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# Assessment of the status of advanced glycation end products, oxidative stress, and other biomarker parameters in the sera of diabetic patients in Erbil city

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## Abstract

**Background and objective**: Diabetes mellitus (DM) poses a significant threat to human health, especially in developed countries, and is very important for helping us understand advanced glycation end products (AGEs) that contribute to oxidative stress (OS). The purpose of the investigation is to assess the concentration of the serum level of AGE, OS, antioxidants, and lipid profile parameters in diabetic patient. **Methods**: This case-control study comprised 90 participants, including 60 subjects with type 2 diabetes mellitus and 30 healthy subjects, encompassing both genders. Demographic information and medication histories were gathered for each participant. The levels of advanced glycation end products (AGE), oxidative stress (OS) markers, antioxidants, and the parameters of lipid profile were evaluated using Randox Imola and enzyme-linked immunosorbent assay (ELISA) methods.

**Results**: The current investigation demonstrated that serum levels of pentosidine (PEN), peroxynitrite (ONOO•-), and xanthine oxidase (XOD) were significantly elevated, whereas serum levels of glutathione reductase (GR) and vitamin E were significantly reduced in individuals with type 2 diabetes mellitus compared to the healthy group. **Conclusion**: Elevated levels of AGEs and oxidative stress are correlated with diabetes mellitus patients. Glutathione reductase and peroxynitrite may serve as effective indicators for diabetes mellitus.

**Keywords**: Advanced Glycation End Products (AGEs), Antioxidants, Diabetes Mellitus, Lipid Profile, Oxidative Stress (OS).

## Introduction

Diabetes mellitus (DM) is a principal threat to human health and constitutes the foremost challenge to health systems in the twenty-first century (1). The International Diabetes Federation (IDF) estimates in its 2019 report (IDF Diabetes Atlas) that 463 million individuals aged 20–79 globally have diabetes, with an expected rise to 700 million by 2045 (2). Around 90% of all diabetes cases are classified as type II diabetes. Its etiopathogenesis is affected by environmental as well as

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hereditary variables. Type 1diabetes constitutes 5 to 10% of cases and is determined by the autoimmune-mediated destruction of beta cells in the pancreas (3).

Diabetes mellitus is a metabolic condition marked by hyperglycemia, which occurs from the insufficiency of the body to secrete and response to insulin (4). Over time, it can result in major consequences, such as macrovascular (cardiovascular disease) and microvascular (diabetic retinopathy, nephropathy, and neuropathy) issues, which are the main causes of mortality and morbidity among diabetics (5). Moreover, heart failure is also a common early manifestation of cardiovascular illness in subjects with type two diabetes mellitus (T2DM). It confers a substantial risk of mortality in patients with T1DM or T2DM (6). Longer diabetes duration and poor glucose control raise the chances of this consequence (7). One of the primary reasons for the established pathophysiology of diabetes complications is hyperglycemia. It plays a key role by producing advanced glycation end products (AGEs) (8).

Advanced glycation end product (AGEs) are various substances created by the Maillard reaction, which is a non enzymatic interaction between lipids, proteins, reducing sugars, or nucleic acids (9). AGEs are categorized as both endogenous and exogenous (10). Exogenous AGEs are introduced through dietary sources or environmental factors like cigarette smoke (11). Endogenous AGEs, formed within the body (12). The accumulation of AGEs linked various chronic diseases, including diabetes, cardiovascular, renal, and neurodegenerative disorders, and obesity (13).

An imbalance between the body's capacity to neutralize free radicals and their production leads to oxidative stress (14), which is an essential component in the progress of diabetes and its consequences (15). Free radicals include reactive oxygen species (ROS) and reactive nitrogen species (RNS) that may destroy DNA, lipids, and proteins in cells, resulting in chronic illness (16). There are several different types of free radicals, including. Reactive oxygen species (ROS), including hydroxyl (•OH), hydrogen peroxide ($H_2O_2$), superoxide ($O_2$•–), and hypochlorous acid (HOCl). Furthermore, peroxynitrite (ONOO•-) and nitric oxide (NO) are examples of reactive nitrogen species (RNSs) (17).

Antioxidants are chemical compounds with diverse applications and functions. They are regarded as advantageous to human health due to their presence in meals including fruits, vegetables, tea, and red wine (18). To reduce harmful oxidative processes and the negative effects of reactive oxygen species (ROS) in food, using nutritional antioxidant molecules can prevent lipid peroxidation and the formation of harmful byproducts, which helps keep

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the food's flavor, color, and texture intact (19). Antioxidants are classified into enzymatic and non-enzymatic groups. The enzymatic group comprises enzymes which comprising catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPse), and glutathione reductase (G-reductase) (20). Non-enzymatic antioxidants include beta-carotene, uric acid, vitamin E, C, and A. Hyperglycemia is a direct and potentially fatal consequence of untreated diabetes (21). Over time, DM patients experience elevated lipid profiles, which result in plaque formation and accumulation of cholesterol in the arteries (21, 22).

The main purpose of the investigation is for assessment the level of AGEs, OS, and antioxidants in the sera of type II diabetes patients and identify the relationships between AGEss and oxidative stress, antioxidants, and lipid profiles. The parameters involved in the estimation include pentosidine, peroxynitrite, xanthine oxidase, glutathione reductase, and vitamin E. The parameters also include total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, and very high-density lipoprotein.

## 2. Materials and Methods

### Design of the study

The case-control study was designed to investigate advanced glycation end products and additional biochemical marker, such as oxidative stress, antioxidants, and lipid profiles, in diabetes mellitus patients and the healthy group.

### Study population and sample collection

For this study, blood samples of 90 subjects with type 2 diabetes mellitus at the Galiawa Teaching Center for Diabetes and Endocrinology in Erbil were collected. The study comprised of 60 individuals with type 2 diabetes (29 males and 31 females) who had (HbA1C ≥ 7) diabetes mellitus as the patient group. Although blood samples of 30 individuals (13 males and 17 females) as a healthy group were obtained who had no diabetes. The patients and healthy groups ranged in age from 34 to 72. The process of collecting blood samples started in October 2024 and was completed in December 2024. A venous blood sample of approximately 7 ml was obtained from both patients and healthy individuals. The blood samples were maintained at room temperature to facilitate clotting; thereafter, they were centrifuged at 2500 rpm for 10 minutes to isolate the serum, which was then stored at -40°C until analysis.

### Questionnaire and data collection

An in person interview was used to fill in a questionnaire that was designated for matching the study's needs. The interviews were conducted face-to-face with a questionnaire that included personal information, health and medical history (age, weight, height, gender, and family history of DM,

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medications, and other diseases in relation to diabetes mellitus).

**Biochemical analysis**

All biochemical parameters, AGEs, antioxidants, and oxidative stress were determined by using (SUNLONGBIOTECH) ELISA kits. The serum lipid profile determination was performed using RandoxImola.

**Statistical Analysis:**

We used GraphPad Prism (version 10) to perform statistical analyses. Categorical variables were represented as percentages, and comparisons were assessed using the chi-square test. Continuous data were expressed as means with standard errors for normally distributed datasets, or this analysis used medians along with ranges to represent non-normally distributed data. The Student's t-test was used for comparisons of normally distributed continuous data, whereas the Mann–Whitney U test was performed for nonparametric continuous variables. The Pearson test was employed for correlation analysis. The power of serum AGEs to diagnose type II diabetes was evaluated using receiver operating characteristic (ROC) curves in MedCalc statistical software. An area under the curve (AUC) greater than 0.70 indicates diagnostic efficacy, with statistical significance determined at P <0.05.

**Results**

The study involved 90 subjects from both groups. The Case group comprised individuals diagnosed with type two diabetes mellitus, while the control group consisted of healthy individuals. The case group consisted of 60 participants, it composed of 29(48.33%) males and 31 (51.67%) females, the (Mean ± SE) of age was (56.27± 1.24) years, and control group consisted of 30 participants, it composed of 13(43.33%) males and 17 (56.67%) females, the (Mean ± SE) of age was (50.93± 2.03) years. The (Mean ± SE) of HbA1c was 8.567±0.21 in diabetic patients, while in control group was 5.279±0.05. The current study showed significant difference between case and control, P-value <0.00. The (Mean ± SE) BMI was (27.30± 0.44) Kg/$m^2$ and (27.33± 0.60) Kg/$m^2$ in diabetic and healthy group respectively. The majority of diabetic patients had a familial history of type 2 diabetes mellitus, which was about 45(75.00%) and about 14 (46.67%) of control group had family history of T2DM. The main characteristics of the investigation's participant appear in Table 1.

**Determination of advanced glycation end product in DM patients and control group**

The results of this study show that the serum mean levels of pentosidine in DM patients was 66.47± 9.798, while in control group was 61.44± 3.736 ng/ml.

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The P-value is 0.0428. This result exhibited that the mean level of pentosidine is significantly higher in DM Patients compared to non-diabetic group, as shown in Table 2 and Figure 1.

**Determination of Oxidative stress parameters in sera of DM patients and healthy groups**

The findings of the present investigation demonstrated that the serum mean levels of peroxynitrite and XOD in DM patient are 15.20± 3.588 ng/ml and 880.5± 148.6pg/ml, respectively, whereas they are 13.04± 0.7199 ng/ml and 828.8± 31.45 pg/ml, respectively, in control group. The P-values are 0.0014 and 0.0310. These results exhibit that the mean level of peroxynitrite and XOD are significantly higher than those of healthy group. As shown in Table 2 and Figure 1.

**Determination of antioxidant parameters in sera of DM patients and control groups**

The results of this study show that the serum mean levels of GR and Vitamin E in DM patient are 442.0± 68.15 and 5.676± 0.18, respectively, whereas they are 504.7± 73.21 and 6.703± 0.3171pg/ml, respectively, in control group. The P-values are 0.0002 and 0.0121. These results exhibit that the mean level of GR and Vitamin E are significantly lower than those of DM patients, as present in Table 2 and Figure 1.

**Table 1.** The main characteristics of the study participants

| Variables | Case group Mean ± SE | Control group Mean ± SE | P-value |
|---|---|---|---|
| **Age (in years)** | 56.27± 1.24 | 50.93± 2.03 | <0.0001* |
| **BMI(Kg/m)²** | 27.30± 0.44 | 27.33± 0.60 | 0.9136* |
| **Gender** | | | |
| Male | 29(48.33%) | 13(43.33%) | 0.6540** |
| Female | 31(51.67%) | 17(56.67%) | |
| **Family History** | 45(75.00%) | 14(46.67%) | 0.0077** |
| **HbA1c (%)** | 8.567± 0.21 | 5.279± 0.05 | <0.0001* |

* By student's t-test. BMI: body mass index

** By Chi-square test for qualitative samples.

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**Table 2.** Comparison of biochemical parameters in sera of Type 2 patients and control group

| Biochemical Parameters | Case group Mean ± SE | Control group Mean ± SE | P-value |
|---|---|---|---|
| Pentosidine (ng/ml) | 66.47± 9.798 | 61.44± 3.736 | 0.0428 |
| Peroxynitrite (ng/ml) | 15.20± 3.588 | 13.04± 0.7199 | 0.0014 |
| XOD (Pg/ml) | 880.5± 148.6 | 828.8± 31.45 | 0.0310 |
| GR (Pg/ml) | 442.0± 68.15 | 504.7± 73.21 | 0.0002 |
| Vitamin E (Pg/ml) | 5.676± 0.1771 | 6.703± 0.3171 | 0.0121 |

Data were analyzed by t-test for two independent samples express and mean and standard error

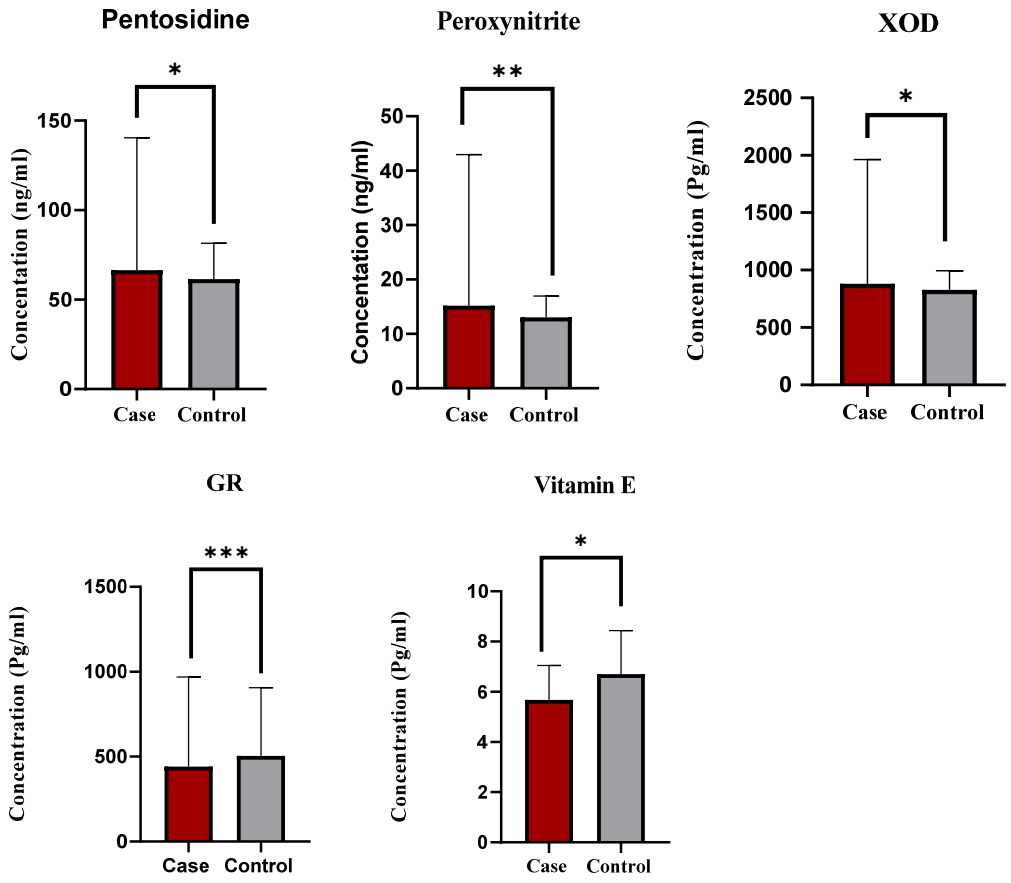


**Figure 1.** The serum levels of biochemical parameters petosidine, peroxynitrite, XOD, GR, and vitamin E in type 2 diabetes patients and controls

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**Determination of Lipid profile parameters in sera of DM patients and control groups**

The results of this study show that the serum mean levels of TC, TG, LDL, VLDL and mean level of HDL in DM patient are 202.4±6.901,198.4± 20.06,122.5± 5.771,38.62± 3.966 and 37.20± 0.7677, whereas they are 149.3± 5.246,101.2± 7.244,93.92± 4.658,18.79± 1.485 and 42.32± 1.938mg/dl in control group, as shown in Table 3 and Figure 2. The P-value are <0.0001, <0.0001, 0.0010, <0.0001, 0.0479. These results show that the mean level of TC, TG, LDL and VLDL in DM patients is significantly higher as compared to control group, and the mean level of HDL in DM patients is significantly lower compared to control group.

**Table 3.** Serum level Lipid profile between type 2 diabetes patients and control group

| Variables | Case group | Control group | |
|---|---|---|---|
| | Mean± SE | Mean± SE | P-value |
| TC (mg/dl) | 202.4±6.906 | 149.3±5.246 | <0.0001 |
| TG (mg/dl) | 198.4±20.06 | 101.2±7.244 | <0.0001 |
| HDL-C (mg/dl) | 37.20±0.7677 | 42.32±1.938 | 0.0479 |
| LDL-C (mg/dl) | 122.5±5.771 | 93.92±4.658 | 0.0010 |
| VLDL-C (mg/dl) | 38.62±3.966 | 18.79±1.485 | <0.0001 |

Data were analyzed by t-test for two independent samples express a mean and standard error

TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; and HDL-C: high-density lipoprotein cholesterol

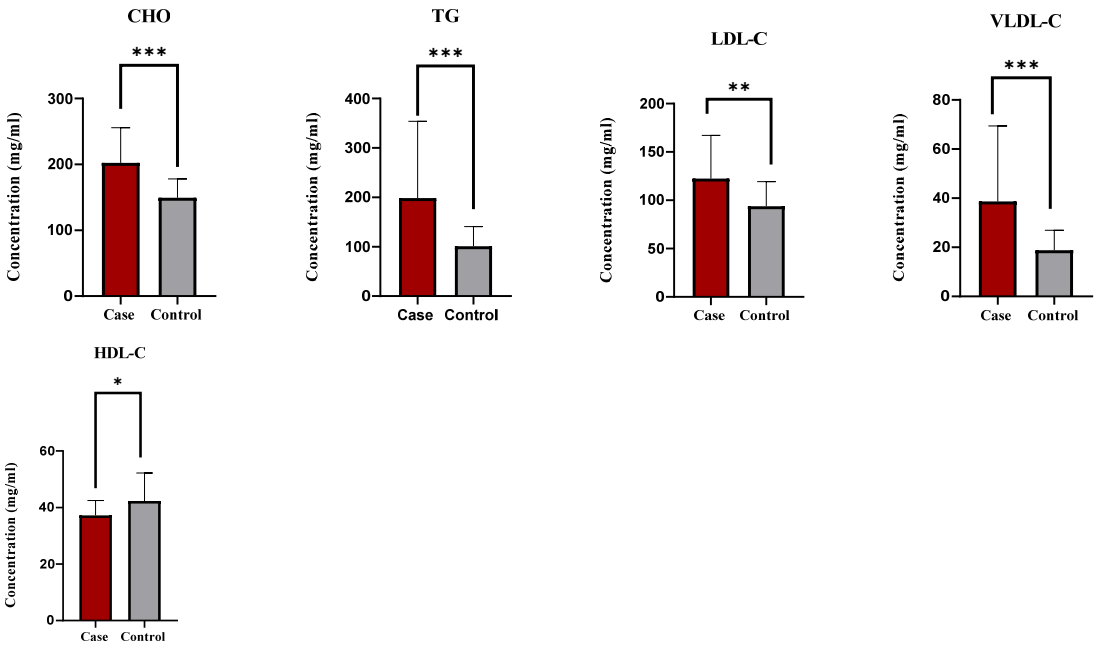


**Figure 2**. The serum levels of Cholesterol, TG, HDL, LDL, and VLDL in DM patients and control group

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**Correlation between serum AGEs (Pentosidine) with oxidative stress in Type two diabetes.**

Correlation analysis of pentosidine with xanthine oxidase and peroxynitrite in type two diabetic patients showed that there are positive and significant correlations of pentosidine with xanthine oxidase and peroxynitrite r=0.7797, r=0.6915) P-values are (≤ 0.05), as show in Table 4 and Figure 3.

**Table 4.** Correlation analysis between serum AGE (Pentosidine) with OS (Oxidative stress) parameters in Type two diabetes

| Oxidative stress | Pearson Correlation | Pentosidine (ng/ml) |
|---|---|---|
| Peroxynitrite (ng/ml) | R | 0.7797 |
| | P-value | <0.0001 |
| Xanthine oxidase (Pg/ml) | R | 0.6915 |
| | P-value | <0.0001 |

AGE: Advance glycation and product.

OS: Oxidative stress

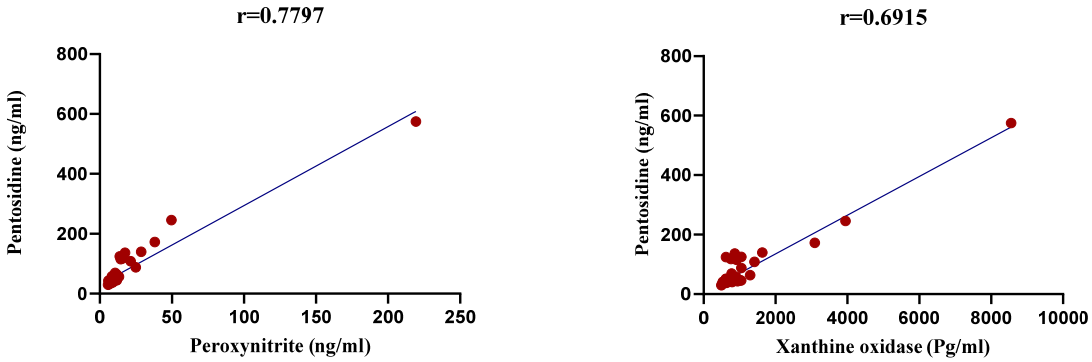


**Figure 3.** Correlation between serum AGE (Pentosidine) with OS (Oxidative stress) in Type II diabetes patients

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**Correlation between serum AGEs (Pentosidine) with Antioxidants in Type II diabetes**

Correlation analysis of pentosidine with Glutathione reductase and vitamin E in type two diabetic patients showed that there are positive and significant correlation of pentosidine with Glutathione reductase and vitamin E (r=0.6513 and r=0.3028), P-values are (≤0.05), as showed in Table 5 and Figure 4.

**Table 5.** Correlation analysis between AGE (Pentosidine) with Antioxidants in Type 2 diabetes

| Antioxidants | Pearson Correlation | Pentosidine (ng/ml) |
|---|---|---|
| **Glutathione reductase (Pg/ml)** | R | 0.6513 |
| | P-value | <0.0001 |
| **Vitamin E (Pg/ml)** | R | 0.3028 |
| | P-value | 0.0187 |

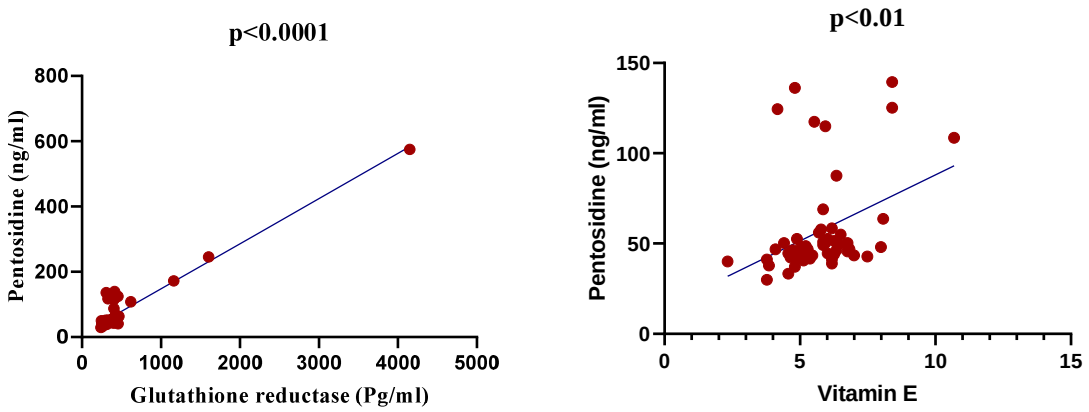


**Figure 4.** Correlation analysis between AGES (Pentosidine) with Antioxidants in Type 2 diabetes

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## Discussion

Diabetes mellitus plays a significant role in the formation of advanced glycation end products induced by oxidative stress. The current study showed that the serum level of pentosidine was significantly higher in type II diabetic patients when compared with the healthy group, as shown in Table 2 and Figure 1. This result is consistent with some previous studies, which reported the serum level of pentosidine is significantly higher in DM patients as compared with the control group (23-25).

Oxidative stress happens when the body makes more reactive oxygen species (ROS) than it can handle with its antioxidants, causing harm to cells. This imbalance contributes to beta cell disability, insulin resistance, lipid peroxidation, and chronic inflammation, exacerbating diabetes progression (26). The current study demonstrated that the serum level of oxidative stress was significantly higher in type II diabetic patients when compared with the control group, as shown in Table 2 and Figure 1. These results were similar to a previous study, which reported the serum levels of xanthine oxidase and peroxynitrite were significantly higher in DM patients as compared with the control group (27).

Natural compounds called antioxidants can prevent or postpone specific forms of cell damage. Additionally, they are essential for antioxidant defense and could be indicators of oxidative stress (28). These results illustrate that serum levels of vitamin E and glutathione reductase are significantly lower in type two diabetes mellitus when compared with the healthy group, as presented in Table 2 and Figure 1. These results are similar to some previous report investigations, which reported that the serum levels of vitamin E and glutathione reductase are lower in type II DM as compared to the health subjects (29, 30).

Dyslipidemia is a significant contributing factor to chronic non-infectious illnesses (31, 32). The current study indicated that the serum levels of total cholesterol, triglycerides, LDL, and VLDL were significantly higher and HDL significantly lower in diabetic patients as compared to the control group, as presented in Table 3 and Figure 2. These results are consistent with some previous report investigations, which reported that the serum levels of total cholesterol, triglycerides, LDL, and VLDL are higher and HDL lower in type II diabetes mellitus as compared to the healthy group (33-35).

The correlation analysis was performed to find the relationship between pentosidine with Xanthine oxidase and peroxynitrite. The results of this study show there are positive and significant correlations between pentosidine, xanthine oxidase and peroxynitrite. The Correlation coefficient (r) for xanthine oxidase is (0.6915) and peroxynitrite

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(0.7797). And there is a positive and significant correlation between pentosidine, glutathione reductase and vitamin E. The Correlation coefficient (r) for glutathione reductase is (0.6513) and for vitamin E (0.3028) as presented in Table 4 and Table 5.

## Conclusions

According to the result of the current investigation the serum level of pentosidine, xanthine oxidase and peroynitrite were significantly higher in type II diabetes mellitus compared with health group. The serum level of GR and vitamin E were significantly lowered in patients as compared to control group. Furthermore, the finding of this investigation demonstrated that the serum level of cholesterol, triglycerides, LDL and VLDL were significantly elevated in DM patients compared with control group, while serum level of HDL significantly lowered in type II diabetes compared with control group. There is positive significant correlation between pentosidine and XOD, peroxynitrite, GR and vitamin E.

## Competing interest

The authors declare that they have no competing interests.

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