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Impact Of Oxidative Stress On Level Of Biomarkers Associated To Type 1 Diabetes

Qatar-Al-Nada M. Mahdi[1] , Thaer M. M. Al-Rammahi[*1] , Ali Mejbel .M .Alkhafaji[2] and Haneen H. Shablawi[1]

[1]Department of Chemistry, College of Science, University of Kerbala, Karbala, Iraq

[2]Department of Anaesthesia, Al safwa University College, Karbala, Iraq

Abstract

Type1 diabetes mellitus (T1DM) is characterized by hyperglycaemia since insulin secretion is inhibited by pancreatic islets. Oxidative stress is a generally recognized participant in the incidence and development of T1DM and its complications. The study aims to investigate the effect of oxidative stress including H_2O_2 and NO on the biomarkers related to the diagnosis of T1DM disease. The study has involved 80 participants within age (5-25 years); 50 T1DM patients (24 boys and 26 girls) and 30 healthy volunteers (18 boys and 12 girls).The level of fasting blood glucose (FBG), glycated haemoglobin (HbA1c),lipid profile (total cholesterol, triglyceride, LDL, and HDL), liver function test (AST, ALT), renal function test (B.urea, S.creatinine), Ca^{+2},Fe^{+2},H_2O_2 and NO were determined. The data for this study were analysed and presented as mean ± standard deviation, Pearson's correlation test was applied to examine various correlations. Two-tailed P-values were used and statistical significance has been considered as $P < 0.05$. The results have shown significantly higher levels ($P>0.0001$) of H_2O_2 inpatients with type1 diabetes compared to control, which is in the patient's group was (75.31±25.61), and in the healthy group was (50.51±17.00). However, there were non-significant differences in the levels of NO between Type1 diabetic patients and the healthy group which is (36.05±20.86µmol/L),(32.18±8.83 µmol/L) respectively. Pearson's correlation test has shown no correlations between oxidant markers and biomarkers. As a result, it can be concluded that the levels of oxidative stress such as H_2O_2 and NO have not a direct impact on the level of other biomarkers related to the diagnosis of T1DM.

Keywords Type1 diabetes mellitus, Oxidative Stress, H_2O_2, NO.

Introduction

Diabetes mellitus (DM) is the most common metabolic endocrine disorder in the human, which can be characterized by hyperglycaemia since insulin secretion is inhibited by pancreatic islets, diabetes is considered a common disorder by affecting over 100 million people worldwide (1).According to the International Diabetes Federation (IDF), there are 425 million people worldwide, who had diabetes in 2017, with the number expected to rise to 629 million by 2045 (2). The inhibition of insulin secretion leads to other metabolic abnormalities, which include carbohydrates, lipids, and proteins. In addition,

diabetes has a remarkable impact on further human organisms, particularly the blood vessels, eyes, kidneys, heart, and nerves (3,4).

Type 1 diabetes mellitus is caused by autoimmune loss or absence of pancreatic islets of Langerhans (beta cells). These pancreatic islet beta cells (β-cells) secrete insulin, which is a hormone led to stimulate the cell's body to transport glucose out of the bloodstream. Decreased insulin secretion contributes to decreased protein synthesis when amino acids use insulin to move through the body. Accumulation of glucose in the bloodstream causes hyperglycaemia (5,6).

Oxidative stress (OS) is a phenomenon that takes place when oxidants and antioxidants in a biological system are out of balance (imbalance). The imbalances occur as a result of an increase of reactive oxygen species (ROS) or the antioxidant system's failure to work properly (7). free radicals and antioxidants imbalance lead to oxidative damage of protein, nucleic acid, lipid, and carbohydrates (8).

Oxidative stress is a state in which the increased production of free radicals overwhelms the available antioxidant capacity in the body, and it is associated with many disorders, such as cardiovascular, atherosclerosis, and neurological diseases as well as aging (9). Oxidative stress is also a generally recognized participant in the incidence and development of T1DM and its complications. The disruption of physiological free radical homeostasis has been linked to a compromised beta-cell function (10).

The crucial role of molecular oxygen in biology cannot be overstated, as it is essential for optimal cellular function and the survival of all organisms. Although oxygen is necessary for life and plays a role in signal transduction, gene transcription, and other cellular processes, it also harms biomolecules in the form of free radicals and reactive oxygen species (ROS). The harmful effect of oxygen results from a univalent metabolic reduction state, which is responsible for the production of ROS (11). Reactive oxygen species (ROS) production by aerobic systems is linked to the specific chemical features of $O_{2^{\cdot}}$, Superoxide ($O_{2^{\cdot-}}$) and hydrogen peroxide (H_2O_2) are the proximal ROS generated by mitochondria and can damage cell components at high levels (12). Nitric oxide (NO) is the other most significant species, which regulates vascular smooth muscle cell relaxation and proliferation, leukocyte adhesion, angiogenesis, platelet aggregation, thrombosis, vascular tone, and hemodynamic, among other status (11).

Untreated diabetes can lead to significant complications; however, early detection of diabetes can help to avoid serious complications. High blood glucose levels over an extended length of time, frequent urination, increased thirst, and polyphagia (constant hunger) are all common diabetes symptoms (13).

This study has focused on understanding the relationship between the level of oxidative stress (free radicals) and other biomarkers related to T1DM. In addition, our finding has suggested the formation mechanism of free radicals to investigate if it's the outcome from high blood sugar levels (mean after T1DM) or if they cause and develop diabetes type1.

Research Design and Methods

Design of Study

A case-control study was conducted between March 2019 to October 2020, and it was carried out at the postgraduate laboratory, department of chemistry, college of science, University of Kerbala, and cooperative with Imam Al-Hassan (As) Diabetes and Endocrinology Specialized Center, Imam Hussain Medical City, Karbala, Iraq.The study has involved 80 participants within age (5-25 years); 50 T1DM patients (24 boys and 26 girls) and 30 healthy volunteers (18 boys and 12 girls).All participants and their parents were informed and verbal consent to participate in the study was taken.

Collection of Blood Sample

In this study, 80 blood samples were collected from children who have type 1 diabetes (n=50) and from healthy volunteers (n=30). The venous blood samples were collected from the time between 9 a.m. to 12 p.m. from fasting diabetic patients and healthy volunteers, five ml of blood was obtained from each participant, 2 ml was placed intoethy lenediamine tetra acetic acid(EDTA) tubes and the remaining (3 ml) pushed slowly into a disposable gel containing tubes SSGT(serum separator gel tube) to obtain plasma and serum after centrifugation at 4000 g for 10 minutes. The aliquots of plasma and serum were stored at -20 C^0 until being used in further tests.

Biomarkers and Oxidative Parameters Test

The following tests: fasting blood glucose (FBG), glycated haemoglobin(HBA1c), lipid profile (Total cholesterol, triglyceride, LDL, and HDL), liver function test (AST, ALT), Renal function test(Blood urea, S.creatinine), S.Ca and iron were examined by using a spectro photometric method using Cobas biochemical analyser (Roche, Bern, Switzerland), the procedure follows manufacturer's recommendations at a laboratory of biochemistry.

In addition, the H_2O_2 and NO levels were determined using the enzyme-linked immunosorbent assay (ELISA) kits.

Statistical Study

The data for this study were analysed and presented as mean ± standard deviation, then analysed using the Graph Pad Prism-8 provided by the University of California San Diego. Mean differences between subjects with T1DM and subjects healthy volunteers' groups with normal distributions were analysed with Student's t-test for independent samples. Pearson's correlation test was applied to examine various correlations. Two-tailed P-values were used and statistical significance has been considered as $P < 0.05$.

Results and Discussion

Oxidative Parameters

Hydrogen Peroxide (H_2O_2)

The results have shown significantly higher levels ($P>0.0001$) of serum H_2O_2 in a patient with type1 diabetes compared to control, which is in the patient's group is (75.31±25.61), and in the healthy group is (50.51±17.00), as shown in Fig.1, these results were agreement with a prior study that showed that

there were significantly higher levels in H_2O_2 concentration in T1DM than in controls. The higher values of H_2O_2 observed in patients with T1DM were probably due to the metabolic disturbances (14).

Probably that the high levels of H_2O_2 concentration belong to the high levels of blood glucose, it has been proven that hyperglycaemic conditions induce the overproduction of reactive oxygen species (ROS), which comprises several chemically reactive molecules derived from oxygen, such as H_2O_2 (15).

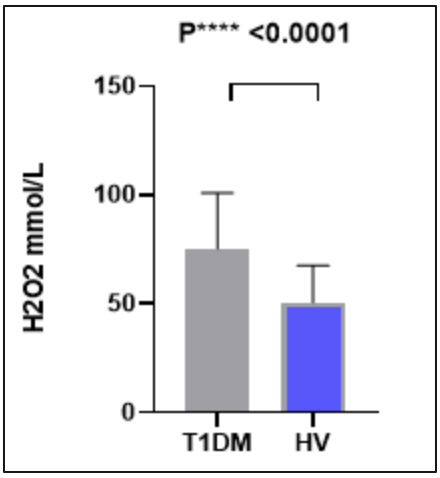

Figure 1. The levels of H_2O_2 in both T1DM patients and healthy groups.

Nitric Oxide (NO)

The results of the current study showed statically no significant differences (p = 0.3) between mean averages in studied groups, as notified Fig. 2. In which, there were non-significant differences in the levels of NO between T1DM patients and the healthy group which is (36.05±20.86µmol/L), (32.18±8.83 µmol/L) respectively.

A recent study has estimated nitric oxide (NO) serum level in type1 diabetes patients, observed a lower significant difference concentration of serum NO in patients compared to control (16). Other studies also founding there was a reduced in serum NO level in both types of diabetes (T1DM, T2DM) compared to control (17, 18), while other research found the opposite effect (19, 20) Our present study is agreement with previous studies showing that hyperglycaemia may stimulate nitric oxide production (19, 21, 22). Glucose autoxidation is a major source of free radicals in chronic hyperglycaemia which is a chemical transition metal-catalysed process that produces deleterious free radical and ketoaldehyde compounds during hyperglycaemia, generating hydrogen peroxide, and other biomarkers of oxidative stress (23).

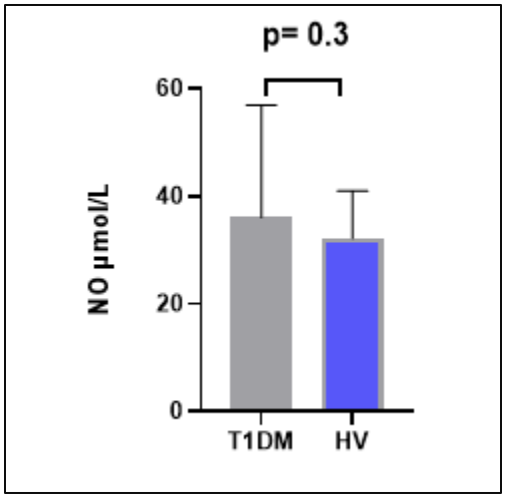

Figure 2. The levels of NO in both T1DM patients and healthy groups.

The Correlation Between Oxidative Parameters and Biomarkers

The current study showed that there was a negative significant correlation between oxidative parameters (H_2O_2, NO) and other biomarkers (FBS, HbA1c, TG, TC, LDL, HDL, ALT, AST, urea, creatinine, Ca^{+2}, Fe^{+2}), as shown in Figures (3) to (14).

A recent study has performed on 150 cases of Type2 diabetes to find whether there was a correlation between serum nitric oxide and serum lipid profile, recorded that there was a poor correlation between NO and lipid profile(24).

A further study had assessed the levels of serum NO among DM patients and its correlation with lipid profile in north Indian. This study illustrated that there was a poor correlation (25). Various studies had discovered that the subjects with diabetes mellitus have lower nitric oxide production, which was linked to confounding factors such as age, body mass index, and lipid profile. According to researchers, subjects with diabetes have an unfavourable lipid profile and changed plasma levels of oxidative stress biomarkers such as nitric oxide, and the nitric oxide levels were lower than in control participants (26, 27).

A previous study discussed the relationship between NO and the level serum of urea and creatinine in patients with chronic renal disease, the study showed that there was a positive significant correlation between NO and serum urea and creatinine, where the correlation between serum nitric oxide and serum creatinine was more significant as compared to that between serum nitric oxide and serum urea nitrogen. This impact was most probably caused by a shared influence on their removal via the renal tract. As a result, changes in serum nitric oxide will be accompanied by changes in renal function as shown by variations in creatinine concentration, there was a quadratic association between serum nitric oxide and serum creatinine, indicating that after a specific level of creatinine (8 mg/dl), serum nitric oxide increased sharply (28). This may be attributed to the decreased renal function and the insufficient purification of the blood (28).

Generally, there is no previous study that showed there was a correlation between free iron and H_2O_2, but several researchers reported that iron-catalysed oxidations could occur in some diabetic subjects (29). Other researchers reported that oxidative stress, perhaps initiated by a transition metal like (Cu, Fe) may contribute to the pathogenesis of DM and its complications (30).

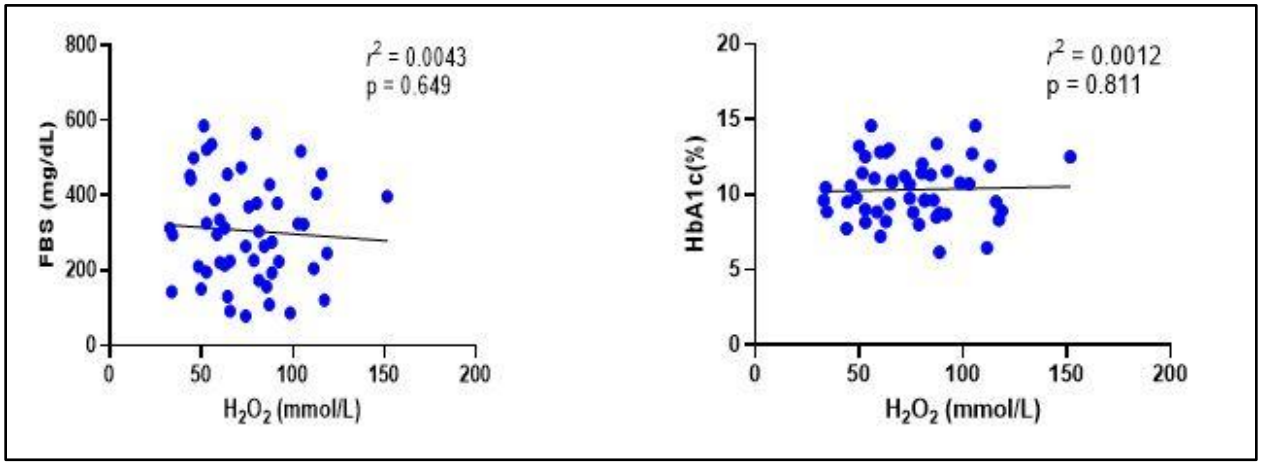

Figure 3. Correlation of H_2O_2 with FBS and HbA1c in T1DM.

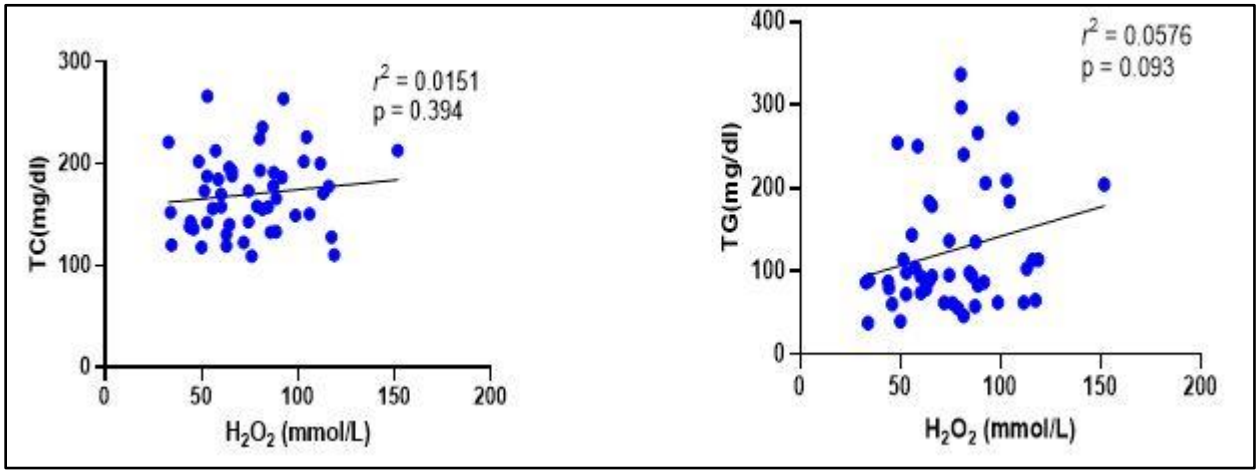

Figure 4. Correlation of H_2O_2 with TG and TC in T1DM.

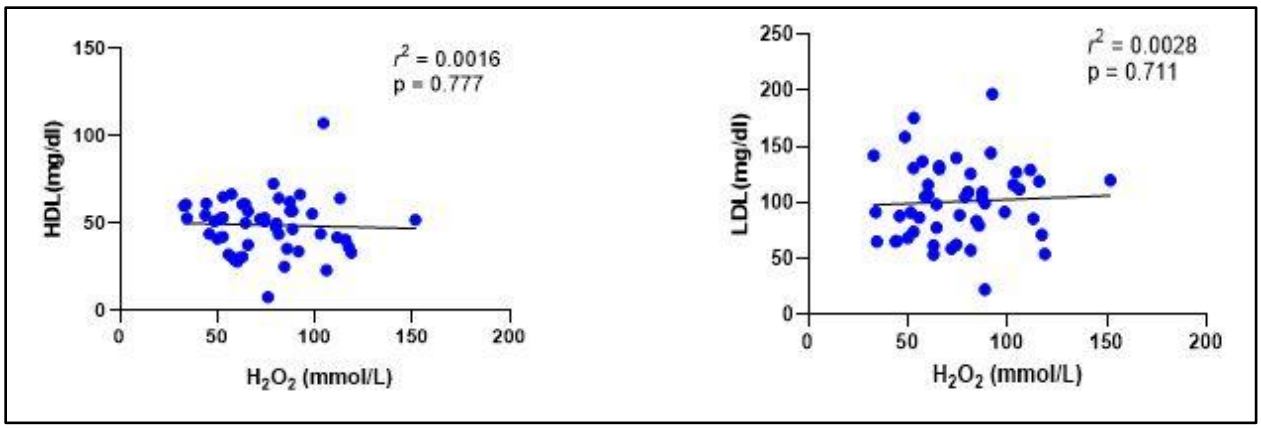

Figure 5. Correlation of H_2O_2 with HDL and LDL in T1DM.

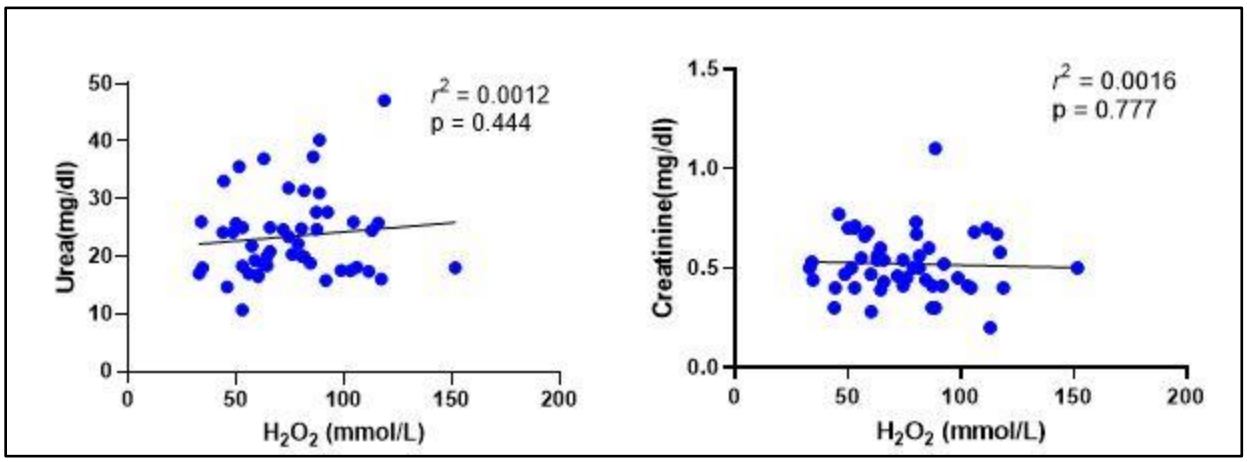

Figure 6. Correlation of H_2O_2 with Urea and Creatinine in T1DM.

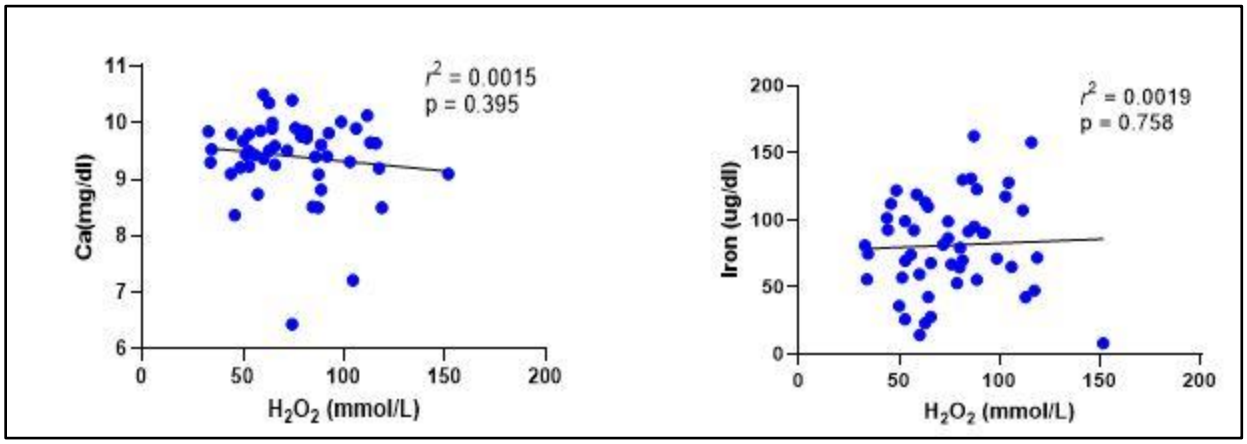

Figure 7. Correlation of H_2O_2 with Ca and Iron in T1DM.

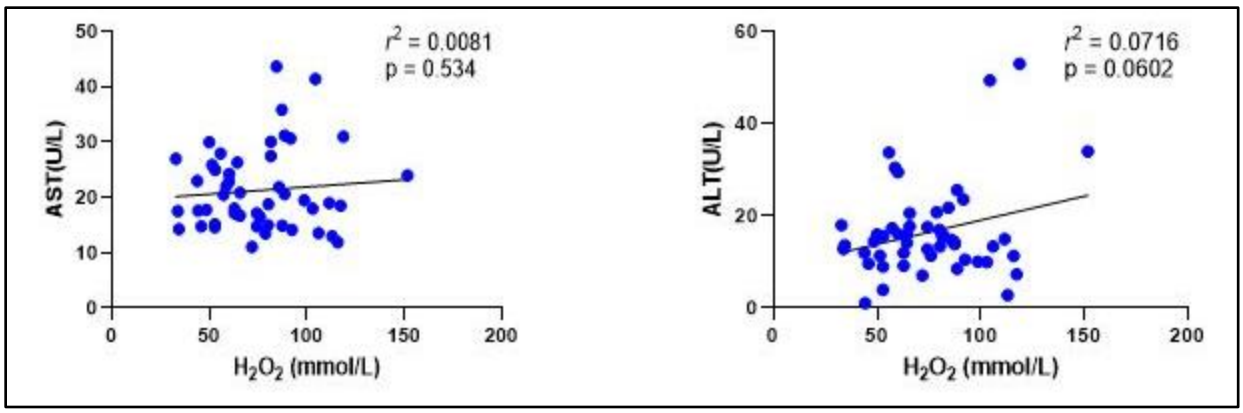

Figure 8. Correlation of H_2O_2 with AST and ALT in T1DM.

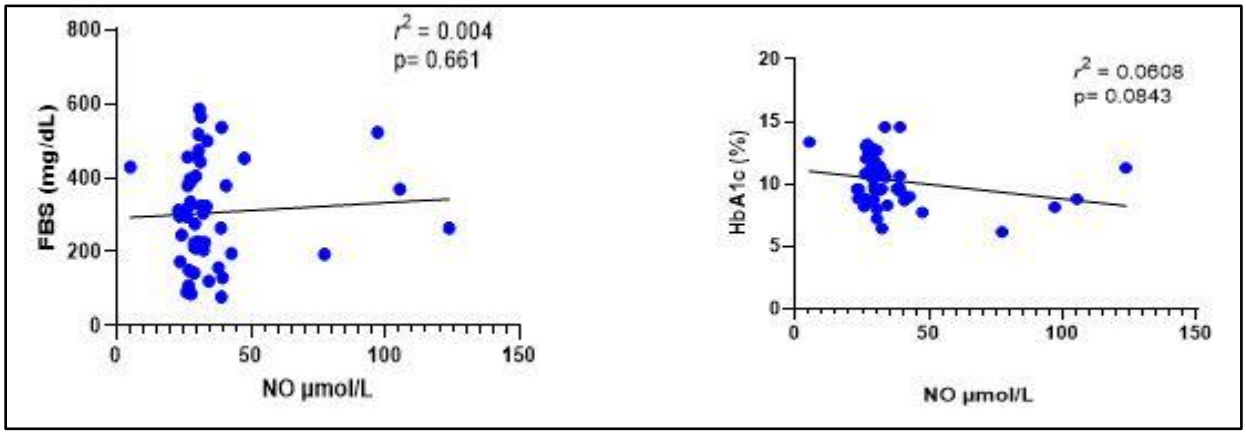

Figure 9. Correlation of NO with FBS and HbA1c in T1DM.

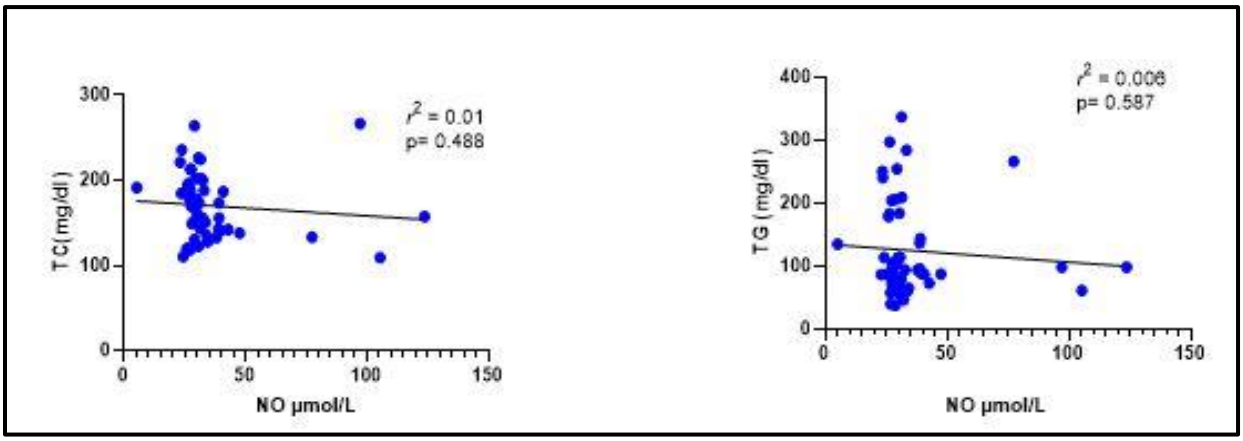

Figure 10. Correlation of NO with TG and TC in T1DM.

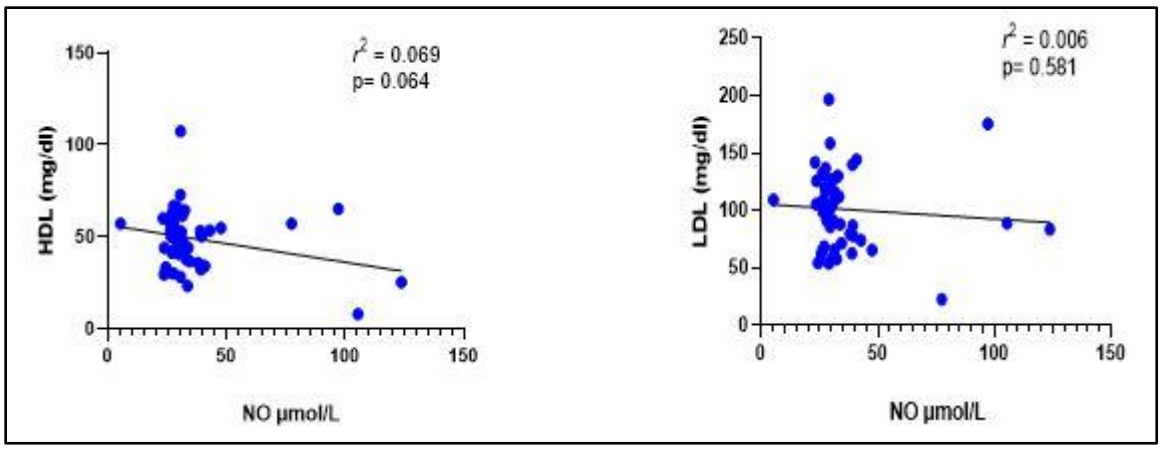

Figure 11. Correlation of NO with HDL and LDL in T1DM.

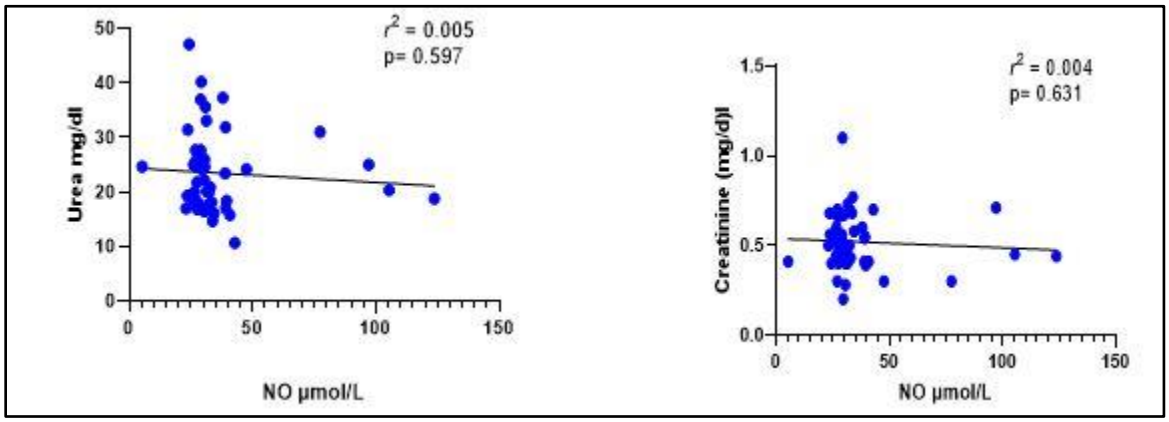

Figure 12. Correlation of NO with Urea and Creatinine in T1DM.

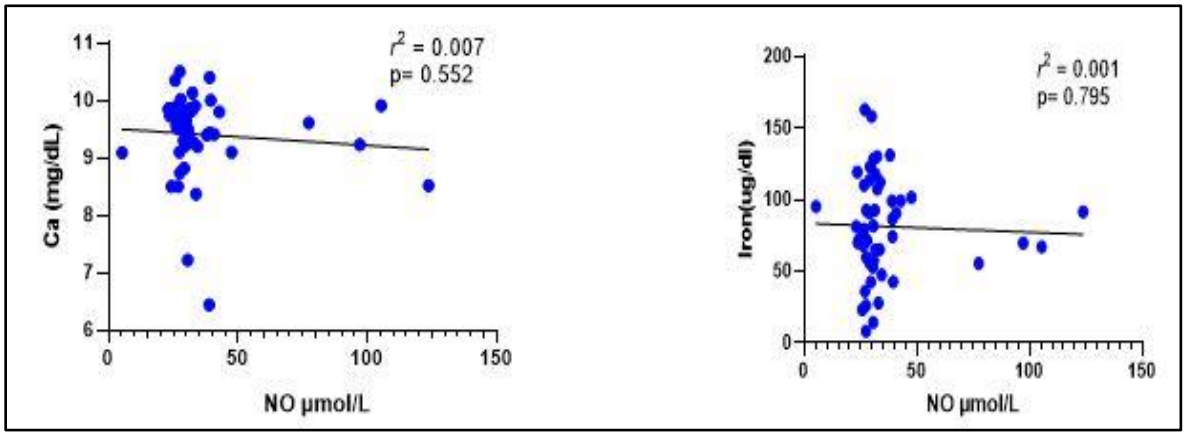

Figure 13. Correlation of NO with Ca and Iron in T1DM.

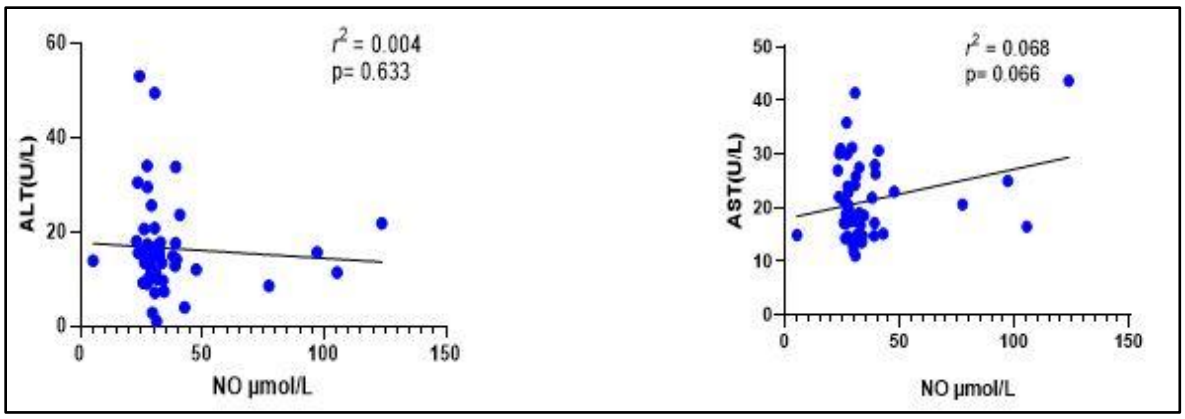

Figure 14. Correlation of NO with ALT and AST in T1DM.

Conclusions

Type1 diabetes mellitus is an autoimmune disease that targets young categories. Acute and chronic T1DM leads to dangerous complications. The current study has applied to patients with T1DM; Age(years) (mean ± SD) (11.44 ±2.351), and (20.48± 3.530), in holy Karbala city. The results of this study have shown that T1DM has clearly impact on the metabolism of other dietary components such as lipids and proteins.

The distinguish in the levels of oxidative parameters between T1DM and HV show unique results. The statistical analysis of H_2O_2 data exhibit a highly significant difference between T1DM and HV (P>0.0001), which is similar to the previous study (14). However, there is no significant difference for NO data between T1DM and HV (p = 0.3), and that is corresponding with a recent study (15).

The associations of oxidative parameters (H_2O_2 and NO) with other biomarkers (FBG, HbA1c, total cholesterol, triglyceride, LDL, HDL, ALT, AST, Urea, Creatinine, Calcium, and Iron) in T1DM display weak correlations with no significant differences. Consequently, the levels of oxidative stress such as H_2O_2 and NO have not a direct impact on the level of other biomarkers related to the diagnosis of T1DM.

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Conflict of Interest

The authors declare no conflict of interest.

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