Ministry of Higher Education and
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College of Pharmacy

Study of antioxidant effect in patients with type 2 diabetes

Research project

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By:

Duaa Adel Hamza

Ghadeer Ahmed Hamza

Alaa Faek Obaies

Supervised by:

Assist Lec. Ataa Khalil Al-Shamari

Assist Lec. Hajir Bassim Aljaryan

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بسم الله الرحمن الرحيم

(وقل ربي زدني علما)

صدق الله العلي العظيم

Dedication

Dedication To my family especially
Who supported me
Since the beginning of my life.
I dedicate this work

The students

Abstract

Background: Diabetes is the most common cause of chronic renal disease globally. Oxidative stress is one of the problems that can encounter of diabetic patients. Antioxidants system serves to protect cells from damage that may occur due to the actions of these species. In this work, we attempt to investigate the biochemical parameters including enzymatic and non-enzymatic antioxidants in patients with type 2 diabetic mellitus.

Material and method: Blood samples were collected from 40 subjects they were classified into two groups, the first group represents the patients group, which includes 20 patients. The second group consists of 20 healthy persons as a control group. Random Blood sugar, Catalase, Superoxide Dismutase, and Malondialdehyde were assessed.

Results: Results showed a significant increase in levels of RBS and an increase in activity of the MDA patients group related to the control group. While there is no significant difference in the activity of CAT and SOD between patients and control groups.

Conclusion: Free radical generation increase in diabetic cases, which leads to a decrease in antioxidant levels of CAT and SOD. The level of MDA is the better indicator to evaluate oxidative stress and can be used as a marker for the early detection of diabetes.

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1. INTRODUCTION

One of the most prevalent endocrine disorders that are caused by deficiencies in insulin action or insulin secretion is DM [1]. The primary sign of diabetes is hyperglycemia in the blood, caused by insufficient pancreatic insulin secretion or low insulin-directed fostering of the glucose via target cells, DM could be classified into several types, T1DM and T2DM are the two most common types, For T1DM, insulin renewal therapy is the backbone, while in T2DM, there should be lifestyle modification and a control diet [2].

Type 2 Diabetes Mellitus (T2DM) has been referred to for a long time as non-insulin dependent diabetes, or adult-onset diabetes characterized by insulin resistance, which could progressively worsen to absolute resistance, but in the past decade, reduced β-cell function has been recognized as a key problem in T2DM [3]. Indeed, in the past two decades, T2DM emerged as a new and very serious health problem also in children [4]. According to the World Health Organization (WHO), diabetes mellitus is a chronic, metabolic disease characterized by elevated levels of blood glucose, which leads over time to damage to the heart, vasculature, eyes, kidneys, and nerves. Over 90% of diabetes mellitus cases are T2DM, a condition marked by deficient insulin secretion by pancreatic islet β-cells, tissue insulin resistance (IR), and an inadequate compensatory insulin secretory response [5]. The progression of the disease makes insulin secretion unable to maintain glucose homeostasis, producing hyperglycemia. Patients with T2DM are mostly characterized by being obese or having a higher body fat percentage, distributed predominantly in the abdominal region. The main drivers of the T2DM epidemic are the global rise in obesity, sedentary lifestyles, high-caloric diets, and population aging, which have quadrupled the incidence and prevalence of T2DM. The organs involved in T2DM

development include the pancreas (β-cells and α-cells), liver, skeletal muscle, kidneys, brain, small intestine, and adipose tissue [6].

The pathogenesis that underlies T2DM is complex and multifactorial. One of the most important components is the role of glucose autoactivation following persistently elevated circulating glucose, resulting in prooxidant production. Oxidative stress is caused by a perturbation of prooxidants (reactive oxygen species ((ROS)) and the antioxidant micro ecosystem that favors excess production of prooxidants relative to antioxidant defense [7]. The ROS are formed by multiple overlapping and interacting mechanisms that highlight their biological complexity and their effects on individuals' genetic backgrounds. These enzymatic and non-enzymatic pathways include mainly oxidative phosphorylation, plasma membrane proteins such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOXs), lipid metabolism within the peroxisomes, and cyclooxygenases. The abnormality of these two pathways leads to oxidative stress, which accelerates the development of diabetes complications, both microvascular and cardiovascular. Free radicals are associated with oxidative stress. Cells are protected from the damage that can occur by oxidants. Antioxidants are molecules present in low concentrations that function to neutralize oxidants through certain mechanisms [8]. Antioxidants can be classified into two major groups. Enzymatic antioxidants, which include different enzymes such as catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase, and non-enzymatic antioxidants that consist of glutathione, lipoic acid, selenium, zinc, and the dietary supplements [9].

1.1. Aim of study

This study aims to estimate some enzymatic and non-enzymatic antioxidants in the blood sera of patients with diabetes as biochemical markers that can be used in the early detection of diabetes.

2. Literatures Review

2.1. Diabetes Mellitus:

Diabetes is a metabolic disorder in which there are high levels of sugar in the blood, a condition called hyperglycemia. Under normal conditions, food is broken down to glucose, which then enters the bloodstream and acts as fuel for the body. The pancreas produces a hormone called insulin, which helps to carry glucose from the bloodstream into muscle, fat, and liver where it can be used as fuel. Diabetics are not able to move this sugar out of the bloodstream because of two primary reasons: their pancreas does not produce enough insulin and/or their cells do not respond normally to insulin, a condition called insulin resistance. This is why people with diabetes have high blood sugar levels [10].

2.1.1. Types of diabetes mellitus:

I. Type 1 DM: is characterized by loss of insulin in the islets of Langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune diabetes is of an immune-mediated nature, in which an attack leads to the loss of beta cells and thus insulin [11]

II. Type 2 DM: is one of the most common metabolic disorders worldwide and its development is primarily caused by a combination of two main factors: defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond to insulin. Insulin release and action have to precisely meet the metabolic demand; hence, the molecular mechanisms involved in the synthesis and release of insulin, as well as the insulin response in tissues must be tightly regulated. Therefore, defects in any of the mechanisms involved can lead to a metabolic imbalance that leads to the pathogenesis of T2DM [12]. The organs involved in T2DM development include the pancreas (β-cells and α-cells), liver, skeletal muscle, kidneys, brain,

small intestine, and adipose tissue. Evolving data suggest a role for adipokine dysregulation, inflammation, and abnormalities in gut microbiota, immune dysregulation, and inflammation have emerged as important pathophysiological factors [13].

2.1.2. Signs and symptoms:

The classic symptoms of untreated diabetes are weight loss, polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes. Several other signs and symptoms can mark the onset of diabetes, although they are not specific to the disease. In addition to the known ones above, they include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye [14].

2.1.3. Complications of Diabetes

Diabetes is justly recognized as an emerging global epidemic, representing one of the leading causes of morbidity and mortality worldwide. Hyperglycemia, the common characteristic of both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), has the potential to cause serious complications due to its insidious and chronic nature. All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10 -20), but may be the first symptoms in those who have otherwise not received a diagnosis before that time. The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease [15]. Other "macrovascular" diseases are stroke and peripheral vascular disease. The primary microvascular complications of diabetes

include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and potentially blindness. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney

disease, sometimes-requiring dialysis or kidney transplant damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle wasting and weakness. There is a link between cognitive deficit and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5-fold greater rate of decline in cognitive function [16].

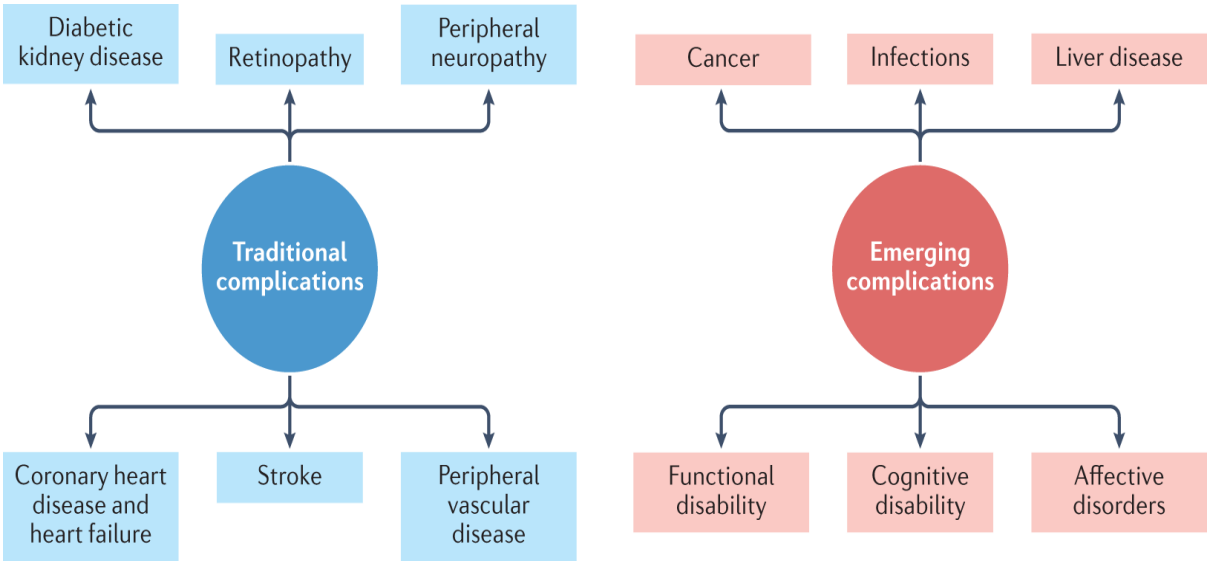

Fig. 2.1 Diabetes mellitus complications

2.1.4. Prevention

Type2 diabetes can often be prevented by a person being a normal body weight, physical exercise, and following a healthy diet Dietary changes known to be effective in helping to prevent diabetes include a diet rich in whole grains and fiber, and choosing good fats, such as polyunsaturated fats found in nuts, vegetable oils, and fish. Limiting sugary beverages and eating less red meat and other sources of saturated fat can also help in the prevention of diabetes. Active smoking is also associated with an increased risk of diabetes, so smoking cessation can be an important preventive measure as well [17].

2.2.Antioxidants

Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent. Oxidation reactions are known to produce free radicals. These free radicals are highly reactive species that contain one or more unpaired electrons in their outermost shell. Once they are formed, the chain reaction starts. Antioxidant reacts with these free radicals and terminate this chain reaction by removing free radical intermediates and inhibiting other oxidation reactions by oxidizing themselves. Though oxidation reactions are crucial for life, they can also be damaging. Plants and animals have a complex system of multiple types of antioxidants, such as vitamin C and vitamin E, as well as enzymes, such as catalase (CAT), superoxide dismutase (SOD), and various peroxidases. Oxidative stress plays a key role in causing various human diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorder, Parkinson’s dementia, Alzheimer’s disease, inflammatory disease, muscular dystrophy, liver disorder and even aging [18]. Antioxidants can be classified into three lines of defense according to their mechanism of action shown in (2.2).

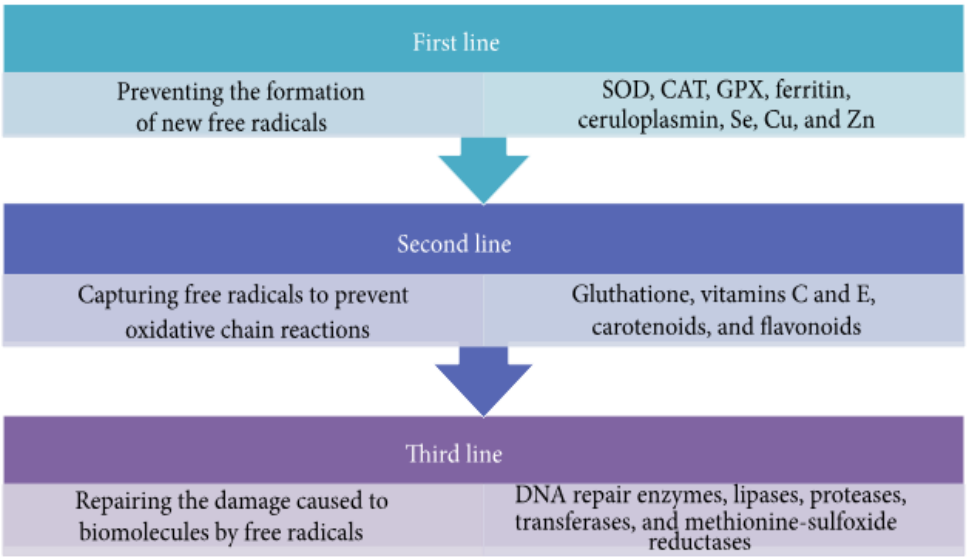

Figure 2.2: The antioxidant defense.

2.2.1. Catalase

Catalase is an antioxidative enzyme present nearly in all living organisms. It plays an important role against oxidative stress-generated complications such as diabetes and cardiovascular diseases. Catalase one of the most studied enzyme. The overall reaction for catalase can simply be described as the degradation of two molecules of hydrogen peroxide to water and oxygen. This catalytic reaction occurs in two distinct stages, but what each of the stages includes is mainly based on the kind of catalase [19]. The first stage involves oxidation of the heme using first hydrogen peroxide molecule to form an oxyferryl species in which one-oxidation equivalent is taken off from the iron and one from the porphyrin ring to make a porphyrin cation radical. In the second stage, this radical intermediate, known as compound I, is reduced by a second hydrogen peroxide to regenerate the resting state enzyme, water and oxygen [20]. Catalase acts as main regulator of hydrogen peroxide metabolism. Hydrogen peroxide is a highly reactive small molecule formed as the natural by-product of

energy metabolism. Excessive concentration of hydrogen peroxide may cause significant damage to proteins, DNA, RNA, and lipids. The deficiency of this enzyme leads, in the β-cell, to an increase in oxidative stress and ultimately to a failure of this cell type β-cells are rich in mitochondria, and thus this organelle might be a source of ROS. Catalase protects pancreatic β-cells from damage by hydrogen peroxide. Low catalase activities, which have been reported in patients with schizophrenia and atherosclerosis, are consistent with the hypothesis that long-term oxidative stress may contribute to the development of a variety of late-onset disorders, such as type 2 diabetes [21].

2.2.2. Malondialdehyde (MDA):

MDA is one of the most frequently measured biomarkers of oxidative stress in plasma and serum. [22]. MDA is the principal and most extensively studied compound derived from lipid peroxidation, known to possess mutagenic and toxic effects. Additionally, MDA can be enzymatically produced as a side product during the biosynthesis of thromboxane A2 [23]. Once generated, MDA can be metabolized by various enzymes, particularly at the mitochondrial level by aldehyde dehydrogenase, or it can covalently interact with proteins and nucleic acids, leading to the formation of DNA-protein crosslinks and various adducts that damage biomolecules [24]. Moreover, a portion of MDA is excreted in the urine. As a result of its interactions, additional modifications occur, leading to the formation of various MDA epitopes that interact with the innate immune system. Furthermore, the detection of such products in inflammatory disorders suggests that lipid peroxidation plays a significant role in this type of disease [25]. The relationship between type 2 diabetes and malondialdehyde (MDA) involves oxidative stress. MDA is a byproduct of lipid peroxidation, which is a process that occurs when free radicals damage cell membranes containing lipids. In type 2 diabetes, there is often an

imbalance between the production of free radicals and the body's ability to neutralize them, leading to increased oxidative stress. Elevated levels of MDA have been found in individuals with type 2 diabetes, indicating increased lipid peroxidation. This oxidative stress can contribute to the development and progression of diabetes-related complications, such as cardiovascular disease, nephropathy, and neuropathy. Overall, MDA levels can serve as a marker for oxidative stress in type 2 diabetes and may be used to assess the effectiveness of antioxidant therapies aimed at reducing oxidative damage in diabetic patients [24].

2.2.3. Superoxide dismutase (SOD):

SOD is a class of enzymes that restrict the biological oxidant cluster enzyme system in the body, which can effectively respond to cellular oxidative stress, lipid metabolism, inflammation, and oxidation. SOD enzymes (SODs) could maintain a dynamic balance between the production and scavenging of biological oxidants in the body and prevent the toxic effects of free radicals, and be effective in anti-tumor, anti-radiation, and anti-aging. Diabetes is a relatively common disease and is hereditary. More and more evidence revealed that SOD enzymes play an important role in the prevention and treatment of diabetes [26]. Maternal diabetes-induced autism-like behavior through hyperglycemia-mediated sustained oxidative stress and inhibition of SOD2. The SOD2 enzyme alleviated obesity, inflammation, and insulin resistance induced by high-fat diet .SOD3 was upregulated in the serum and placenta of physically active pregnant women. [27]. Indeed, SODs play an indispensable role in improving diabetes by lipid metabolism.

2.3.Antioxidants and DM

Oxygen is one of the important components of life. However, in some circumstances, this oxygen may be a killer of cells when it generates reactive species

that cause necrosis, organ damage [28], and ultimately cell death. Reactive nitrogen and carbon species also cause oxidation by the generation of certain mechanism that interferes with the normal physiological processes inside the cell [29]. Oxidative stress can be defined as a disturbance in the balance between oxidants and antioxidants due to different factors such as aging, drug actions and toxicity, inflammation, and/or addiction. It is in general, excess formation or/and insufficient removal of highly reactive molecules. Oxygen is a highly reactive species that can become part of potentially harmful and damaging molecules; free radicals. Oxidative stress causes healthy cells of the body to lose their function and structure by attacking them. It is when the antioxidant level is limited that this damage can become debilitating and cumulative leading to several diseases. Oxidative stress is increased in diabetes because of multiple factors. Among these factors, is glucose autoxidation which is a dominant factor leading to the production of free radicals. Other factors include cellular oxidation/reduction imbalances and reduction in antioxidant defenses including decreased cellular antioxidant levels and a reduction in the activity of antioxidant enzymes that dispose of free radicals. Moreover, levels of some pro-oxidants such as ferritin and homocysteine are elevated in diabetes. Another important factor is the interaction of Advanced Glycation End Products (AGEs) with specific cellular receptors called AGE Receptors (RAGE).Elevated levels of AGE are formed under hyperglycemic conditions. Their formation is initiated when glucose interacts with specific amino acids on proteins forming a compound that then undergoes further chemical reactions. Glycation of protein alters protein and cellular function, and binding of AGEs to their receptors can lead to modification in cell signaling and further production of free radicals [30]. It is believed that oxidative stress plays important role in the development of vascular complications in diabetes particularly type2 diabetes [31]. ROS level elevation in diabetes may be due to perturbations in antioxidant defense system. The variation in

the levels of antioxidant enzymes makes the tissues susceptible to oxidative stress leading to the development of diabetic complications. According to epidemiological studies, diabetic mortalities can be explained by an increase in vascular diseases other than hyperglycemia [31].

Much evidence from experiments have given a link between diabetes and oxidative stress by measuring various biomarkers include DNA damage biomarkers and lipid peroxidation products. It is believed that in the onset and progression of late diabetic complications, free radicals have got a major role due to their ability to damage lipids, proteins and DNA [32]. Varieties of pathological conditions are induced by oxidative stress such as Rheumatoid arthritis, Diabetes mellitus and cancer. Biomarkers of oxidative stress in diabetes mellitus include proteins, lipids, and vitamins, enzymatic and non-enzymatic antioxidants [33].

2.4.Free Radicals

Free Radicals are molecules with an unpaired electron and are important intermediates in natural processes involving cytotoxicity, control of vascular tone, and neurotransmission. Free radicals are very unstable and react quickly with other compounds, and try to capture the needed electron to gain stability. A chain reaction thus gets started. Once the process is started, it can cascade, and inally results in the disruption of a living cell. Generally, harmful effects of reactive oxygen species on the cell are most often like damage of DNA, oxidations of fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivate specific enzymes by oxidation of co-factors. Free radicals cause many human diseases like cancer Alzheimer's disease, cardiac reperfusion abnormalities, kidney disease, fibrosis, etc. The free radicals formed in our body are combated by antioxidants that safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Excessive exercise has been found to increase the free radical level in the

body and causes intense damage to the Regular physical exercise enhances the antioxidant defense system and protects against exercise induced free radical damage. Apart from the destructive effects of free radical they are also responsible for some vital actions like destroy the bacteria and other cells of foreign matter, kill cancer cells, turning on and off of genes and fight infection, to keep our brain alert and in focus. Human body is continuously exposed to different types of agents that results in the production of reactive species called as free radicals (ROS/RNS) which by the transfer of their free unpaired electron causes the oxidation of cellular machinery. In order to encounter the deleterious effects of such species, body has got endogenous antioxidant systems or it obtains exogenous antioxidants from diet that neutralizes such species and keeps the homeostasis of body. Any imbalance between the RS and antioxidants leads to produce a condition known as "oxidative stress" that results in the development of pathological condition among which one is diabetes. Most of the studies reveal the inference of oxidative stress in diabetes pathogenesis by the alteration in enzymatic systems, lipid peroxidation, impaired Glutathione metabolism and decreased Vitamin C levels. Lipids, proteins, DNA damage, Glutathione, catalase and superoxide dismutase are various biomarkers of oxidative stress in diabetes mellitus. Oxidative stress-induced complications of diabetes may include stroke, neuropathy, retinopathy, and nephropathy [32].

2.5. Oxidative Stress-Induced Alterations in Diabetes

Oxidative stress in diabetes mellitus causes several adverse effects on the cellular physiology. This is particularly relevant and dangerous for the islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses. Multiple biochemical pathways and mechanisms of action have been implicated in the deleterious effects of chronic hyperglycemia and oxidative stress on the function of vascular, retinal, and renal tissues [34]. Here we have described the oxidative stress-

induced alterations in major biomolecules in the cell and status of plasma antioxidant potential during type 2 diabetes (Figure 2.3).

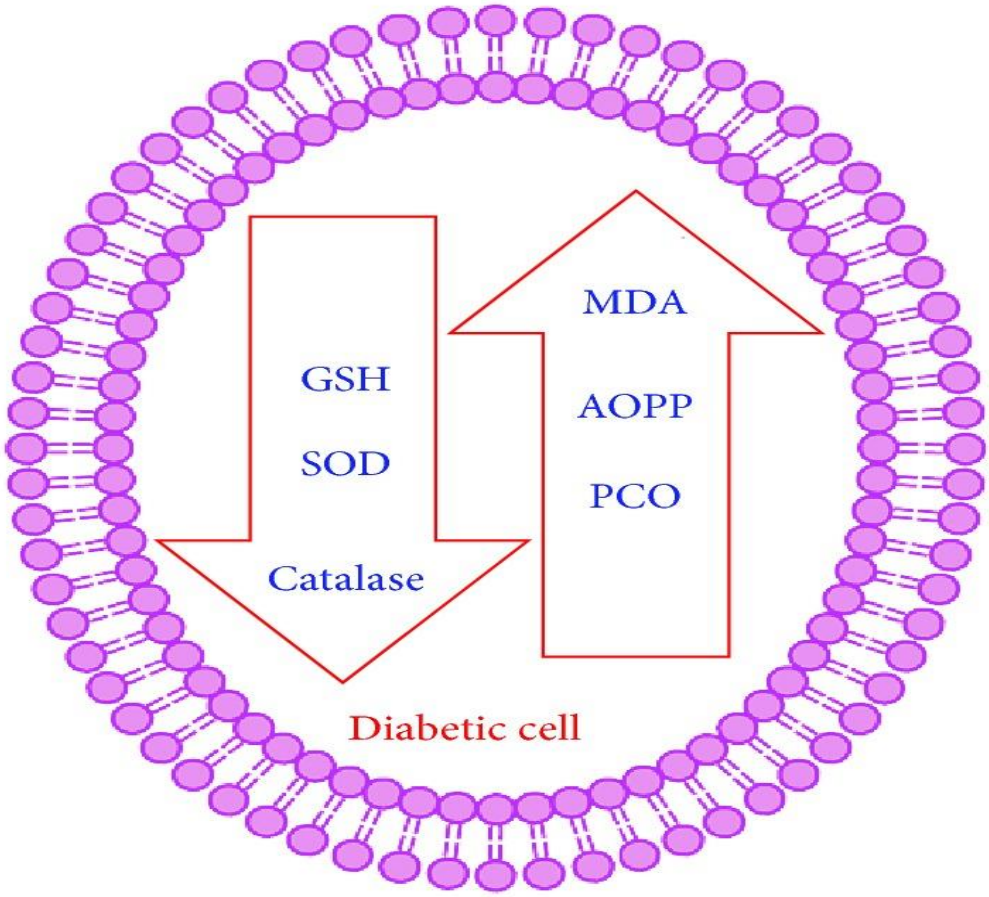

Figure 2.3 Schematic representation of the status of oxidative stress markers during diabetes. MDA: malondialdehyde, AOPP: Advanced oxidation protein products, PCO: protein carbonyls, GSH: reduced glutathione, and SOD: superoxide dismutase.

3. Material and Methods

3.1. Study Design

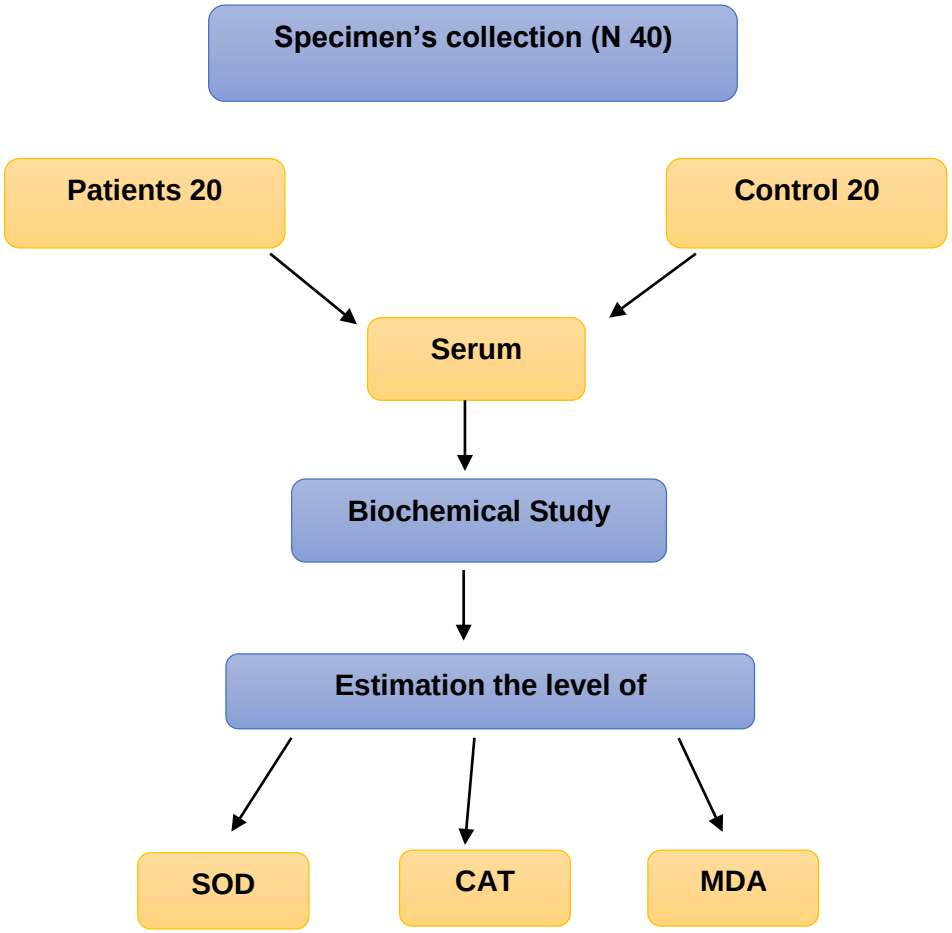

Figure (3.1): Scheme general steps for the research project

3.3. Material and Methods

3.3.1 Study Population

3.3.1.1 Case Group

Samples collection and practical work of this study were done from January 2024 to March 202024. The study subjects comprised 20 patients with Diabetes selected from Merjan Teaching Hospital in Babylon-Iraq.

3.3.1.2 Control Group

Blood samples of 20 healthy individuals were collected to compare with case patients.

3.3.1.3 Data Collection

A questionnaire was taken from the subjects included in the study. It included age, sex, weight, family history, and duration of disease.

3.3.1.4. Blood Samples

Five milliliters of venous blood were taken from both patients and control and then used to separate the serum after clotting the blood by centrifugation at 5000 rpm for 10 min then kept in Eppendorf tubes at -20 ºC until use.

3.4. Biochemical Analysis

3.4.1 Antioxidants

3.4.1.1 Superoxide dismutase (SOD)

The activity of Superoxide dismutase was determined by using a simple and rapid method, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol according to Marklund and Marklund, (1974)

50µL from Serum was taken, then mixed with 1000 µL of Tris buffer, and added 1000 µL of Pyrogallol solution, mixed by inversion. While the control consisted of 1000 µL of pyragallol, 1000 µL tris-buffer with 50µL distal water. Absorbance was measured at 420 nm against Tris-EDTA buffer at zero time and after 1 minute of the addition of pyrogallol.

3.4.1.2 Catalase (CAT)

Catalase assay was measured according to procedure of Goth et al, [35]. 0.2 ml serum was incubated in 1 ml of substrate (65 μmoL per ml H_2O_2 in 60 mmol/L sodium-potassium phosphate buffer, pH 7.4) at 37 °C for 1 min. The enzyme activity was suspended by adding 1 ml of 32.4 mM ammonium molybdate. The yellow absorption value of the molybdate complex and hydrogen peroxide was measured at 405 nm using a spectrophotometer.

Catalase enzyme activity was calculated according to the following equation:

C.A (KU/l) =(S-B1/B2-B3) * 271

C.A: Catalase activity (KU/l)

S: Sample reading.

B1: Blank 1 reading contained 1.0 ml substrate, 1.0 mL molybdate and 0.2 mL sample.

B2: Blank 2 reading contained 1.0 ml substrate, 1.0 ml molybdate, and 0.2 ml of 60 mmol/L sodium-potassium phosphate buffer, pH 7.4.

B3: Blank 3 reading contained 1.0 ml of 60 mmol/L sodium-potassium phosphate buffer pH 7.4, 1.0 mL molybdate, and 0.2 ml of 60 mmol/L sodium-potassium phosphate buffer pH 7.4.

3.4.1.3. Malondialdehyde (MDA)

Malondialdehyde was estimated by Thiobarbituric acid (TBA) assay method of Buege & Aust, [36], on spectrophotometer .

To 0.4 ml of serum, 0.6 ml TCA-TBA-HCl reagents were added. It was mixed well and kept in a boiling water bath for 10 minutes. After cooling 1.0 ml freshly prepared 1N NaOH solution was added. This absorbance of pink colour was measured at 535 nm against a blank which contained distilled water in place of serum. In blank 0.4 ml distilled water and 0.6 ml TCA-TBA-HCl reagent was mixed and boiled Blank was always taken..

Calculation:

$$\text{Malondialdehyde}(\mu\text{mol/l}) = \frac{\text{Absorbance of sample}}{E_o \times L} \times D$$

Where:

E_o = Extinction coefficient 1.56×10^5 M^{-1} cm^{-1}

L= light path cm.

D = dilution factor = 6.7×10^6

3.5. Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 26 and a p-value <0.05 was set as a cutoff value of statistical significance.

4. Results

According to the results in Table (4-1) summarize the demographic characteristics of the two studied groups of 20 patients with DM and 20 healthy individuals. Among all the participants in the different groups, females were more represented than males. Body weight did not significantly differ between the two groups. While the RBS levels in DM patients was significant ($P \leq 0.05$) than controls, the mean of glucose for DM patients and controls is (5.495±0.90 mm/l).

Table (4-1): Comparison between according to demographic data.

Demographic Data	Patient	Control	P value
Age	56.5±8.41	43.20±15.37	*P ≤0.05
Sex **Males** **Females**	 6(%30) 14(%70)	 8(%40) 12(%60)	 P <0.05
Body weight	79.9±15.11	73.70±12.17	P <0.05
RBS mm/l	9.49±3.88	5.49±0.92	*P ≤0.05

*Significant

Figure 4.1 displays the percentages of individuals who had diabetes in the study according to family history. The diabetes prevalence for individuals with a family history was more than the prevalence for individuals without a family history.

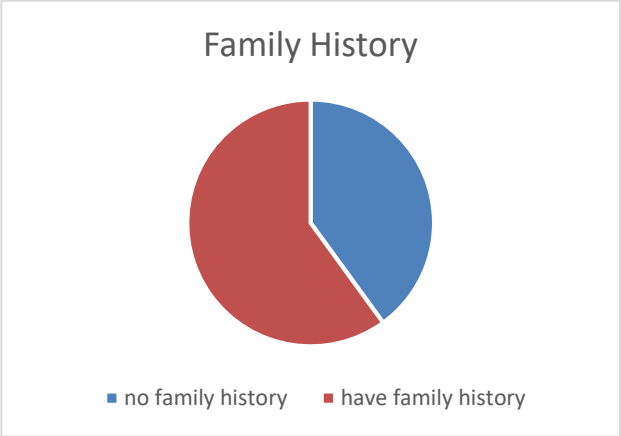

Figure (4.1): The distribution of DM in patients according to family history

Figure 4.2 shows the percentages of complications in individuals who had diabetes in the study. The eye complications prevalence for individuals with DM was (75%), followed by heart disease (45%) and foot complications (35%).

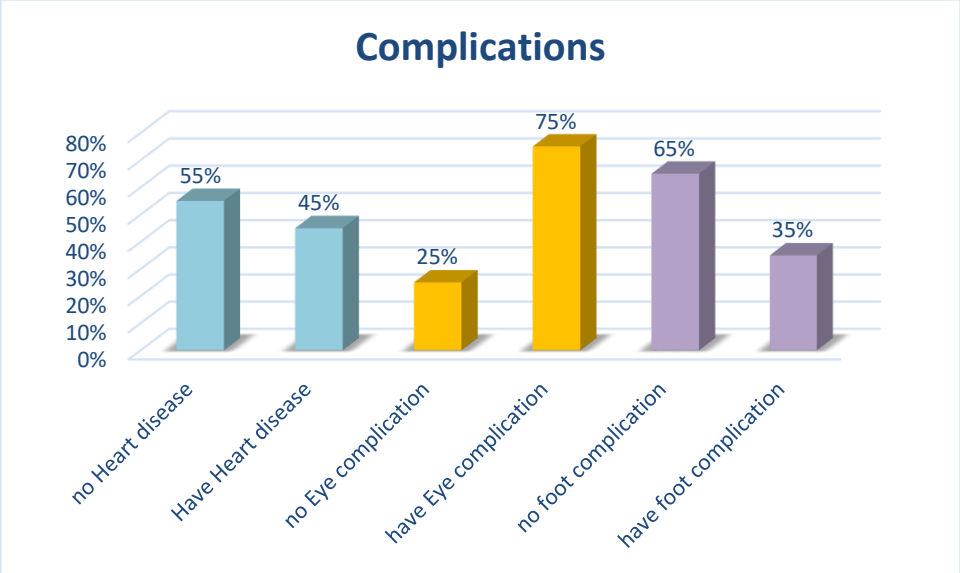

Figure (4.2): The distribution DM complications among patients

This current study was conducted to measure serum MDA, CAT, and SOD levels in type 2 diabetes patients as a reliable marker and control group. The results of antioxidants illustrated in Table (4-2) show the serum levels of some measures in the two studied groups. The activities of SOD did not exhibit any significant difference ($P > 0.05$) in patients with diabetic participants (45.91±7.51 U/ml) as compared to the control group (47.19±12.73 U/ml). In addition, there are non-significant differences in the serum CAT activity in patients with diabetic participants (31.71±10.87 KU/l) as compared to the control group (33.81±8.49 KU/l) ($P > 0.05$) observed. While MDA level was significantly ($p< 0.05$) in patients with diabetic participants (30.84±6.10 µmol/l) as compared to the control group (20.09±2.03 µmol/l).

Table 4.2.Comparison of the level of MDA, SOD, and CAT in the patient and control groups

Variables	**Patient**	**Control**	**P value**
SOD	45.91±7.51	47.19±12.73	$P > 0.05$
CAT	31.71±10.87	33.81±8.49	$P > 0.05$
MDA	30.84±6.10	20.09±2.03	*$P \leq 0.05$

*Significant

Discussion

Type II diabetes mellitus is associated with multiple metabolic derangements which can cause secondary pathophysiological changes in multiple organ systems. This in turn can impose a heavy burden of morbidity and mortality from micro- and macro-vascular complications [37].

Our study was conducted on 20 patients who were type 2 diabetics and 20 were healthy subjects as a control group.

Oxidative stress has been investigated in metabolic disorders in several studies. Evidence suggests that oxidative stress is associated with DM, both in the pre-diabetes state and clinical phase. According to various studies, oxidative stress is a crucial factor in DM2 pathogenesis as well as in the development of diabetic complications [38].

Although diabetes is associated with an increased production of ROS, the reports about the antioxidant defense in diabetes are not conclusive. Our study revealed decreased activity of CAT and SOD in patients with DM2 in comparison with the control group. These results are in agreement with some studies, high blood glucose levels cause an increase in reactive species production as a result of oxidative stress, which leads to a decrease in enzymatic antioxidant levels like SOD and CAT. Gilani, et al., observed that lower activity of non-enzymatic antioxidants SOD, and CAT. Kumawat, et al., [40] showed that decrease activity of SOD levels in serum in patients with DM2. While Bandeira, et al. [41] revealed that the total SOD activity was higher in diabetics compared to non-diabetics.

The reduction in serum SOD activity levels could be due to excessive consumption in the autoxidation procedure and increased excretion from the

inflammatory kidney in nephropathy. About 50% of SOD in erythrocytes of diabetic patients is glycated, resulting in low activity of SOD [39].

We observed that, in diabetic individuals, the levels of MDA were significantly elevated in serum when compared with control individuals as in Table 2. This

was in accordance with the studies done by Bikkad et al. [42] proposed that the increase in the MDA levels may be due to the increased activity of the free radical formation and prolonged exposure to hyperglycemia which in turn leads to increased oxidative stress. Also, agree with Al-Rawi, Kumawat et al., Mahadevan and Velavan, and Padalkar et al., [43,40,44,,45].

An increased level of MDA in diabetics suggests that peroxidative injury may be involved in the development of diabetic complications. The increase in lipid peroxidation is also an indication of a decline in the defense mechanisms of enzymatic and non-enzymatic antioxidants [46].

Conclusions and Recommendations

Conclusions

1- There is an association between diabetes and age, as it was observed that most of the patients with T2DM were in older groups.

2- Most of those diagnosed with T2DM are female, as the number of females more the number of males.

3- Increase in the MDA concentration, which means the rate of oxidative stress, is high; MDA is more commonly used as a biomarker of oxidative stress.

4- Decrease in the antioxidant concentration of CAT and SOD in patients with T2DM.

Recommendations

1- Comparison of study parameters with diabetic patients who had controlled glucose levels and those who had an uncontrolled glucose level.

2- Study variant parameters of oxidative stress for patients with DM

References

1. Muhsen, R. D., Abdullah, J. A., & Al-Kutubi, H. M. (2022). Investigation Role of Hormones and Lipid Profile in Diabetes Mellitus Type I Patients. HIV Nursing, 22(2), 2892-2899.

2. Yaribeygi, H., Farrokhi, F. R., Butler, A. E., & Sahebkar, A. (2019). Insulin resistance: Review of the underlying molecular mechanisms. Journal of cellular physiology, 234(6), 8152-8161.

3. Udler, M. S. (2019). Type 2 diabetes: multiple genes, multiple diseases. Current diabetes reports, 19, 1-9.

4. Caulfield, J. I., Aizenbud, L., Perdigoto, A. L., Meffre, E., Jilaveanu, L., Michalek, D. A., ... & Kluger, H. (2023). Germline genetic variants are associated with development of insulin-dependent diabetes in cancer patients treated with immune checkpoint inhibitors. Journal for Immunotherapy of Cancer, 11(3).

5. Perng, W., Conway, R., Mayer-Davis, E., & Dabelea, D. (2023). Youth-onset type 2 diabetes: the epidemiology of an awakening epidemic. Diabetes Care, 46(3), 490-499.

6. Mambiya, M., Shang, M., Wang, Y., Li, Q., Liu, S., Yang, L., ... & Liu, W. (2019). The play of genes and non-genetic factors on type 2 diabetes. Frontiers in public health, 7, 349.

7. Tiwari, B. K., Pandey, K. B., Abidi, A. B., & Rizvi, S. I. (2013). Markers of oxidative stress during diabetes mellitus. Journal of biomarkers, 2013.

8. Kunwar, A., & Priyadarsini, K. I. (2011). Free radicals, oxidative stress and importance of antioxidants in human health. J Med Allied Sci, 1(2), 53-60.

9. Razack, S., Hemanth Kumar, K., Nallamuthu, I., Naika, M., & Khanum, F. (2015). Antioxidant, biomolecule oxidation protective activities of Nardostachys jatamansi DC and its phytochemical analysis by RP-HPLC and GC-MS. Antioxidants, 4(1), 185-203

10. Mukhtar, Y., Galalain, A., & Yunusa, U. (2020). A modern overview on diabetes mellitus: a chronic endocrine disorder. European Journal of Biology, 5(2), 1-14.

11. Massoud, A., & Massoud, A. H. (2012). Immunologic and genetic factors in type 1 diabetes mellitus. In Autoimmune Diseases-Contributing Factors, Specific Cases of Autoimmune Diseases, and Stem Cell and Other Therapies. IntechOpen.

12. Roden, M., & Shulman, G. I. (2019). The integrative biology of type 2 diabetes. Nature, 576(7785), 51-60.

13. Schwartz, S. S., Epstein, S., Corkey, B. E., Grant, S. F., Gavin III, J. R., & Aguilar, R. B. (2016). The time is right for a new classification system for diabetes: rationale and implications of the β-cell–centric classification schema. Diabetes care, 39(2), 179-186.

14. Thomas, S. J., McDougall, C., Brown, I. D., Jaberoo, M. C., Stearns, A., Ashraf, R., ... & Kelly, I. G. (2007). Prevalence of symptoms and signs of shoulder problems in people with diabetes mellitus. Journal of shoulder and elbow surgery, 16(6), 748-751.

15. Papatheodorou, K., Banach, M., Edmonds, M., Papanas, N., & Papazoglou, D. (2015). Complications of diabetes. Journal of diabetes research, 2015.

16. Papatheodorou, K., Banach, M., Bekiari, E., Rizzo, M., & Edmonds, M. (2018). Complications of diabetes 2017. Journal of diabetes research, 2018.

17. Asif M. (2014). The prevention and control the type-2 diabetes by changing lifestyle and dietary pattern. Journal of education and health promotion, 3, 1. https://doi.org/10.4103/2277-9531.127541

18. Kunwar, A., & Priyadarsini, K. I. (2011). Free radicals, oxidative stress and importance of antioxidants in human health. J Med Allied Sci, 1(2), 53-60

19. Chelikani, P., Fita, I., & Loewen, P. C. (2004). Diversity of structures and properties among catalases. Cellular and Molecular Life Sciences CMLS, 61, 192-208.

20. Nicholls, P., Fita, I., & Loewen, P. C. (2000). Enzymology and structure of catalases.

21. Kodydková, J., Vávrová, L., Kocík, M., Žák, A., Ohsaka, Y., Nishino, H., ... & Da Silva, L. B. Human catalase, its polymorphisms, regulation and changes of its activity in different diseases.

22. Del Rio, D., Stewart, A. J., & Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition, metabolism and cardiovascular diseases, 15(4), 316-328.

23. Sharma, R. A., Gescher, A., Plastaras, J. P., Leuratti, C., Singh, R., Gallacher-Horley, B., ... & Plummer, S. M. (2001). Cyclooxygenase-2, malondialdehyde and pyrimidopurinone adducts of deoxyguanosine in human colon cells. Carcinogenesis, 22(9), 1557-1560.

24. Busch, C. J., & Binder, C. J. (2017). Malondialdehyde epitopes as mediators of sterile inflammation. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1862(4), 398-406.

25. Silva, S. B. D., Costa, J. P., Pintado, M. E., Ferreira, D. D. C., & Sarmento, B. (2010). Antioxidants in the prevention and treatment of diabetic retinopathy–a review. Journal of Diabetes & Metabolism, 1(3).

26. Younus, H. (2018). Therapeutic potentials of superoxide dismutase. International journal of health sciences, 12(3), 88.

27. Kusuyama, J., Alves-Wagner, A. B., Conlin, R. H., Makarewicz, N. S., Albertson, B. G., Prince, N. B., ... & Goodyear, L. J. (2021). Placental superoxide dismutase 3 mediates benefits of maternal exercise on offspring health. Cell metabolism, 33(5), 939-956.

28. Kusuyama, J., Alves-Wagner, A. B., Conlin, R. H., Makarewicz, N. S., Albertson, B. G., Prince, N. B., ... & Goodyear, L. J. (2021). Placental superoxide

dismutase 3 mediates benefits of maternal exercise on offspring health. Cell metabolism, 33(5), 939-956.

29. Weseler, A. R., & Bast, A. (2010). Oxidative stress and vascular function: implications for pharmacologic treatments. Current hypertension reports, 12, 154-161.

30. Penckofer, S., Schwertz, D., & Florczak, K. (2002). Oxidative stress and cardiovascular disease in type 2 diabetes: the role of antioxidants and pro-oxidants. Journal of Cardiovascular Nursing, 16(2), 68-85.

31. Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. International journal of biomedical science: IJBS, 4(2), 89.

32. Ayepola, O. R., Brooks, N. L., & Oguntibeju, O. O. (2014). Oxidative stress and diabetic complications: the role of antioxidant vitamins and flavonoids. Antioxidant-antidiabetic agents and human health, 923-931.

33. Tiwari, B. K., Pandey, K. B., Abidi, A. B., & Rizvi, S. I. (2013). Markers of oxidative stress during diabetes mellitus. Journal of biomarkers, 2013.

34. Fiorentino T. V., Prioletta A., Zuo P., Folli F. Hyperglycemia-induced oxidative stress and its role in diabetes mellitus-related cardiovascular diseases. Current Pharmaceutical Design. 2013;19(32):5695–5703.

35. Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. Clinica chimica acta, 196(2-3), 143-151.

36. Buege, J. A., & Aust, S. D. (1978). [30] Microsomal lipid peroxidation. In Methods in enzymology (Vol. 52, pp. 302-310). Academic press.

37. Gilani, S. J., Bin-Jumah, M. N., Al-Abbasi, F. A., Nadeem, M. S., Afzal, M., Sayyed, N., & Kazmi, I. (2021). Fustin ameliorates hyperglycemia in streptozotocin-induced type-2 diabetes via modulating glutathione/Superoxide dismutase/Catalase expressions, suppress lipid peroxidation and regulates histopathological changes. Saudi Journal of Biological Sciences, 28(12), 6963-6971.

38. Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., ... & Martín, C. (2020). Pathophysiology of type 2 diabetes mellitus. International journal of molecular sciences, 21(17), 6275.

39. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010; 107:1058–1070. https://doi.org/10.1161/CIRCRESAHA.110.223545 PMID: 21030723

40. Kumawat, M., Sharma, T. K., Singh, I., Singh, N., Ghalaut, V. S., Vardey, S. K., & Shankar, V. (2013). Antioxidant enzymes and lipid peroxidation in type 2

diabetes mellitus patients with and without nephropathy. North American journal of medical sciences, 5(3), 213.

41. Bandeira, S. D. M., Guedes, G. D. S., Fonseca, L. J. S. D., Pires, A. S., Gelain, D. P., Moreira, J. C. F., ... & Goulart, M. O. F. (2012). Characterization of blood oxidative stress in type 2 diabetes mellitus patients: increase in lipid peroxidation and SOD activity. Oxidative medicine and cellular longevity, 2012.

42. Bikkad MD, Somwanshi SD, Ghuge SH, Nagane NS. Oxidative stress in type II diabetes mellitus Biomed Res. 2014;25:84–7

43. Al-Rawi, N. H. (2011). Oxidative stress, antioxidant status and lipid profile in the saliva of type 2 diabetics. Diabetes and Vascular Disease Research, 8(1), 22-28.

44. Padalkar, R. K., Shinde, A. V., & Patil, S. M. (2012). Lipid profile, serum malondialdehyde, superoxide dismutase in chronic kidney diseases and type 2 diabetes mellitus. Biomedical Research, 23(2), 207-210.

45. Mahadevan, A., Moningi, S., Grimm, J., Li, X. A., Forster, K. M., Palta, M., ... & Herman, J. M. (2021). Maximizing tumor control and limiting complications with stereotactic body radiation therapy for pancreatic cancer. International Journal of Radiation Oncology* Biology* Physics, 110(1), 206-216.

46. Mahboob, M., Rahman, M. F., & Grover, P. (2005). Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. Singapore medical journal, 46.