. Prof. Dr. Şevki ADEM, for his patience, guidance and understanding.

Abdulhameed Mote Abdullah ABDULLAH

Çankırı-2022

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LIST OF SYMBOLS

µL	Microliter
g	Gram
mg/dL	Milligrams per decilitre
mL	Milliliter
mM	Millimeter
nm	Nanometer

LIST OF ABBREVIATIONS

A	Absorbance
CAT	Catalase
CETP	Cholesterol ester transfer protein
CETP	Cholesterol ester transport protein
DM	Diabetes mellitus
EDTA	Ethylenediaminetetraacetic acid
ERK	Extracellular signal-regulated kinase
FG	Fasting glucose
GPx	Glutathione peroxidase
GPX	Glutathione Peroxidase
GSH	Gluthatione
GSH-Px	Glutathione peroxidase
H2O2	Hydrogen Peroxide
HDL	High density lipid
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IR	Insulin resistance
LDL	Low density lipid
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
ROS	Reactive oxygen species
SOD	Superoxide
SST	Serum separator tubes
T1DM	Type 1 diabetes mellitus
T2DMM	T2DM mellitus
TBA	Thiobarbituric Acid
TC	Total cholesterol
TGs	Triglyceride
VLDL	Very low density lipid

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1. INTRODUCTION

One of the most frequent and serious metabolic diseases, diabetes mellitus type 2 (T2DMM) is a worldwide pandemic and a major issue for the health care system. In 2013, people number with DM around the world was expected to be 382 million. The use of T2DMM is increasing in frequency. This disease will be found in more than 590 million individuals by 2035, and that figure is anticipated to rise (Guariguata *et al.* 2014).

"Diabetes is a metabolic disease with many different causes," the World Health Organization says. It's characterized by "persistent hyperglycemia" and "disorders in carbohydrate, lipid, and protein metabolism" because of problems with insulin production, insulin action, or both. 90 percent of people with diabetes are thought to have T2DMM, and T1DM is the most common type for the other 10 percent. There are also other types of diabetes that are very rare (Thompson *et al.* 2013).

Diabetes type 2 (T2DMM) is largely caused by reduced insulin production and secretion by beta-cells in the pancreas and insulin resistance in other organs (Leahy *et al.* 2010, Kahn *et al.* 2014). Given that 90% of T2DMM persons are fat or overweight at the time of diagnosis, etiology is thought to be related to high-nutrient, low-energy-output diets (Chen *et al.* 2012).

In subjects with T2DMM, there are many effective ways to lower their blood sugar levels, all of which work by increasing insulin production or reducing insulin resistance in their bodies (Raz et al. 2013). Regardless, post-diagnosis problems, especially long-term consequences, are common all over the world. People who have diabetes are more likely to get blind, end-stage renal disease, lose their lower limbs, and have heart disease (Thompson *et al.* 2013).

imbalance between of free radicals production, which can be boosted by malfunctioning mitochondria, and ability of body to fight them off leads to oxidative stress (Small *et al.*

2012). It has been found that oxidative stress is linked to both T2DM and its long-term effects (Odum *et al.* 2012, Shi and Pan 2012). T2DM patients have metabolic problems that make their bodies more prone to oxidative stress and weaken their antioxidant defense system (Lima *et al.* 2011). In people who have T2DMM, there seems to be a problem with the oxidative state. These people are thought to be in danger (Peerapatdit and Sriratanasathavorn 2010).

1.1 Objectives of Study

- Elevate the concentration of lipid profile in pateints with T2DMM
- Elevate the concentration of uric in pateints with T2DMM
- Elevate the concentration of MDA in pateints with T2DMM
- Elevate the concentration of some antioxidant enzymes in pateints with T2DMM.

2. LITERATURE REVIEW

2.1 T2DM Mellitus (T2DMM)

Diabetes mellitus (DM) is a long-term metabolic disorder that causes high blood sugar levels to stay the same. If you don't produce enough insulin, your body might not be able to use it properly, or both. Around 415 million subjects between 20 to 79 years had diabetes in 2015 by the International Diabetes Federation (IDF). By 2040, an extra 200 million people are expected to have DM, making it a global public health issue that needs to be taken care of. (Zheng *et al.* 2018)

For a long time, T2DMM was called non-insulin dependent, or adult-onset diabetes with insulin resistance that could lead to total insulin resistance (IR). In the last decade, however, diminished -cell function has been recognized as a major concern in T2DMM (Saisho *et al.* 2014).

T2DMM has become a new and very dangerous health condition for young people in the last two decades. (Rosenbloom et al. 1999). Obesity, IR and cell damage were found to be linked in children's research, just like they were in older people with T2DM (Sinha *et al.* 2002). (Figure 2.1) illustrates the relationship.

2.1.1 Classification of T2DMM

There is now a new name for T2DMM: Diabetes that is not insulin-dependent. There has been a name change at the American Diabetes Association. A variety of factors may be considered in order to arrive at an independent diagnosis: Two-hour values of 200 mg/dL or higher on a 75-gram oral glucose tolerance test; a random glucose of 200 mg/dL or higher with typical diabetes symptom; and numerous days with a fasting glucose level that is more than or equal to 126 mg/dL (Wingard *et al.* 1995).

Figure 2.1 T2DM pathophysiology (Sinha *et al.* 2002)

Because of its ease of use, repeatability, and connection to a higher risk of microvascular problems, fasting glucose levels are preferred because they can be used again and again. 110 mg/dL or less in the blood is considered impaired fasting glucose, and so is glucose level of 110 mg/dL or less in the blood when you wake up (Wingard *et al.* 1995). When you have a 2-hour glucose concentration of 140 mg/dL or higher but not more than 200 mg/dL on an oral glucose tolerance test, you have impaired glucose tolerance. This occurs when you consume a lot of sweets (American Diabetes Association 1997).

2.1.2 Etiology

There are a lot of things that can make you more likely to get T2DM. When body suffering from obese, risk of T2DMM up 90 time, and most patients are overweight or obese. T2DM risk rises rapidly with a BMI above 30 and is strongly linked to an increase in BMI, which is why it's important to keep your weight under 30 (Reed *et al.* 2021). People with T2DM in Western countries are thought to have a BMI of more than

30. People with a BMI of 25–30 are thought to have a BMI of less than 30. In some Asian countries, however, less than half of the people who go to the doctor are obese. A lot of people with T2DM aren't getting enough food, which is surprising (Nguyen *et al.* 2011, George *et al.* 2015).

Diazygotic twin concordance is greater in T2DMM than in T1DM, which has a prevalence of between 20% and 30%. Even with a 1.57 odds ratio, there may be additional untested variants at the T2DM risk locus that increase a person's risk of the disease. because they eat the same foods and do the same things, and because they share their genes, it might be more likely that relatives of type 2 diabetic people get the disease as well (Poulsen *et al.* 2009).

Some people are more likely to get T2DM even though they don't eat a lot of sugar, even though both groups had the same average BMI scores and standard deviation values. One study found that genetics play a role in T2DM susceptibility, even though both groups had the same average BMI scores and standard deviation values. People who drank alcohol and smoked were more likely to get T2DM, even if they had low-risk diets and a lot of exercise, according to a study of people who lived together (Mozaffarian *et al.* 2009).

Adult men are more likely to get T2DM than women, which is thought to be because men have different ways of storing fat. Men with type 2 diabetes (T2DM) are more likely to develop cardiovascular disease, according to 81 research (CVD). There was a poorer prognosis for women with T2DM who develop cardiovascular disease (CVD) than for those without diabetes. This may be due to the fact that males with type 2 diabetes are more likely than women to achieve their medical objectives (such as optimal glucose control and blood pressure) (Kautzky *et al.* 2016).

As of now, it isn't clear if what people are exposed to can cause diabetes. Type 1 herpes simplex virus and hepatitis C virus are both associated with an increased risk of developing type 2 diabetes, however the reason for this is unclear. Because Hepatitis C

has been shown to make people more insulin resistant in their bodies, this is likely to raise the risk of T2DM (Wang *et al.* 2007, Bose *et al.* 2014).

2.1.3 Epidemiology

In 2011, the number of people with diabetes was expected to reach 366 million. This number is expected to climb to 552 million by 2030. (GBD 2011). More individuals in low- and middle-income nations are being diagnosed with Type 2 Diabetes Mellitus (T2DM). DM killed 4.6 million people worldwide in 2011. By 2030, 439 million people around the world are expected to have T2DM. People in different parts of the world are more likely to get T2DM because of factors like the environment and how they live their lives (Zimmet *et al.* 2001).

Investigate: T2DM is a rare occurrence in Africa as a whole, according to available data. Both rural and urban regions in Africa have experienced a significant increase in the number of individuals becoming ill in recent years. Both sexes are affected by this. Only around 10% of diabetes occurrences in Africa are caused by type 1 diabetes; T2DM seems to be the most frequent form of diabetes in the continent. More than 10% of the American population had diabetes in 2015, says the International Diabetes Federation (IDF). There were seven million of them. As people get older, the number of people with diabetes rises. In the United States, more than a quarter of people over the age of 65 have diabetes. (Carrillo-Larco and Colleagues 2019).

Diabetics affected about 25.8 million people (7.8% of the population) in 2010, according to a 2011 CDC report. 90% to 95% of them had T2DM (DHHS 2011). During the next two decades, the number of adults with T2DM, especially in developing countries, is expected to rise. Most of the rise will happen in those countries, where most T2DM patients are between 45-64 years (Wild and Colleagues 2004).

2.1.4 Pathophysiology and risk factors

T2DMM is define as metabolic condition that causes high blood sugar, insulin resistance, and less insulin production. T2DMM is caused by a complicated mix of social, and environmental risk factors which affect people who are genetically predisposed, adult studies show. Many parts of the puzzle about how genes play a role in T2DM development haven't been solved, but multiple genes seem to play a role, which means there is a strong genetic link. When someone has T2DM, their close relatives are more likely to get it, too. A monozygotic twin study says that, if one twin has diabetes, the other has a 90% chance of having diabetes, too (Barnett *et al.* 1981).

There are also studies that show that more than half of kids with T2DM have at least one parent who has it. (Fagot-Campagna et al. 2000). Parents who have T2DM have a 3.5-fold higher risk of their children developing diabetes than parents who don't have T2DM. This risk rises to 6 times if both parents have T2DM (Meigs *et al.* 2000).

It's another "risk factor" for T2DM that has been genetically determined. Teenage girls are 1.3 to 1.7 times more likely to get the disease than boys, but why isn't clear. It also seems that puberty has an important part to play in the development of T2DM because of the biologically high insulin resistance of adolescence. It's not a surprise that 40% of T2DMM cases are found in children between the ages of 10 and 14. The other 60% are found in adolescents between the ages of 15 and 19 (Pinhas-Hamiel *et al.* 1996).

A lot of these things, as well as other things that are common in our obesogenic environment, make it more likely that kids will become overweight and have visceral fat buildup. Increased visceral fat has been shown to make people more sensitive to insulin through a number of different ways (Hardy *et al.* 2012).

In the beginning, the pancreatic cells produce more insulin to keep blood sugar levels normal. Prediabetic disorders like IGT and IFG happen in people who are genetically vulnerable and have a sedentary lifestyle and become overweight. Hyperglycemia and elevate in the production of free fatty acids are bad for cells (gluco- and lipotoxicity), and over time, insulin production decreases, which means that insulin resistance is becoming more common, too (Valaiyapathi 2020).

2.1.5 Diabetes complications

Diabetes is affects the heart, kidney tissues, the eyes, nerves, and the teeth, as well as many other parts of the body. In high-income countries, diabetes is the main cause of chronic heart disease, kidney failure and blindness (IDF 2015). Diabetes can cause a lot of problems, like the ones below:

Cardiovascular diseases: Atherosclerosis is caused by long-term high blood sugar and low cholesterol levels in the blood arteries, which can lead to a fatal heart attack or brain stroke. In people with diabetes, cardiovascular problems are the main cause of death (IDF 2015)

Diabetic nephropathy: Glucose and blood flow changes that happen because of diabetes can cause glomerular sclerosis and fibrosis. Diabetic nephropathy can cause albuminuria to get worse, blood pressure to rise, and kidney failure to get worse. People with T2DM have kidney failure about 20% to 30% of the time, but it happens more often in people who have had the disease for a long time (typically 10 years) (Merk 2015).

Diabetic eye disease: As a result of diabetes, a number of vision problems can happen, such as: Diabetic retinopathy is a condition in which the vessels in the retina are damaged, causing blurry vision or even blindness. This can happen because of diabetes. Diabetes speeds up the development of cataracts, which are cloudy lenses in the eyes. Glaucoma is a condition in which the pressure in the vitreous fluid rises, causing damage to the optic nerve, retinal detachment, and vision loss. Diabetes is thought to be the cause of 39 million cases of blindness around the world, with 248 million diabetics also having trouble seeing (Courtright and Lewallen 2011).

Pharmacological treatment: Dietary changes and oral hypoglycemic medicines have been found to be ineffective in treating this long-term, progressive illness, while insulin therapy only temporarily helps. Patients still have problems with their macro- and microvascular systems, even with the most recent drugs. Diabetes raises the risk of heart attack and stroke, blindness, and 60% of non-traumatic lower-limb amputations that aren't caused by an accident. (National diabetes fact sheet Atlanta 2011).

2.2 Free Radicals

Free radicals are chemical entities with one or more unpaired electrons that can be very dangerous. They have a short life span. If you want to communicate with other organisms, they can also be thought of as a bad thing. By passing an unpaired electron across cells, free radicals cause oxidation of cell parts and molecules, which can be bad for the cells (Bansal and Bilaspuri 2011).

2.2.1 Biological roles of free radicals

In the genesis and development of life, free radicals are seen as a necessary evil since they perform a function. Gene expression and cell death both depend on the cell's many signaling pathways being activated. These include the MAPK and ERK pathways, which are responsible for gene expression and cell death, respectively. As stated in a 2003 paper, (Cho and Wolkenhauer 2003). There are two types of RNS: neurotransmitters and immunological mediators. These proteins control leukocyte adhesion, thrombosis, angiogenesis, and the tone of the blood vessels. Oxygen radicals (ROS) play a big role in how genes are turned on and how other cell activities are regulated (Fang *et al.* 2002).

2.2.2 Production and scavenging of free radicals

In cells and their surroundings, both outside and inside chemicals make free radicals. Ionizing radiations as well as non-enzymatic interactions between organic molecules

and oxygen may lead to these reactions (Pham-Huy et al. 2008). Oxidative phosphorylation may be a part of this process in the mitochondrion. Radioactive particles, reactive nitrogen species (RNS), the production of neutrophils and macrophages, chemicals, cigarette, beedi, and cigar smoking as well as industrial waste are all examples of different sources of radiation. These are just a few examples (Sen *et al.* 2010).

The body has a variety of ways to make antioxidants, either internally or externally, that can fight off free radicals and protect cells from their harmful effects. This helps prevent disease, which is why these antioxidants are important. (Pham-Huy et al. 2008).

2.3 Oxidative Stress in Diabetes Mellitus

oxidative stress is thought to play a role in the development of vascular problems in people with diabetes, especially people with T2DM (Pham-Huy 2008). Rising production of ROS by antioxidants like catalase, superoxide dismutase, and GSH–Px may be a factor in the rise in ROS levels in people with diabetes, as these antioxidants make more ROS. Variations in the levels of these enzymes make cells more vulnerable to oxidative stress, which can cause diabetes problems. (Lipinski, 2001). According to epidemiological research, diabetes deaths are mostly caused by a rise in vascular diseases that aren't caused by high blood sugar (Pham-Huy 2008).

3. MATERIALS AND METHODS

You may see a list of the instruments utilized in this research in Table 3.1.

Table 3.1 A list instruments used in the present study

Instruments and glasses	Company	Country
ELISA	Labon	China
Spectrophotometer	Biobase	India
Centrifuge	Memmert	Germany
Light microscope	Olympus	Japan
Oven	Memmert	Germany
Water bath	Memmert	Germany
Shaking water bath	Memmert	Germany
Sysmex device	Sysmax	Japan
Pipette	BioSan	Germany
Different glasses	-----	China
Refrigerator	BEKO	Turkey

3.1 Patients

120 people, both men and women, who had diabetes mellitus type II and 30 people who didn't have diabetes were used in this study. Everyone who took part is between 30 and 70 years old. There were a lot of different hospitals in Kirkuk city that were used for the study. It took place from October 2021 to January 2020.

3.2 Blood Samplings

People who took part in the study had to go without food for 10-12 hours before they had their blood and urine checked (patients and healthy people). They were stored in tubes with Gold-top serum separator tubes (SST) and tubes with Ethylenediaminetetraacetic acid (EDTA). Centrifuged for 10 minutes at 3500 rpm to separate the serum samples, which were kept in Eppendorf tubes. After the sample collection process was over, all of the samples were dissolved so that they could be used for biochemical analysis.

3.3 Glucose

Glucose oxidase enzyme converts glucose to D-gluconate in the Trinder reaction with the generation of hydrogen peroxide.

Procedure: At reaction temperature (37°C), the working reagent, samples, and standard were incubated. The additions were then made in accordance with the Table 3.2.

Table 3.2 Steps of glucose procedure

Addition sequence	Blank	Sample	Standard
Working reagent	1 mL	1 mL	1 mL
Sample	-	10 µL	-
Standard	-	-	10 µL
For 10 minutes at room temperature, or 5 minutes at 37 oC, combine the ingredients and let them to stand in the test tubes. Take a reading at 500 nm of each sample and the standard and compare it to that of the blank reagent.			

Calculation: The glucose level is calculated according to the Equation 3.1:

Results= A sample/ Astandard * C Standard = mg/dL total glucose A standard (3.1)

3.4 Uric acid

When uricase reacts with uric acid, allantoin, carbon dioxide, and hydrogen peroxide are produced.

Procedure: At reaction temperature (37°C), the working reagent, samples, and standard were incubated. The additions were then made in accordance with the Table 3.3.

Table 3.3 Steps of uric acid procedure

Sample	25 µL
Reagent R	1000 µL
Mix. Allow 5 minutes at ^{o}C to stand. Using a reagent blank, measure absorbance at 505 (495-505) nm. For 30 minutes, the color remains steady.	

Calculation: The concentration of uric acid is calculated according to the Equation 3.2:

(Sample / Standard) x c Standard (3.2)

3.5 Lipid Profile

3.5.1 Total cholesterol

Using a BioLabo kit from France and a spectrophotometer, Allain wrote about how to measure cholesterol in the blood.

The Equation 3.3 was used to figure out the total cholesterol level.

$$\text{Cholesterol levels} = \frac{\text{absorbance (Test)}}{\text{absorbance (Standard)}} * \text{Standard} \quad (200\text{mg/dL}) \tag{3.3}$$

3.5.2 Triglyceride

The enzymatic method for measuring triglyceride in the blood was described by Allain as follows (Tietz 1999) and using (BioLabo kit/ France/ spectrophotometer):

The triglyceride concentration was calculated according to the Equation 3.4.

$$\text{Triglyceride levels} \quad \frac{\text{absorbance (test)}}{\text{absorbance (Blank)}} \quad \frac{\text{Standard}}{\text{(200 mg/dL)}} \tag{3.4}$$

3.5.3 High density lipid (HDL)

The enzymatic method for measuring triglyceride in the blood was described by Allain as follows (Tietz 1999) and using (BioLabo kit/ France/ spectrophotometer):

The triglyceride concentration was calculated according to the Equation 3.5.

$$\text{HDL} = \frac{\text{absorbance (test)}}{\text{absorbance (Blank)}} \quad \frac{\text{Standard}}{\text{(200 mg/dL)}} \tag{3.5}$$

3.5.4 Low density lipid (LDL)

LDL concentration was calculated according to the Equation 3.6.

$$\text{LDL= total cholesterol – HDL – VLDL} \tag{3.6}$$

3.5.5 Very low density lipid (VLDL)

VLDL concentration was calculated according to the Equation 3.7.

$$\text{VLDL= triglyceride/5} \tag{3.7}$$

3.6 Oxidative Stress and Antioxidant Enzymes

3.6.1 Malondialdehyde (MDA) levels estimation

Hydration makes MDA, which is made from some primary and secondary lipid peroxidation products that have been broken down. By using the method that was explained, MDA levels were found to be the same. (Rao *et al.* 1998) as follows:

- A glass tube: Add 1 mL of plasma mixed with 0.9 mL of water.
- 0.5 mL of TBA reagent is put in.
- The mixture was heated for an hour in a hot water bath that was on. Before each tube was cooled, it was put in a centrifuge for 10 minutes at 4000 rpm for 10 minutes.
- It's read at 532 nm.
- The concentration is taken out based on the curve shown in the Figure 3.1.

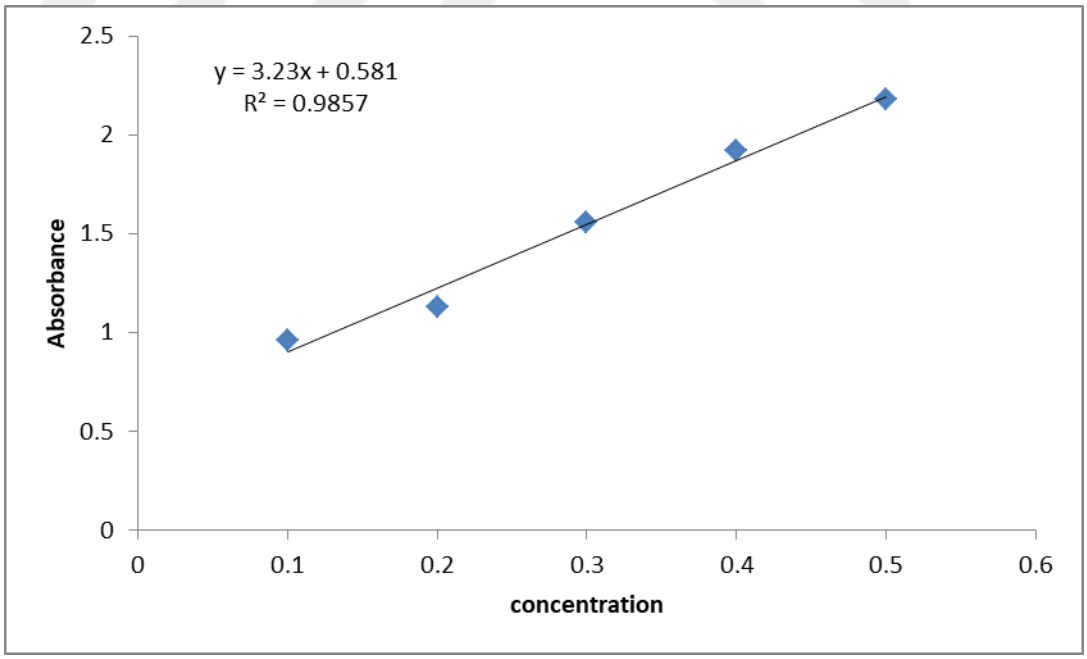

Figure 3.1 The conventional MDA curve

3.6.2 Estimation of glutathione levels

Glutathione levels were checked with the help of DTNB, according to a method. (Moron *et al.* 1979) that went like this:

- The test tube has been filled with 100 l of the sample that is in it.
- It was put into each tube: 1 mL phosphate buffer pH 8.0 and 0.9mL of 0.1 mL of water (DTNB).
- TCA (10%) was added to 10 l of water.
- The test tubes were kept at room temperature for about 5 minutes to make sure they were safe. Then it was spun for 10 minutes at 4000 g.
- At 412 nm, the amount of yellow color that is absorbed is measured.
- The focus is determined by the curve depicted in the Figure 3.2.

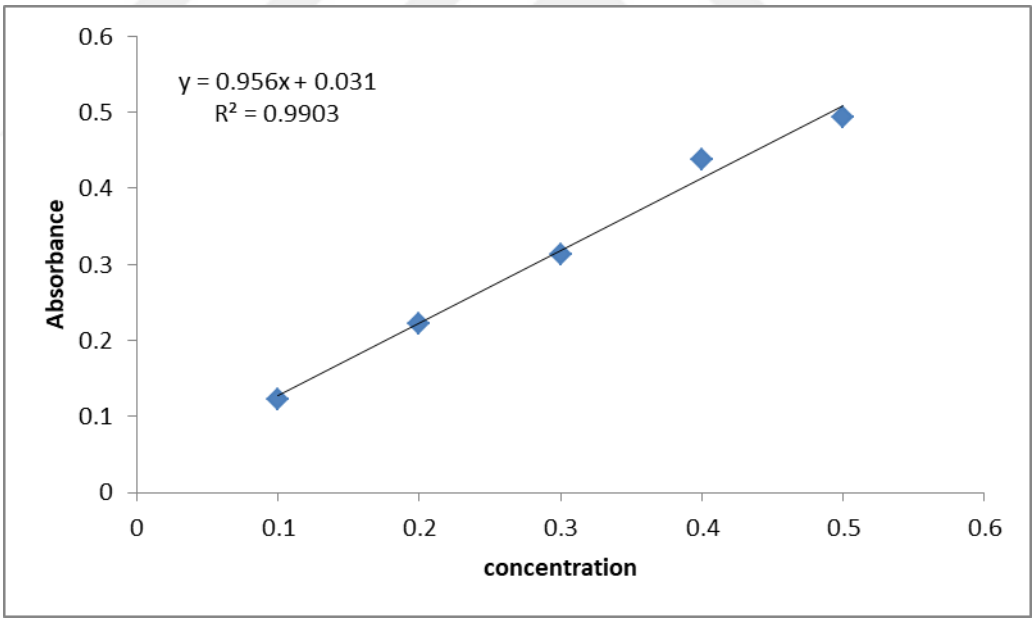

Figure 3.2 The GSH (mmol/L) reference curve

3.6.3 Catalase

Catalase is the enzyme that helps break down H2O into H2O and O2. As a part of the enzyme assay, you can figure out how much residual H2O2 there is by titrating with

KMnO4. These are the ways to measure the levels of catalase in your body. Table 3.4 according to the method (Moron *et al*. 1979):

- The Hydrogen Peroxide (0.065 M) (H_2O_2)
- pH 7.4 Phosphate Buffer.
- Ammonium Molybdate, 32.4 mmol/l

Table 3.4 Shows the steps of performing a catalase enzyme assay

Reagents	S. µL	B. µL	B. µL	B. µL
Serum	50	-	-	-
A foundation (H_2O_2)	1000	1000	1000	-
Buffering with Phosphate	-	-	50	1050
Molybdate of ammonium	-	1000	1000	1000
Serum	-	50	-	-
The tubes are incubated for one minute at a temperature of 37 ^{o}C				
Ammonium molybdate	1000	-	-	-

The concentration of catalase enzyme is extracted according to the equation below:

$$\text{Serum CAT activity (kU/l)} = \frac{A_{(\text{Blank 1})} - A_{(\text{Sample})}}{A_{(\text{Blank 2})} - A_{(\text{Blank 3})}} \times 271$$

3.6.4 SOD activity evaluation

The photochemical Nitroblue Tetrazolium technique was used to assess SOD activity. Bicarbonate, Ethylenediaminet etraacetic acid sodium salt buffer, and Epinephrine are all used in this approach Table 3.5.

Table 3.5 shows the steps of performing a SOD enzyme assay

Sample µL	Carbonate buffer (50 mM, pH 8.00) µL	Ethylenediaminet etraacetic acid sodium salt buffer	Epinephine
100	1800	1000	100
Samples at a wavelength of 480 nm are read immediately (A1) and after 5 minutes (A2)			

Enzyme activity (SOD) calculation: The quantity of enzyme that prevented oxidation by 50% was determined as one unit of SOD. The following Equation 3.9 was used to calculate the SOD's effectiveness:

SOD activity (µmol/min/mL) = (I%/2/5)* 300 (3.9)

3.7 Statistical Analysis

Statistical analysis was performed using SPSS version 21 and GraphPad prism version 8. Statistical tests and bar graphs were expressed using MeanSE. The parameter means of the patient and control groups were compared using an unpaired T-test (Man-Whitney U).

4. RESULTS AND DISCUSSION

4.1 Glucose and Uric Acid

Table 4.1 and Figure 4.1 demonstrate that the glucose level in the blood of diabetes mellitus patients (292.83±29.21 mg/dL) is significantly higher (P<0.05) higher than in healthy subjects (87.27±5.87 mg/dL). Table 4.1 and Figure 4.2 reveal that uric acid levels in diabetes mellitus patients' serum (7.981±0.58 mg/dL) are significant (P<0.05) higher than in healthy subjects (5.164±0.043 mg/dL).

Table 4.1 Glucose and uric acid in studied groups

Groups Variables	Healthy (30)	Subject with T2DM (120)	P-Value
Glucose mg/dL	87.27±5.87	292.83±29.21*	0.01
uric acid mg/dL	5.164±0.043	7.981±0.58*	0.021

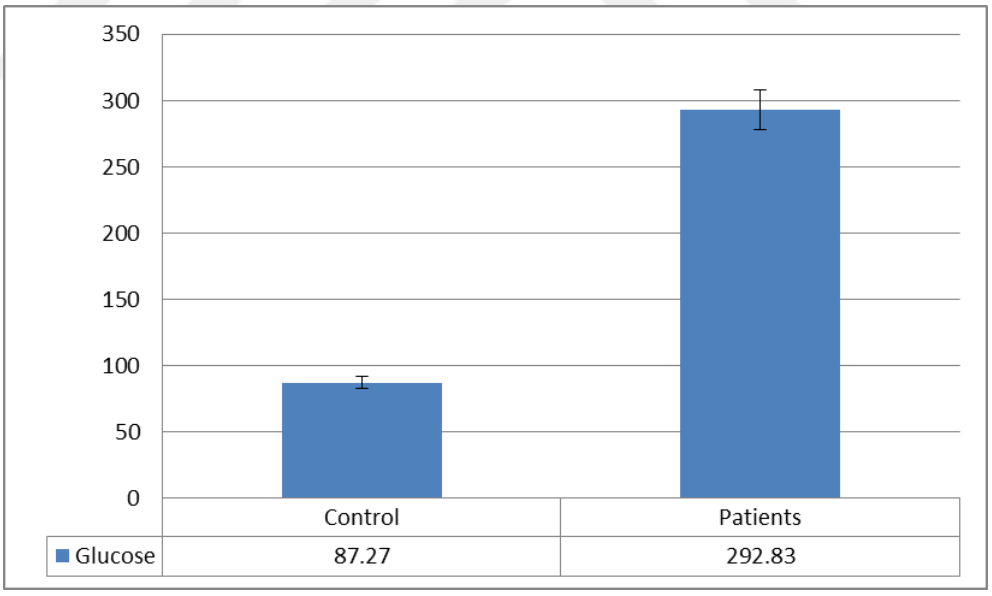

Figure 4.1 Glucose levels in both groups

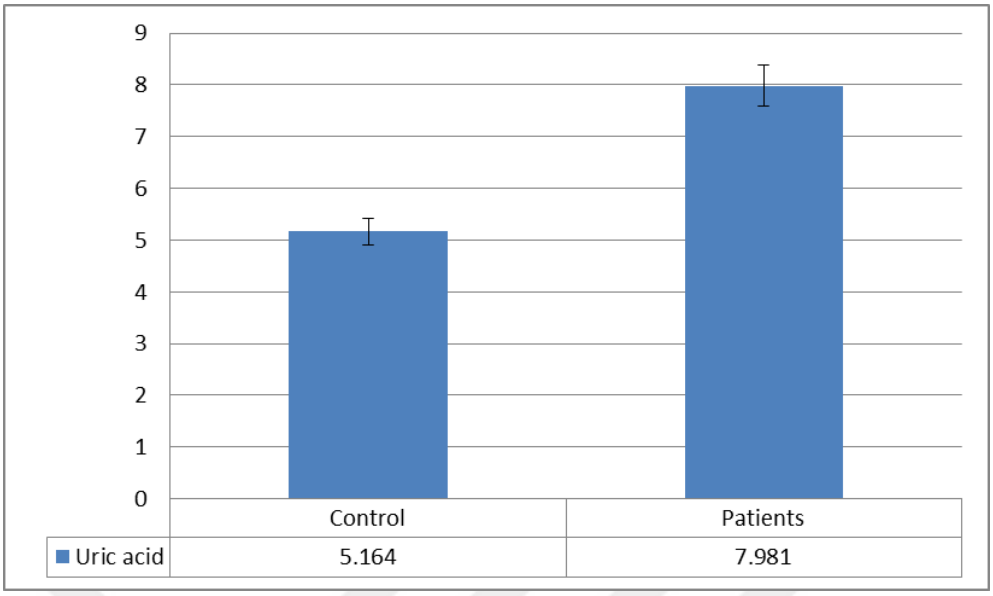

Figure 4.2 Uric acid levels in both groups

A growing body of data from both experimental and clinical investigations shows that hyperglycemia, oxidative stress, and diabetes complications are all linked (Elmarakby and Sullivan 2012).

According to recent studies, blood uric acid levels, regardless of other variables, are linked to T2DM (Chien *et al.* 2008, Kodama *et al.* 2009). In elderly people with impaired fasting glucose levels, uric acid has also been indicated as a prospective predictor of T2DM (Kramer *et al.* 2009). The latest inquiry has yielded similar results. Diabetes individuals reported significantly higher uric acid levels than non-diabetic controls in both genders.

Current results were agree with recent findings on the correlation between UA levels and T2DM risk. the study conducte by (Niskanen *et al.* 2006) found that UA level up (6.4 mg/dL) was associated with a 2-fold elevated in T2DM risk than the lowest of UA (5.2 mg/dL), according to 475 obese subjects with weak glucose tolerance.

Similarly, the Rancho Bernardo Study found that every mg/dL rise in UA level resulted with a 65 percent increase in the risk of T2DM among 566 individuals (mean age 68 years). The Rotterdam trial6 revealed that the risk of acquiring T2DM was 1.68 times

greater in the highest quartile of uric acid (6.2 mg/dL) than in the lowest quartile (uric acid 4.5 mg/dL) (Dehghan *et al.* 2008, Kramer *et al.* 2009).

However, elevated UA levels in the blood, particularly in the kidneys, may suggest the existence of prediabetes. Furthermore, higher insulin levels associated to prediabetes can decrease renal uric acid excretion (Facchini *et al.* 1991, Ter *et al.* 1997).

4.2 Lipid Profile

the total cholesterol level in T2DM patients (238.71±20.41 mg/dL) reveals a significant (P<0.05) higher than in healthy subjects (154.18±11.24 mg/dL), the triglyceride level in the blood of diabetes mellitus patients (320.41±434.57 mg/dL) showed a significant (P<0.05) higher than in healthy subjects (91.52±10.76 mg/dL). HDL levels in diabetes mellitus patients' serum (25.53±1.83 mg/dL) were significantly lower (P<0.05) than in healthy subjects (33.12±1.42). LDL levels in diabetes mellitus patients' serum (153.71±14.93mg/dL) increased significantly (P<0.05). VLDL levels in diabetes mellitus patients' serum (60.13±6.78 mg/dL) were significantly (P<0.05) higher than in healthy subjects (19.08±5.73 mg/dL) Table 4.2, Figure 4.3, Figure 4.4, Figure 4.5, Figure 4.6 and Figure 4.7.

Table 4.2 Lipid profile in studied groups

Groups Variables	Healthy (30)	Subject with T2DM (120)	P-Value
Total cholesterol mg/dL	154.18±11.24	238.71±20.41*	0.036
Triglyceride mg/dL	91.52±10.76	320.41±43.57*	0.019
HDL mg/dL	33.12±1.42	25.53±1.83*	0.044
LDL mg/dL	98.01±4.91	153.71±14.93*	0.038
VLDL mg/dL	19.08±5.73	60.13±6.78*	0.023

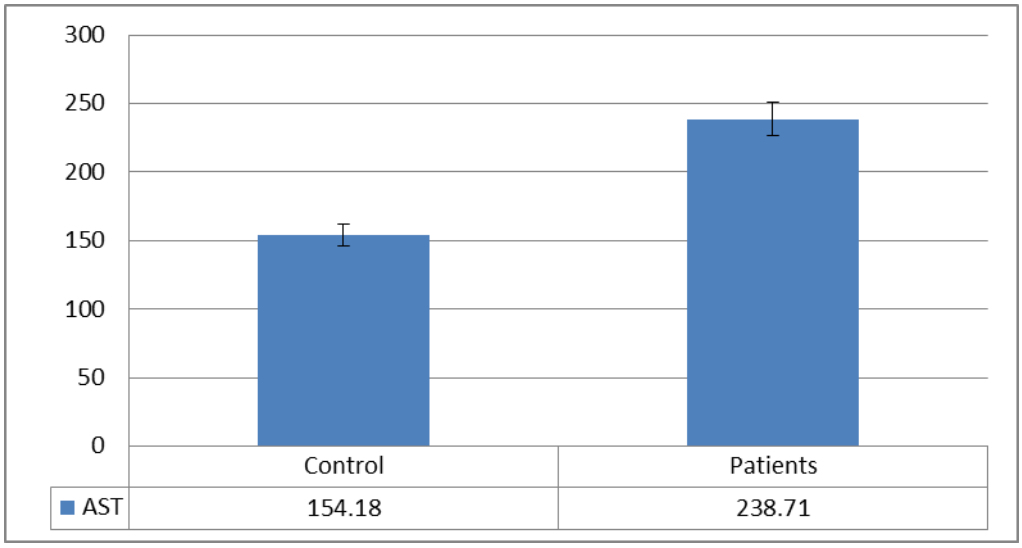

Figure 4.3 TC levels in both groups

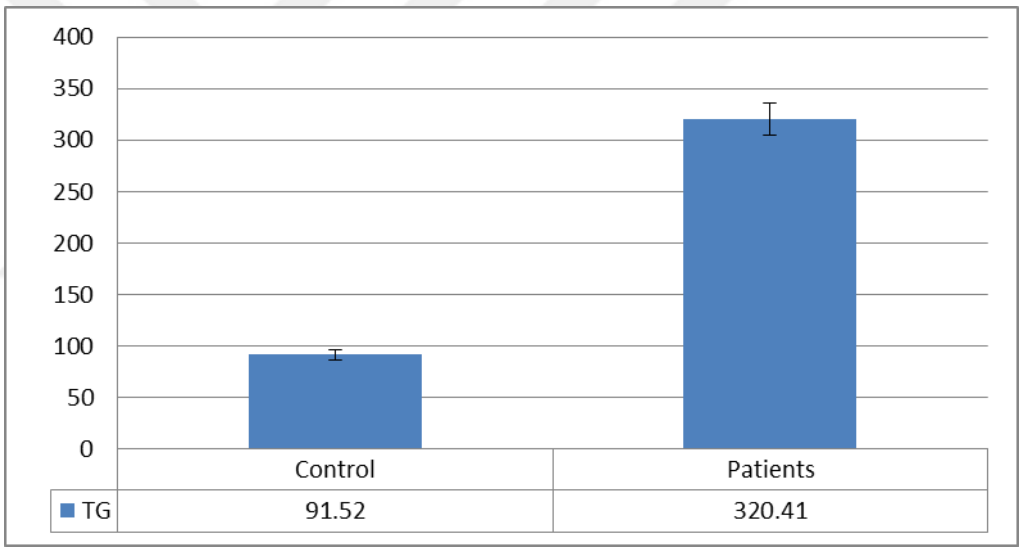

Figure 4.4 TG levels in both groups

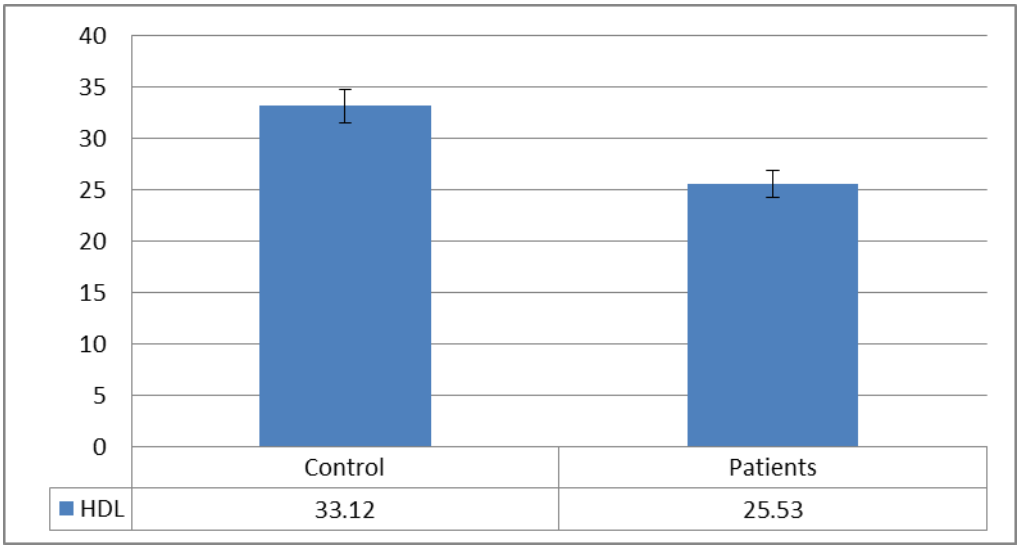

Figure 4.5 HDL levels in both groups

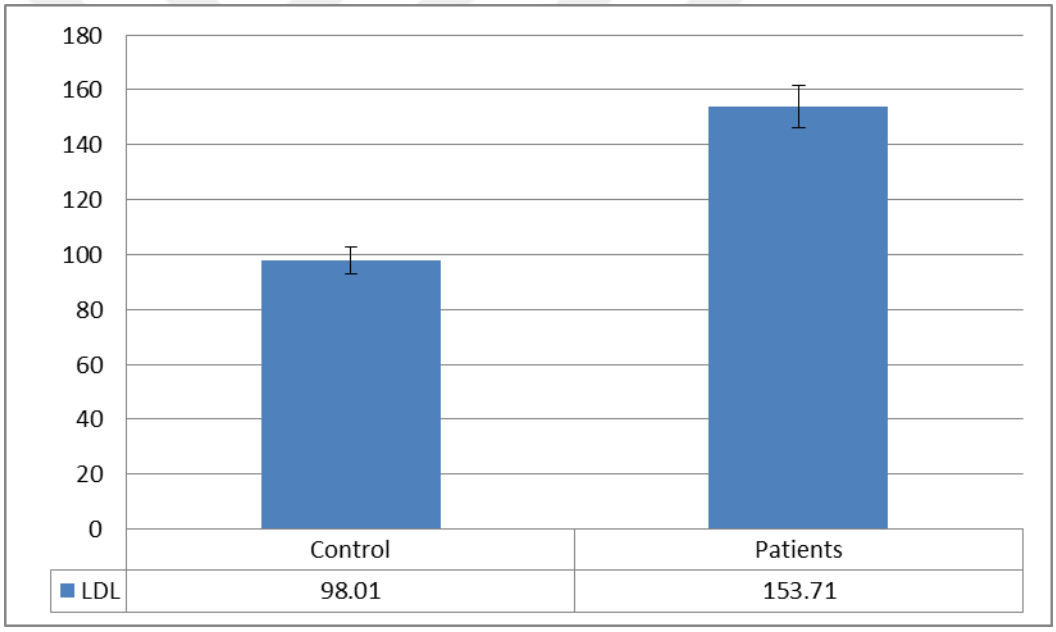

Figure 4.6 LDL levels in both groups

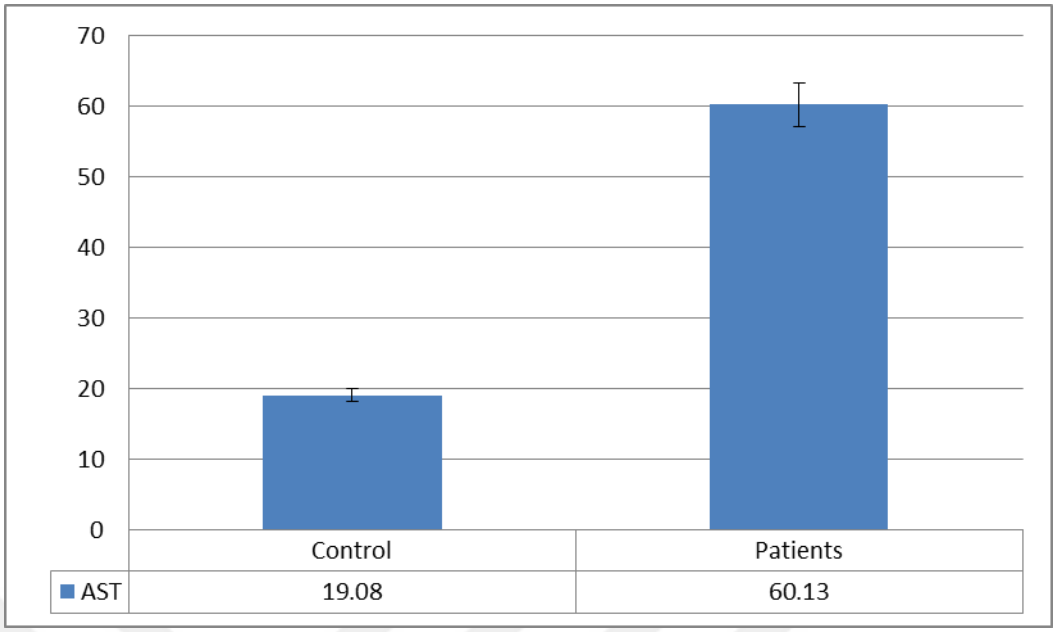

Figure 4.7 VLDL levels in both groups

Diabetic individuals showed substantially higher total cholesterol levels ($P<0.05$) than healthy ones, according to this study. This increase might be attributed to an increase in VLDL and LDL concentrations, which could be driven by increased hepatic VLDL synthesis or decreased clearance of VLDL and LDL from circulation (King *et al.* 1998).

According to the results, a substantial increase in HDL cholesterol ($p=0.001$) increases the number of LDL receptors in diabetic patients, resulting in a rise in LDL cholesterol levels in diabetes mellitus (Sabahelkhier *et al.* 2016). As a consequence of the overproduction of VLDL, triglyceride levels rise, leading in reduced HDL cholesterol levels and an exchange process mediated by CETP, which results in hyperglycemia and the mobilization of fatty acids from fat tissue, causing diabetes. Hyperglycemia occurs when tissue fails to use blood glucose as a consequence of diabetes. The liver stores excess fatty acids as triglycerides, while fatty acids from the adipose tissue are used for energy (Amos *et al.* 1997).

A study of 600 Nigerian patients revealed that 89 percent of the population had dyslipidemia, which was comparable to the findings here, but with a lower number of diabetic patients (n = 100) (Ogbera *et al.* 2009) LDL cholesterol was 74 percent higher,

total cholesterol was 43 percent higher, TG was 13 percent higher, and HDL was 53 percent lower, according to the Nigerian study. These studies matched those done in India, which discovered higher total cholesterol in diabetics with an 86 percent prevalence of dyslipidaemia. HDL dyslipidemia was observed in 71% of diabetic patients in the Indian research, whereas LDL dyslipidemia was found in 64% of diabetic patients and hypertryglyceridemia was reported in 47% of participants (Joffe *et al.* 2004, Ogbera *et al.* 2009).

In a study of 401 diabetic individuals, 33.5 percent had high cholesterol and 38.9% had high triglycerides, with 41 percent and 47 percent having high cholesterol and high triglycerides, respectively (Mengesha *et al*. 2006), According to current study, TC (56 percent), hypertriglycemia (64 percent)and low-density lipoprotein cholesterol (61 percent) are more common than previously thought (65 percent). T2DMM patients have a large dyslipidemia consequence, which may lead to an even greater mortality rate among these patients, as many prior research have demonstrated (Ali *et al.* 2015).

HDL levels in diabetes individuals were substantially lower ($P<0.05$) than in non-diabetic subjects. Lower HDL cholesterol levels are caused by triglyceride enrichment via protein of cholesterol ester transfer and elevate hepatic lipase activity (Fronzo 1988). It's not only the liver that produces HDL particles, but the breakdown of TG-rich lipoproteins also produces HDL. In diabetes, the liver's synthesis of HDL-C is reduced because a protein called CETP moves the cholesterol ester away from HDL in exchange for TG from VLDL particles, resulting in a fall in HDL-C levels. Lowering HDL levels in blood and promoting the formation of LDL particles are the effects of this protein (Chatterjee *et al.* 2005).

As glucose and lipid metabolism are intertwined, any disruption in one causes the other to malfunction. Insulin resistance, which is linked to qualitative changes in TC and TG, is caused by high lipid profile and reduce of HDL levels, with or without hyperglycemia (Del Prato *et al.* 1993).

4.3 Oxidative Stress and Antioxidant Enzymes

MDA levels in diabetes mellitus patients' serum (2.072±0.18 nmol/L) are significantly (P<0.05) higher than in healthy subjects (1.466±0.052 nmol/L). GSH levels in diabetes mellitus patients' serum 0.4043±0.01 nmol/L) are significantly (P<0.05) lower than in healthy subjects (0.4663±0.018 nmol/L). GPX levels in diabetes mellitus patients' serum (0.6032±0.014 nmol/L) were significantly (P<0.05) lower than in healthy subjects (0.6826±0.016 nmol/L). SOD levels in diabetes mellitus patients' serum (0.767±0.021 nmol/L) were significantly (P<0.05) lower than in healthy subjects (0.899±0.035 nmol/L) Table 4.3, Figure 4.8, Figure 4.9, Figure 4.10, Figure 4.11 and Figure 4.12.

Table 4.3 MDA and antioxidant enzymes in studied groups

Groups Variables	Healthy (30)	Subject with T2DM (120)	P-Value
MDA nmol/L	1.466±0.052	2.072±0.18*	0.045
GSH nmol/L	0.4663±0.018	0.4043±0.01*	0.049
GPX nmol/L	0.6826±0.016	0.6032±0.014*	0.044
SOD nmol/L	0.899±0.035	0.767±0.021*	0.046
CAT nmol/L	1.263±0.045	1.063±0.029*	0.034

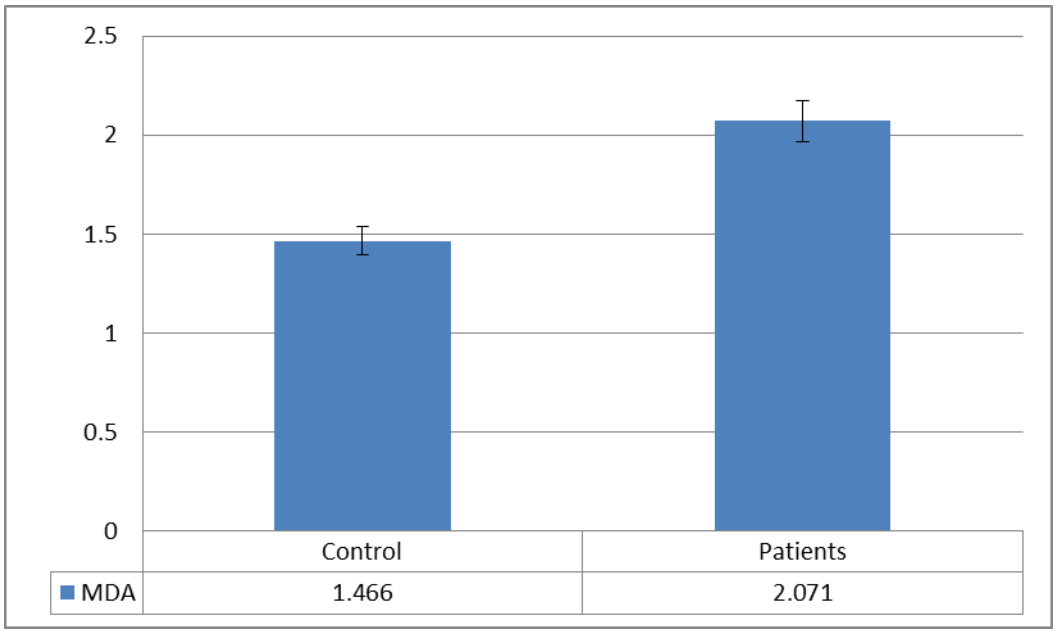

Figure 4.8 MDA levels in both groups

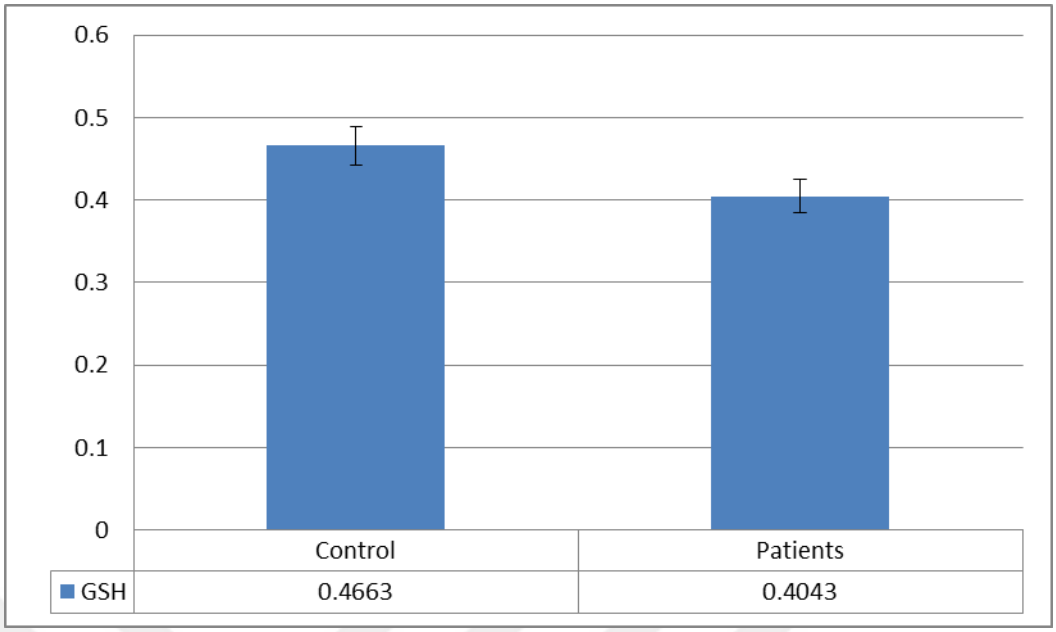

Figure 4.9 GSH levels in both groups

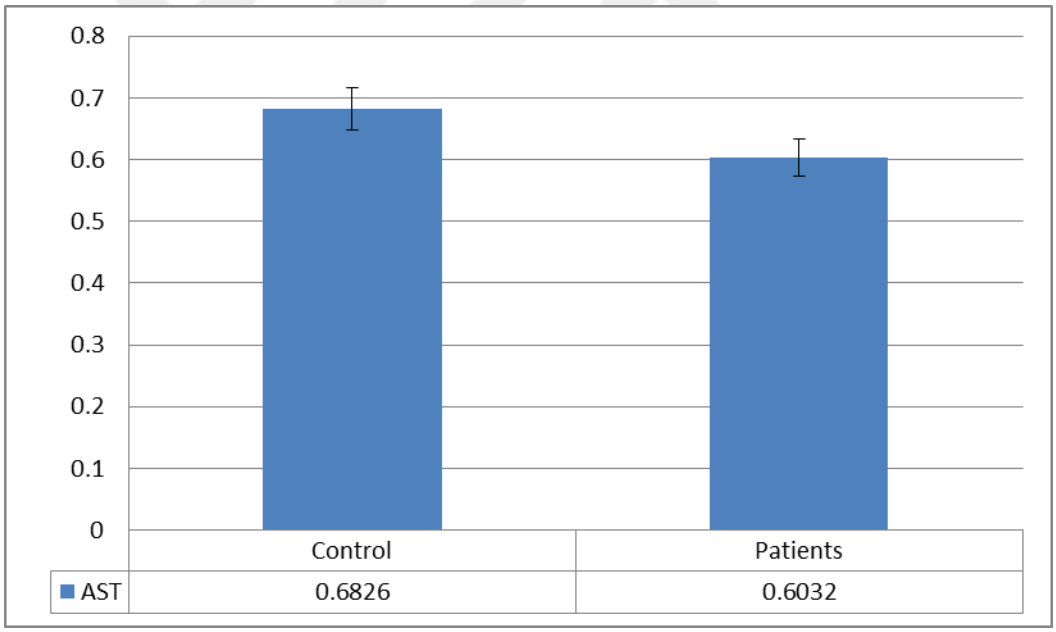

Figure 4.10 GPx levels in both groups

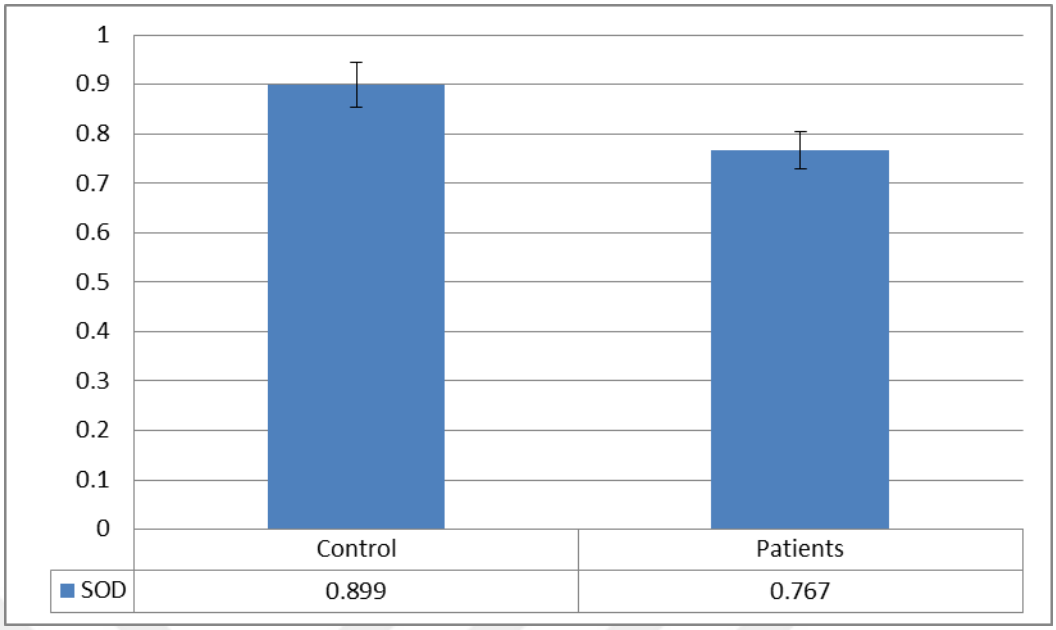

Figure 4.11 SOD levels in both groups

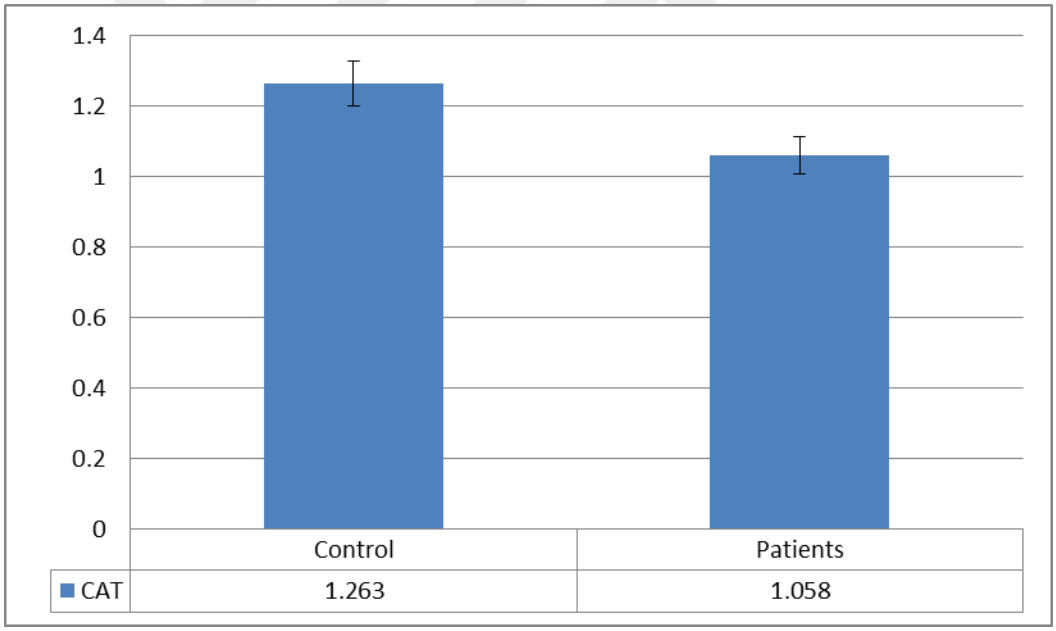

Figure 4.12 CAT levels in both groups

Diabetic individuals have been shown to have increased oxidative stress markers in their blood or a reduction in plasma antioxidant levels. In two investigations, the antioxidative ability of people with Type 2 Diabetes (T2DM) was evaluated. In one study, patients' antioxidant capacity was demonstrated to be lowered; however, in the

other study, those with DM and the control group did not differ (Nuttall *et al.* 1999, Opara *et al.* 1999).

In DM, glucose breakdown, nonenzymatic protein glycation, and subsequent oxidative degradation create an excessive number of free radicals, which may have a role in the development of difficulties in T2DM patients. T2DM patients have significantly higher levels of MDA (as TBARS) (Pasaoglu et al. 2004, Mahboob et al. 2005). The generation of free radicals in diabetes mellitus patients might lead to lipid peroxidation and significant damage (Soliman 2008).

Hyperglycemia causes elevated the levels of free radical. Because GSH is a major antioxidant, its loss causes elevate in the oxidative stress (Seghrouchni *et al.* 2002, Powell *et al.* 2001). Oxidative stress and GSH levels in the vascular straight muscles decreased as a result of hyperglycemia. When we examined the relationship between GSH levels and biochemical indicators, we found a strong negative correlation between fasting glucose and GSH in the present research.

Rani *et al.* (2005) discovered that T2DM had lower total antioxidant status than controls, implying that diabetics face higher oxidative stress than nondiabetics. They also emphasized the need of assessing these indicators for early detection and treatment options (Yang *et al.* 2009) discovered that hyperglycemic mice exhibited greater blood lipid peroxidation as evaluated by MDA, which elevated the risk of myocardial infarction through activating NADPH oxidase.

Oxidative stress complicates the genesis of microvascular disorders (Wierusz-Wysocka *et al.* 1995). Several studies have indicated that the oxidation products of diabetic individuals with chronic issues are greater than those of diabetic people without complications (Bambolkar and Sainani 1995). In diabetic individuals, the antioxidant defense system has been reported to be compromised (Rema *et al.* 1995).

As demonstrated in this study, reduced GPX activity in diabetics without and with MVC might be due to reduced SOD activity, which is required for scavenging superoxide radicals. Reduced SOD activity, which inhibits GPX, results in elevate in superoxide radicals. Diabetic individuals have low glutathione levels, which might explain why GPX activity is low. By functioning as a co-substrate for GPX activity, glutathione acts as a direct free-radical scavenger (Blum and Fridovich 1983, Winterbourn 1993).

Inactivation of the enzyme might be a reason in GPX activity being reduced. Glucose regulation is associated to complications. According to research, hyperglycemia appears to have a function in the generation ROS. Finally, ROS produce oxidative stress in a variety of tissues (Halliwell *et al.* 1995). Many metabolic pathways connected to hyperglycemia, including glucose autoxidation, the pathway called polyol pathway, synthesis of prostanoid, and protein glycation, might result in an increase in free radical generation (Kajanochumpal *et al.* 1997).

In diabetic retinopathy patients, (Hartnett *et al.* 2000) reported elevated in the oxidative stress indicators and a decreacing in the levels of antioxidant enzyme. The mechanism for the decreacing in retinal GSH found in DM is unknown. GSH consumption in DM may increase when GSH synthesis declines, according to certain theories. According to previous studies, there is no failure of GSH production in the retina of diabetic mice, despite reduced in levels of enzyme in cycle of GSH redox (Kowluru *et al.* 1996).

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

- The current investigation discovered a substantial ($P<0.05$) rise in glucose levels in T2DMM patients' blood.
- In both diabetes types, total cholesterol, triglyceride, LDL, and VLDL levels were found to be significantly higher ($P<0.05$) in the current research.
- Both diabetes types had a substantial ($P<0.05$) drop in HDL in the current investigation.
- Both diabetes types had a substantial ($P<0.05$) rise in uric acid levels in the current investigation.
- MDA levels in T2DMM patients' serum were found to be significantly higher ($P<0.05$) in the current investigation.
- In the current investigation, gluthatione, catalase, and SOD levels in T2DMM patients' blood were shown to be significantly lower ($P<0.05$).

5.2 Recommendation

- Determining the concentration of liver enzymes in serum of T2DMM patients.
- Determining the concentration of leptin, adiponectin, and resistin in serum of T2DMM patients.
- Determining the concentration of acid phospitase in serum of T2DMM patients.
- Determining the concentration of Cluster of Differentiation (CD Markers) in serum of T2DMM patients.

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