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**Research Article**

# Evaluation of Reactive Oxygen Species, Antioxidants and Lipid Peroxidation Biomarkers in Diabetics Mellitus Type2

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## Abstract:

This study aims to evaluate the reactive oxygen species ROS, enzyme and non-enzyme antioxidants and peroxidation of lipid biomarkers in diabetic patients in diabetic center of Merjan hospital city. The current study reveals that the concentration of reactive oxygen species, Malondialdehyde MDA, catalase CAT and glutathione S-transferase activities were appeared significant differences at P≤ 0.05 that increased in diabetic disorder when compared with patient without diabetic While the activity of superoxide dismutase, concentration of vitamin C and glutathione were revealed significant differences at P ≤ 0.05 which are decreased in patients when compare with healthy volunteers .

**Keywords: Diabetic type 2, antioxidant; lipid peroxidation and Reactive oxygen species**.

## Introduction

Non-insulin dependent diabetes mellitus, also known as type 2 diabetes mellitus (NIDDM), is a metabolic syndrome with a variety of etiologies that is typified by persistent hyperglycemia brought on by abnormalities in the metabolism of fats and carbohydrates in 2030. It was believed that one person with diabetes affected every ten people (American Diabetes Association, 2012).

Many biological pathways including (glucose autoxidation, pathway of polyol, synthesis of prostanoid and glycation of protein) stimulated by hyperglycemia that leading to increase free radicals production and caused oxidative stress which is factor of pathogenic in diabetes mellitus disorder in humans, the antioxidant enzymes such as superoxide dismutase, Catalase CAT, glutathione and its enzymes as reductase of glutathione GR, peroxidation of glutathione GPx and glutathione-S-transferase, which are a play essential role in protecting human body from the action free radicals (Wei *et al*., 2010 and Jaganjac *et al*., 2013).

During the development of diabetes, hyperglycemia causes an increase in free radical production in tissues by the formation of glycation end products, leading to the stimulation of the production of mitochondrial superoxide and, finally, stimulating cellular damage so that the antioxidant defense systems play an essential role in scavenging free radicals within a controlled concentration in cells (Bansal *et al*., 2013; Ighodaro and Akinloye , 2017).

The free radical production in excessive amounts caused by hyperglycemia leads to cellular damage, alteration in signal cells, inflammation, and development of insulin resistance, influence on metabolic pathways, diabetes, and cardiovascular disorders via impaired function of secretion, insulin, and metabolism. The elevation of MDA concentration reveals adverse physiological effects via altered structure of integrity of the cell membrane, inhibiting membrane binding enzymes and receptors of the cell surface. It causes the formation of foam cells to cause atherosclerosis disease. (Khan *et al*.,2015 and Ngaski, 2018).

## Material and Methods

### Collection of blood samples

A 5ml venous blood sample was collected from each of 30 healthy people and 30 diabetic patients who had fasted for 10 hours; the diabetic patients were chosen over a period of more than ten years without smoking.

### Antioxidant measurements

The SOD activity measured depends on pyrogallol autoxidation (Marklund, 1974), while CAT activity and vitamin C concentration were determined according to procedure (Burtis, ; Ashwood, 1999). GSH concentration has been measured according to (Habig *et al.*, 1979 and the activity of GST has been determined according to the procedure performed by (Habig *et al*., 1979).

### Peroxidation of lipids

Malondialdehyde was measured by using a thiobarbituric acid assay (MDA) concentration according to (Moreno *et al*., 2005) whereas reactive oxygen species were determined by an ELISA kit.

### Statistical analysis

The data of the current study has been analyzed by using SPSS (Version 20) to find the mean, standard deviation SD, and least significant differences by ANOVA.

## Results

### 1-Reactive Oxygen Species (ROS) and Malondialdehyde MDA Markers

The result of the current study revealed, the ROS concentration appeared to have significant differences between control and patients with hyperglycemia at P ≤ 0.05. The ROS mean concentration was (0.231±0.002) P/ml in serum healthy control while in patients, its concentration increased significantly and reached (2.7120.421) P/mL (3). While Malondialdehyde concentrations varied significantly between controls and cases, the mean concentration of MDA in controls was 1.0340.001 mol/ml in serum, while it was 2.9040.24 mol/ml in cases (Figure (2)).

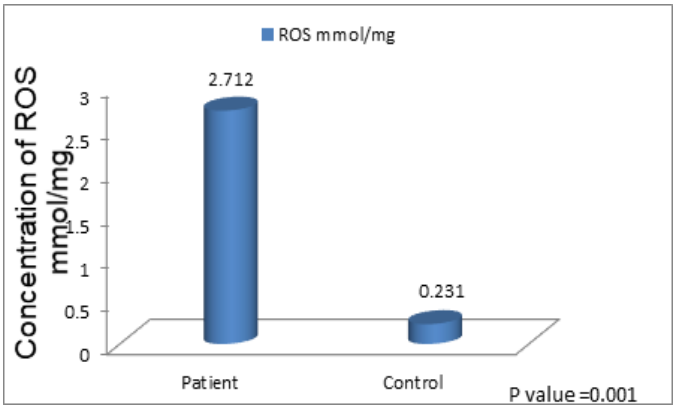


**Figure (1) Concentration of Reactive oxygen species in serum of control and patient with diabetic mellitus type 2**

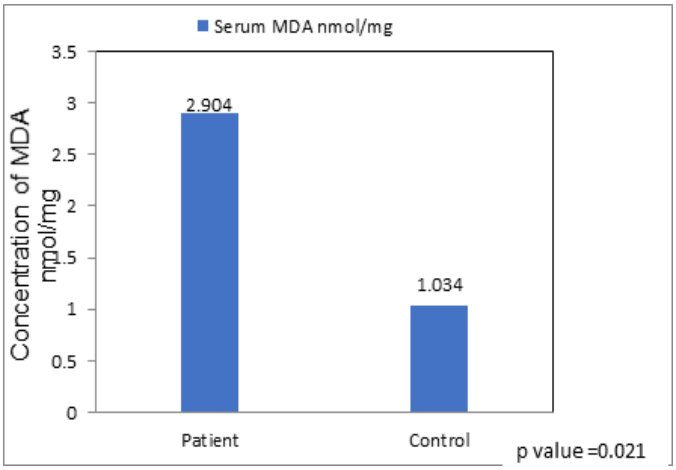


**Figure (2) Concentration of Malondialdehyde in serum of control and patient with diabetic mellitus type 2**

### 2-Antioxidant defense systems

### 2-1-Enzyme antioxidant markers

At P 0.05, all enzyme antioxidants markers showed significant differences between controls and patients. The superoxide dismutase activity in serum of healthy controls was significantly decreased and reached to 39.311±10.021 U/mg as compared with its activity in control, which reached to (52.521±12.011U/mg) Figure (3) Whereas the activity of catalase CAT in the patient was 13.1±2.43 nmol/ml and its activity in control was 8.301±1.012 nmol/ml Figure (4). The glutathione S-transferase GST activity in the patient was 6.856±1.278 µmol/ml as compared with its activity in control, which reached 2.056 ±0.56 µmol/ml Figure (5)

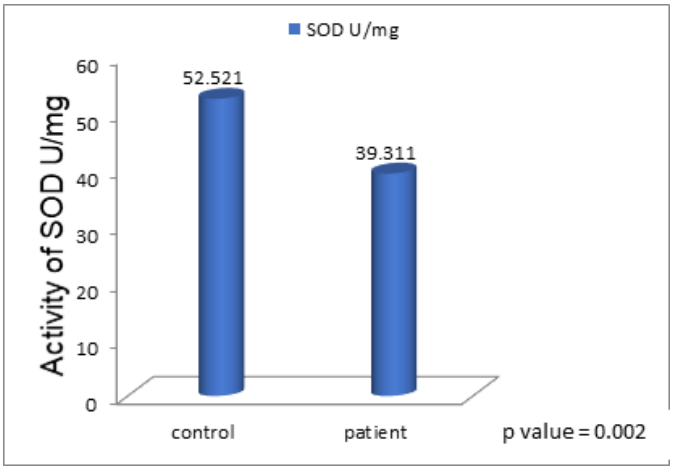


**Figure (3) Activity of Superoxide dismutase in serum of control and patient with diabetic mellitus type 2**

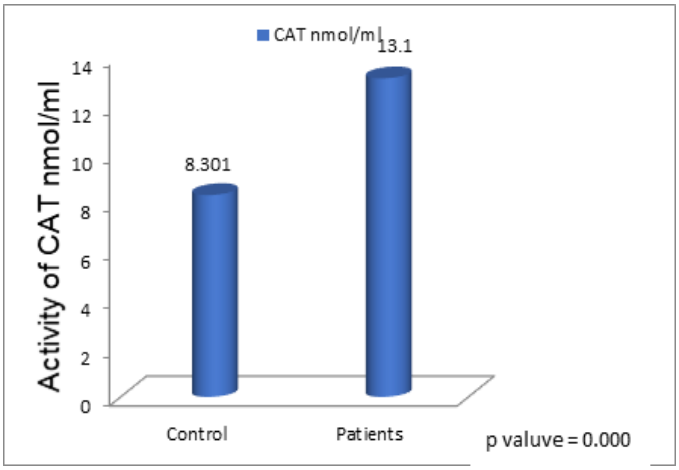


**Figure (4) Concentration of Catalase in serum of control and patient with diabetic mellitus type 2**

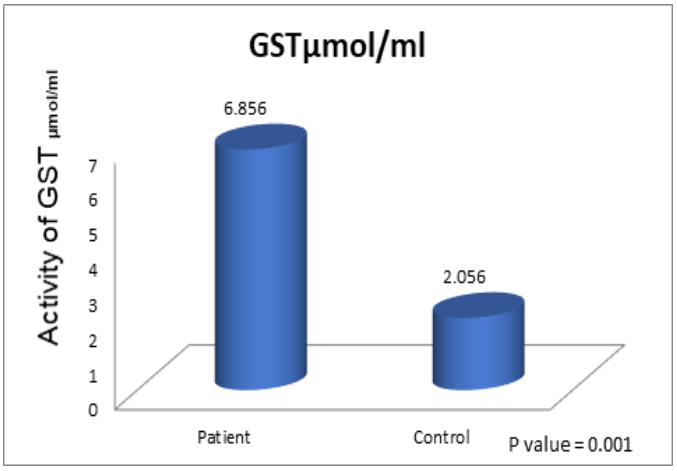


**Figure (5) Activity of glutathione S-transferase in serum of control and patient with diabetic mellitus type 2**

### 2-2-Non-enzyme antioxidants markers

The statistical analysis showed a significant decrease between glutathione and vitamin C in control and patients. The concentration of glutathione in control was 25.19±5.231 nmol/ml and its concentration in patients was 10.212±2.67

nmol/ml. Figure (6) whereas the concentration of vitamin C in patients was decreased and reached to 30.167± 7.989 µmol/ml and its concentration was reached to 60.239 ±12.54 µmol/ml figure (7).

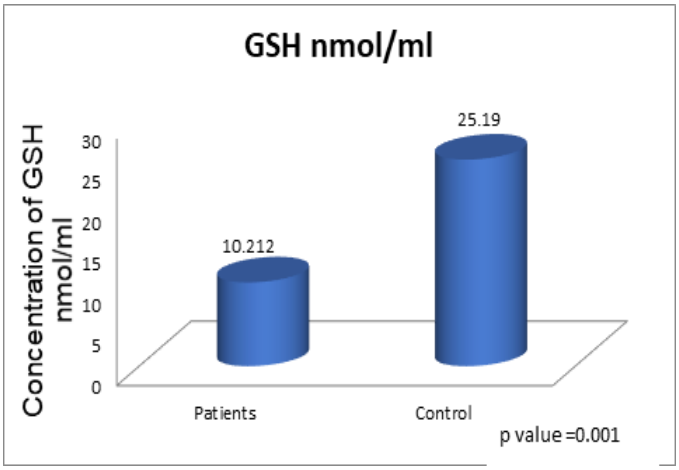


**Figure (6) Concentration of Glutathione in serum of control and patient with diabetic mellitus type 2**

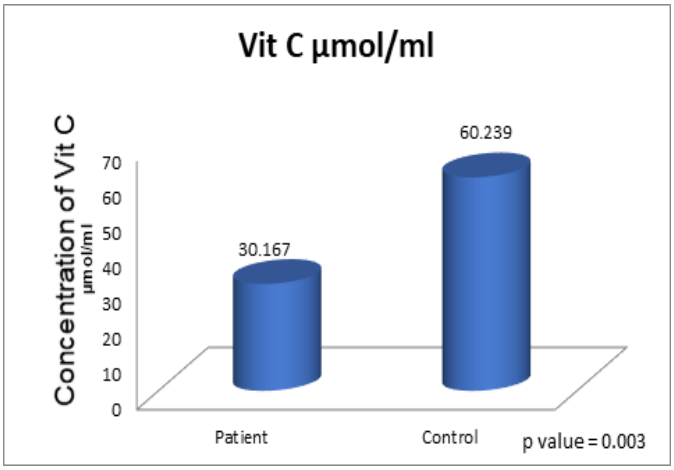


**Figure (7) Concentration of Vitamin C in serum of control and patient with diabetic mellitus type 2**

## Discussion

The ATP source in cells is the mitochondria electron transport chain. Some electrons prematurely leak to oxygen through energy transduction, leading to the formation of oxygen free radical superoxide, such as superoxide anion. This is a result of metabolic processes and can be regarded as primary ROS that generate secondary ROS through direct molecular interactions with other molecules like DNA, proteins, and lipids, as well as through enzyme or metal catalyzed processes. (Biswas *et al*., 2017**)**.

creation of radicals without bounds an atom or molecule with one or more unpaired electrons in the valence shell or outer orbit is regarded as a free radical during normal cellular metabolism. Due to their odd number of electrons, free radicals are extremely reactive, unstable, and have a brief lifespan. Due to their strong reactivity, they are able to extract electrons from other molecules. As a result, the attacked molecule becomes a free radical and loses its electron, which causes oxidative damage to a number of macromolecules, including proteins, DNA, and lipids in diabetes individuals. (Phaniendra *et al.*,2015).

The current study found that ROS concentration appeared to differ significantly between control and hyperglycemic patients, with diabetic patients having significantly higher levels of both ROS and Malondialdehyde when compared to healthy controls. Whereas the concentration of enzyme antioxidant defense systems such as catalase and glutathione S-transferase in the serum of diabetics was significantly increased as compared with its concentration in healthy controls, Whereas superoxide dismutase concentration, glutathione and vitamin C had been significantly decreased in patient with diabetic as compared with healthy control because the patient with hyperglycemia caused stress to cells lead to imbalance in production of antioxidants defense systems and generation of free radical such as reactive oxygen specie, One of the main causes of diabetes mellitus is oxidative stress, which is exacerbated by ROS generation, which also lowers enzymatic antioxidant defenses and raises chronic hyperglycemia in uncontrolled diabetes. (Asmat *et al*., 2016).

Under hyperglycemic situations, ROS production can increase dramatically, which can subsequently stimulate oxidative stress in tissue. ROS and superoxide radicals are substantial contributors to problems that arise from hyperglycemia. Oxidative stress, a major consequence of diabetes mellitus, alters the structure and function of proteins and lipids and causes glycoxidation and peroxidation in chronic hyperglycemia. It is brought on by inflammation (Pieme *et al*., 2017). (Briggs *et al.*, 2014) study antioxidants enzyme and non-enzyme markers, lipid peroxidation such as malondialdehyd and free radicals including reactive oxygen species ROS in diabetic patients, the results showed that the concentration of SOD, GSH and vitamin C significantly decreased as compared with healthy controls. When diabetic patients were compared to healthy people, the concentrations of CAT, ROS, MDA, and GST were significantly higher.

## Conclusion

Patients with diabetic patients revealed that hyperglycemia caused oxidative stress and a decreased antioxidant defense system, resulting in increased complication, so patients must take multivitamins to increase antioxidant biomarkers and reduce oxidative stress caused by hyperglycemia. .

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