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Impact of Glycated Biomarkers, Antioxidant Parameters and TCF7L2 Gene Polymorphism in Type 2 Diabetes Mellitus Patients

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ABSTRACT
Type 2 diabetes mellitus (T2DM) is characterized by dysregulation of carbohydrate, lipid and protein metabolism, and results from impaired insulin secretion, insulin resistance or a combination of both. In this study, 60 sample male and female were collected from patient with T2DM and new case. The statistical analysis showed that the patients with T2DM, glycemic indices considerable increases, except HOMA-β and QUICKI demonstrated significant decreases, and elevated serum levels of these glycemic parameters elucidate their crucial role in the pathophysiology of T2DM. As far as we know this is the first study in Kurdistan region that investigate the effect of T2DM on aflatoxin m1 level, this parameter exhibited a non-significant increase in patients with Type 2 Diabetes Mellitus and is not regarded as a biomarker for the condition. The serum level of oxidative stress and GPX elevated but other antioxidant lowered in the T2DM group relative to the control group. TCF7L2 gene polymorphism was detected in genotype frequencies in Erbil T2DM patients. The heterozygous type of SNP rs7903146 (C > T) was the most frequent genotype. Gene polymorphisms of TCF7L2 (C > T) was considered as a risk factor for T2DM in Kurdish population.
Keywords: *Type 2 DM, Adipsin, Aflatoxin M1, TCF7L2 gene polymorphism*

INTRODUCTION

Diabetes mellitus (DM), commonly referred to as diabetes, is a dangerous and chronic disease marked by consistently elevated blood glucose levels brought on by either insufficient insulin production or the body's inability to use the insulin that is generated. Diabetes is one of the leading causes of death and morbidity worldwide, affecting people of all ages, genders, and geographical locations (Hossain et al. 2024). In 2021, an estimated 537 million individuals had diabetes, with projections indicating an increase to 643 million by 2030 and 783 million by 2045. Furthermore, it is anticipated that 541 million individuals had impaired glucose tolerance in 2021. It is projected that more than 6.7 million individuals aged 20–79 will succumb to diabetes-related complications in 2021 (Sun et al. 2022). In Iraq, DM resulted in 7,279 fatalities, accounting for 4.24% of total mortality (Hussein and Ali 2023). Type 2 Diabetes Mellitus is one of the most widespread metabolic disorders globally (Tahir et al. 2024).

The predominant forms of diabetes mellitus are type 1 and type 2, each possessing established diagnostic criteria. In type 2 diabetes mellitus, there is insufficient insulin synthesis or the insulin generated fails to effectively facilitate glucose entry into the cells, so depriving them of energy. This disease is termed "insulin resistance" as insulin is present but fails to facilitate glucose entry into the cells, resulting in elevated blood glucose levels. This is the primary condition that manifests in the initial phases of type 2 diabetes (Farmaki et al. 2020). Type 2 diabetes constitutes a global public health challenge, representing approximately 90–95% of diabetes mellitus cases (Ahmad et al. 2022).

Hyperglycemia facilitates the gradual increase of glycosylated haemoglobin (HbA1c) (Waheed et al. 2024). Previous study have sought to ascertain whether oxidative stress serves as a causative factor in Type 2 Diabetes Mellitus (T2DM) resulting from hyperglycemia, or if it is a consequence that exacerbates pancreatic dysfunction and insulin receptor impairment, thereby contributing to the onset of T2DM (Li et al. 2023). The impact of carnosine on cognitive impairment generated by type 2 diabetes mellitus and the associated mechanisms remain little elucidated (Wang et al. 2024). Serum concentrations of adipsin are diminished in individuals with type 2 diabetes mellitus and β-cell dysfunction (Lo et al. 2014). Moreover, adipsin enhances glucose absorption and promotes triglyceride synthesis in adipocytes (Litvinova et al. 2014).

The association between aflatoxin M1 (AFM1) exposure and risk factors for Type 2 Diabetes Mellitus (T2DM) indicates that AFM1 exposure induces inflammatory responses and oxidative stress, which may impair insulin secretion and carbohydrate metabolism, ultimately contributing to the development of T2DM and related metabolic disorders (Akash et al. 2021).

Genome-wide association studies (GWAS) have found 143 loci associated with susceptibility to type 2 diabetes mellitus (T2DM). The gene most strongly associated with susceptibility to type 2 diabetes is the Transcription Factor 7-Like 2 gene (TCF7L2), situated on chromosome 10q25.3, with rs7903146 being one of the most common single nucleotide polymorphisms (SNPs) within this gene. (Mustafa and Younus 2021).

Despite the increase prevalence of type 2 diabetes mellitus in Erbil province there is lack of comprehensive local data examining the disease in relation to hematological and biochemical profiles, particularly in patients with genetic variations such as TCF7L2 polymorphism. Must existing studies focus on general metabolic indicators without integrating genetic factor that may influence disease progression and clinical outcomes. There for this study aims to, evaluation of glycemic parameters, determination of antioxidant, estimation of aflatoxin m1 in type 2 diabetes mellitus patient and control group and Detection of Single Nucleotide Gene Polymorphism of TCF7L2 (rs7903146) Using PCR Protocol.

MATERIALS AND METHODS

Research design and study group

This research is founded on a case-control study. The participants in this study were recruited from Shaqlawa Teaching Hospital and Galiawa Centre in Erbil Province between September 22, 2024, and February 25, 2025. The interview and structured questionnaire were developed for the data gathering process (Appendix)*. The study comprised 60 adults, both male and female, diagnosed with Type 2 Diabetes Mellitus (T2DM), aged between 20 and 50 years. The participants were divided into two groups: 30 patients (13 male and 17 female) receiving diabetes medication and 30 patients (13 male and 17 female) who were newly diagnosed and untreated. A control group of 30 healthy individuals (10 male and 20 female), with no history, signs, or symptoms of T2DM, was matched in age to the patient groups.

Determination of glycemic parameter

The concentrations of HbA1c, glucose, and insulin in serum were analyzed using Cobas E411 (Roche, Switzerland) immunoassay analyzers. HOMA-IR, HOMA-β, and QUICKI were computed using the subsequent equation (Winarto et al. 2022):

$$\mathrm{HOMA-IR} = \text{fasting insulin level } \left(\frac{\mu\mathrm{IU}}{\mathrm{ml}}\right) \times \frac{\text{fasting glucose } \left(\frac{\mathrm{mg}}{\mathrm{dl}}\right)}{405}$$

$$\mathrm{HOMA-}\beta = 360 \times \frac{\text{fasting insulin level } \left(\frac{\mu\mathrm{IU}}{\mathrm{ml}}\right)}{\text{fasting plasma glucose } \left(\frac{\mathrm{mg}}{\mathrm{dl}}\right) - 63} \%$$

$$\mathrm{QUICKI} = \frac{1}{\left[\log \text{fasting insulin level } \left(\frac{\mu\mathrm{IU}}{\mathrm{ml}}\right)\right] + \left[\log \text{fasting plasma glucose } \left(\frac{\mathrm{mg}}{\mathrm{dl}}\right)\right]}$$

Determination of toxicological parameter Aflatoxin M1

This kit employs a direct competitive ELISA methodology use a microplate coated with AFT-M1 linked antigen.

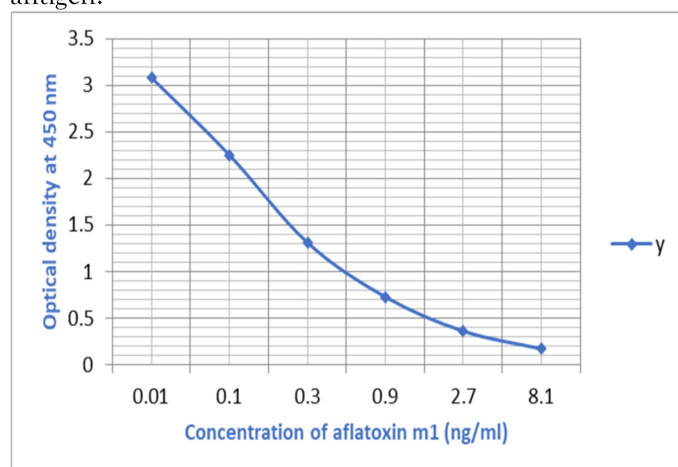


Fig.3.1: standard curve of AFT-M1

Determination of oxidative stress and antioxidants

Serum from patients and controls was evaluated for the concentrations of MDA, adipsin, carnosine, GPX, and catalase using commercially available ELISA kits.

Determination of serum malondialdehyde.
This ELISA kit was used for determination of malondialdehyde.

Table 3.1. Represent the standard dilution preparation for MDA

360 ng/ml	Standard No.1	300µl Original Standard + 150µl Standard Dilution
240 ng/ml	Standard No.2	300µl Original Standard No.1 + 150µl Standard Dilution
120 ng/ml	Standard No.3	150µl Original Standard No.2+ 150µl Standard Dilution
60 ng/ml	Standard No.4	150µl Original Standard No.3+ 150µl Standard Dilution
30 ng/ml	Standard No.5	150µl Original Standard No.4+ 150µl Standard Dilution

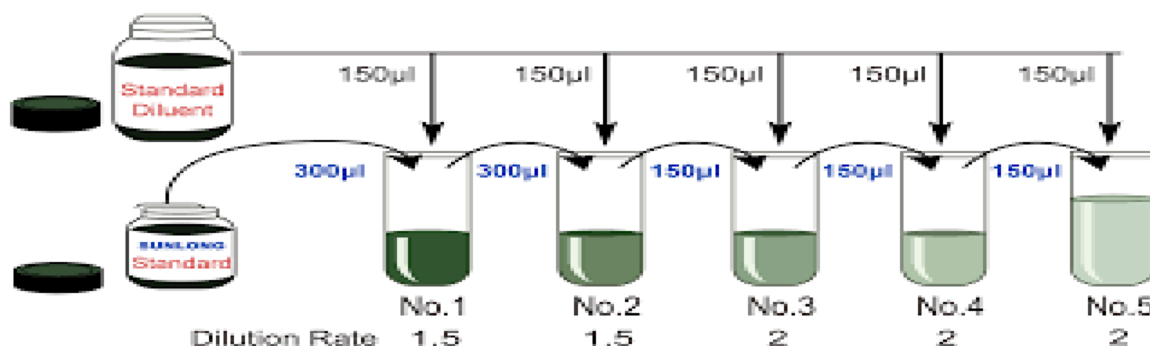


Fig.3.2: A detailed standard solution preparation for MDA

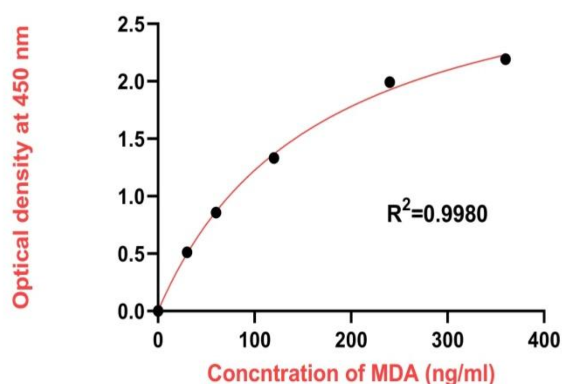


Fig.3.3: Standard curve of MDA

Determination of serum Adipsin

This kit principle of working is the same as the MDA principle of working as mentioned earlier.

Table 3.6. Represent the standard dilution preparation for adipsin.

Standard concentration	Standard No.1	Standard No.2	Standard No.3	Standard No.4	Standard No.5
ADP (2700 pg/ml)	1800 pg/ml	1200 pg/ml	600 pg/ml	300 pg/ml	150 pg/ml

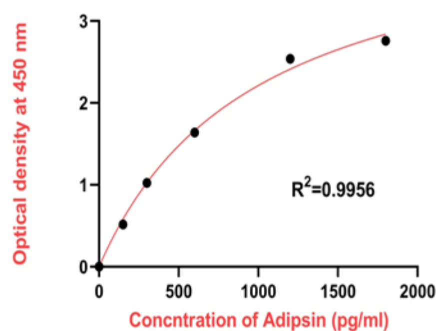


Fig.3.4: Standard curve of adipsin

Determination of serum Carnosine

This kit principle of working is the same as the MDA principle of working as mentioned earlier.

Table 3.7. Represent the standard dilution preparation for carnosine.

Standard concentration	Standard No.1	Standard No.2	Standard No.3	Standard No.4	Standard No.5
CAR (135 pg/ml)	90 pg/ml	60 pg/ml	30 pg/ml	15 pg/ml	7.7 pg/ml

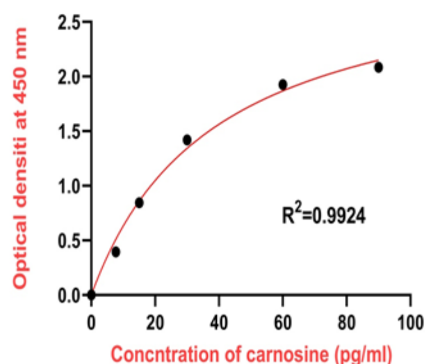


Fig.3.5: Standard curve of CAR

Determination of serum glutathione peroxidase

This kit principle of working is the same as the MDA principle of working as mentioned earlier.

Table 3.8. Represent the standard dilution preparation for GPX.

Standard concentration	Standard No.1	Standard No.2	Standard No.3	Standard No.4	Standard No.5
GSH-PX (27 ng/ml)	18 ng/ml	12 ng/ml	6 ng/ml	3 ng/ml	1.5 ng/ml

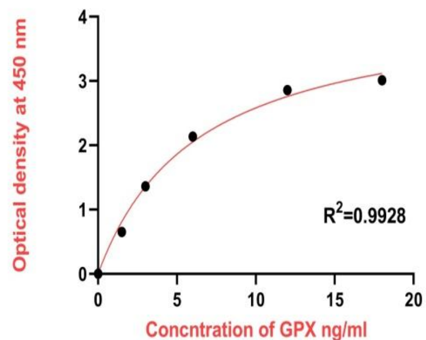


Fig.3.6: Standard curve of GPX

Determination of serum Catalase

This kit principle of working is the same as the MDA principle of working as mentioned earlier.

Table 3.9. Represent the standard dilution preparation for catalase.

Standard concentration	Standard No.1	Standard No.2	Standard No.3	Standard No.4	Standard No.5
CAT (5.4ng/ml)	3.6 ng/ml	2.4 ng/ml	1.2 ng/ml	0.6 ng/ml	0.3 ng/ml

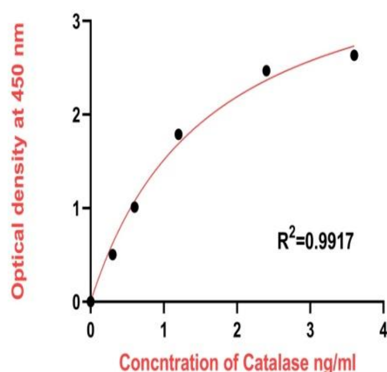


Fig.3.7: Standard curve of catalase

Primers Design

Primers used in this study were as follow: Forward inner primer (FI): 5´- TAG AGA GCT AAG CAC TTT TTA GAT AC -3´ (26mer) Tm (melting temperature) 61.6 C, Reverse inner primer (RI): 5´-CTC ATA CGG CAA TTA AAT TAT ATA A-3´ (25mer) Tm 56.0 C, Forward outer primer (FO): 5´-AAT TTT TTC ACA TGT GAA GAC ATA C-3´ (25mer) Tm 57.6 C, and Reverse outer primer (RO): 5´-TTT ATA GCG AAG AGA TGA AAT GTA G-3´ (25mer) Tm 59.2 C. The product sizes were 211 bp for C allele (C allele replicated by FI and RO primers, F1 primer was specific for the SNP), 272 bp for the T allele (T allele replicated by RI primer where the SNP was included, and FO primer), and 432 bp for the non- allele specific bands (DNA samples amplifying with FO and RO primers).

Genomic DNA extraction and PCR amplification

Genomic DNA was isolated from a peripheral blood sample. The Genomic DNA Extraction kit (Addbio Prep, South Korea) was utilized for this aim. This kit is intended for the fast extraction of genomic DNA from a maximum of 200 µL of whole blood samples. The amplification refractory mutation system, also known as allele specific PCR, is an uncomplicated technique for identifying known mutations involving single base alterations, utilized sequence-specific PCR primers that facilitate DNA amplification solely in the presence of the target allele within the sample. The Amplification Refractory Mutation System (ARMS) approach requires solely PCR amplification and gel electrophoresis of the amplicons. The specific areas of TCF7L2 were amplified. A reaction mixture with a total volume of 20 µl was created, comprising 3 µl of DNA template, 10 µl of Add Start Taq Master, 1 µl of each primer, and 4 µl of double 3830tilized3830 water (ddH2O) in the Applied Biosystems thermocycler. The cycling profile for TCF7L2 included an initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of 30 seconds at 95 °C, 35 seconds at 60 °C, and 55 seconds at 72 °C, concluding with a final extension at 72 °C for 5 minutes.

Gel electrophoresis

Agarose gel (2.5%) was employed to verify the DNA post-extraction; the preparation and casting of the gel and DNA were conducted as follows. To produce 1X TBE (Tris-Borate EDTA) buffer, 10 mL of 10X TBE was pipetted into a flask containing 90 mL of distilled water. 2.5 g of agarose powder was measured and incorporated into 100 mL of 1X TBE buffer. The solution was dissolved in a microwave and heated to boiling for 2 minutes until the agarose was entirely homogenous. Subsequently, 10 µL of Red Safe dye was incorporated and stirred gently. Subsequently, it was permitted to cool to 30 °C. The comb was subsequently secured in the gel tray, and the solution was gradually introduced to the tray to avert the production of air bubbles, assuring complete sealing of the tray's bottom. Air bubbles were extracted with a pipette, and thereafter, the gel tray was allowed to solidify at room temperature for 30 minutes. Upon detaching the gel tray and the comb, the solidified gel was initially placed in the electrophoresis tank and subsequently submerged with 1X TBE buffer (about 3-5 mm above the gel surface). 10 µl of the PCR product (DNA) was introduced into each well. Electrophoresis was conducted at 75 Volts/cm² for 45 minutes, after which the gel was visualised using a UV transilluminator.

Statistical Analysis

The data analysis was conducted using Graph Pad Prism version 9 and MedCalc version 18 software. Chi-square statistics were employed to examine the demographic characteristics. One-Way ANOVA was employed to calculate Mean±SE, with a p value of ≤0.05 deemed significant. The predictive significance of the study was assessed through Receiver Operating Characteristic (ROC) Curve analysis, with results presented as Area Under Curve (AUC) values. The WINPEPI software (version 11.65) was utilized for

the analysis of genotyping data. Genotypes of Transcription Factor 7 The TCF7L2 C/T gene was presented as percentage frequencies, and the relative risk (RR) etiological fraction was calculated to demonstrate the association between genotype and disease. The Hardy-Weinberg Equilibrium (HWE) was calculated with the H-W calculator for two alleles.

Ethical Approval

The participants were fully informed regarding the objectives of the study and ensured them was used only for particular academic purposes, and their participation in the research in voluntary, also we ensured ethics remained a top priority throughout the study (SU2025HREC/10).

RESULTS

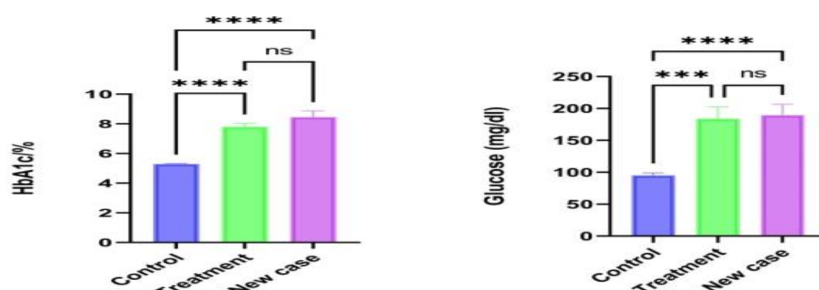
Glycemic parameters

From the total of 90 Kurdish respondents age range between 20-50 years categorized into 3 groups: treated group n=30(1male/17female) which used diabetic medications untreated group n=30(13male/17female) newly diagnosed and control group n=30(10male/20female). The current study showed that the fasting serum level of HbA1c in treated 7.80±0.23 and untreated group 8.45±0.41 was significantly higher than that of control group 5.30±0.03 and increased non-significantly in untreated group compared to treated group. Similarly the serum level of glucose increased significantly which was 189.5±17.12 in treated, 184±18.41 in untreated and in control group was 95.24±3.73.

Serum insulin level in treated group was 27.42±3.90 significantly higher than that of control group 15.69±0.91, but in untreated group increased non-significantly compared to control group, while slightly decreased non-significantly in untreated group when compared to treated group. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) increased significantly in treated 11.50±1.97 and untreated group 11.88±2.16 compared to control group 4.09±0.41, and slightly increased non-significantly in untreated group compared to treated group. Homeostasis Model Assessment of Beta cell (HOMA-β) decreased significantly from 99.11±20.84 in untreated group to 98.46±13.79 in treated group when compared to control group 200.8±15.97. Quantitative Insulin sensitivity Check Index (QUICKI) was significantly lower in both treated 0.28±0.04 and untreated group 0.28±0.04 compared to control group 0.32±0.03 but no deferent between treated and untreated group. According to AUC value HbA1c and glucose can be considered as a potential biomarkers for progression of T2DM, insulin was considered as a satisfactory biomarker for T2DM patients, HOMA-IR, HOMA-B and QUICKI were classified as an important diagnostic values for detection of T2DM disease the AUC value of HbA1c was 0.99, glucose was 0.88, insulin was 0.65, HOMA-IR was 0.79, HOMA-B was 84, QUICKI was 86, and p value= 0.001 for all parameters except insulin p value= 0.006, respectively table 1, figure 4.1, 4.2.

Table 1: Glycemic parameters in studied group.

Variables	Control	Treated	Untreated	p. value
	Mean±SE			
HbA1c %	5.30±0.03	7.80±0.23	8.45±0.41	0.0001
Glucose mg/dl	95.24±3.73	184.0±18.41	189.5±17.12	0.0001
Insulin mg/dl	15.69±0.91	27.42±3.90	26.70±4.11	0.0231
HOMA-IR ug/ml	4.09±0.41	11.50±1.97	11.88±2.16	0.0022
HOMA-β %	200.8±15.97	98.46±13.79	99.11±20.84	0.0001
QUICKI	0.32±0.03	0.28±0.04	0.28±0.04	0.0001



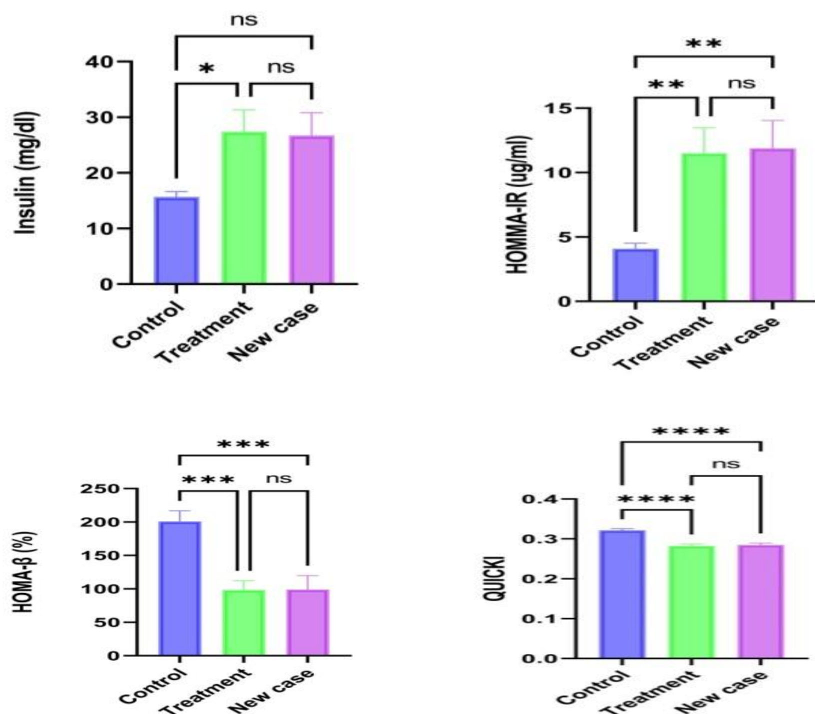


Figure 4.1: Comparison of HbA1c, glucose, insulin levels, HOMA-IR, HOMA-B and QUICKI in studied group.

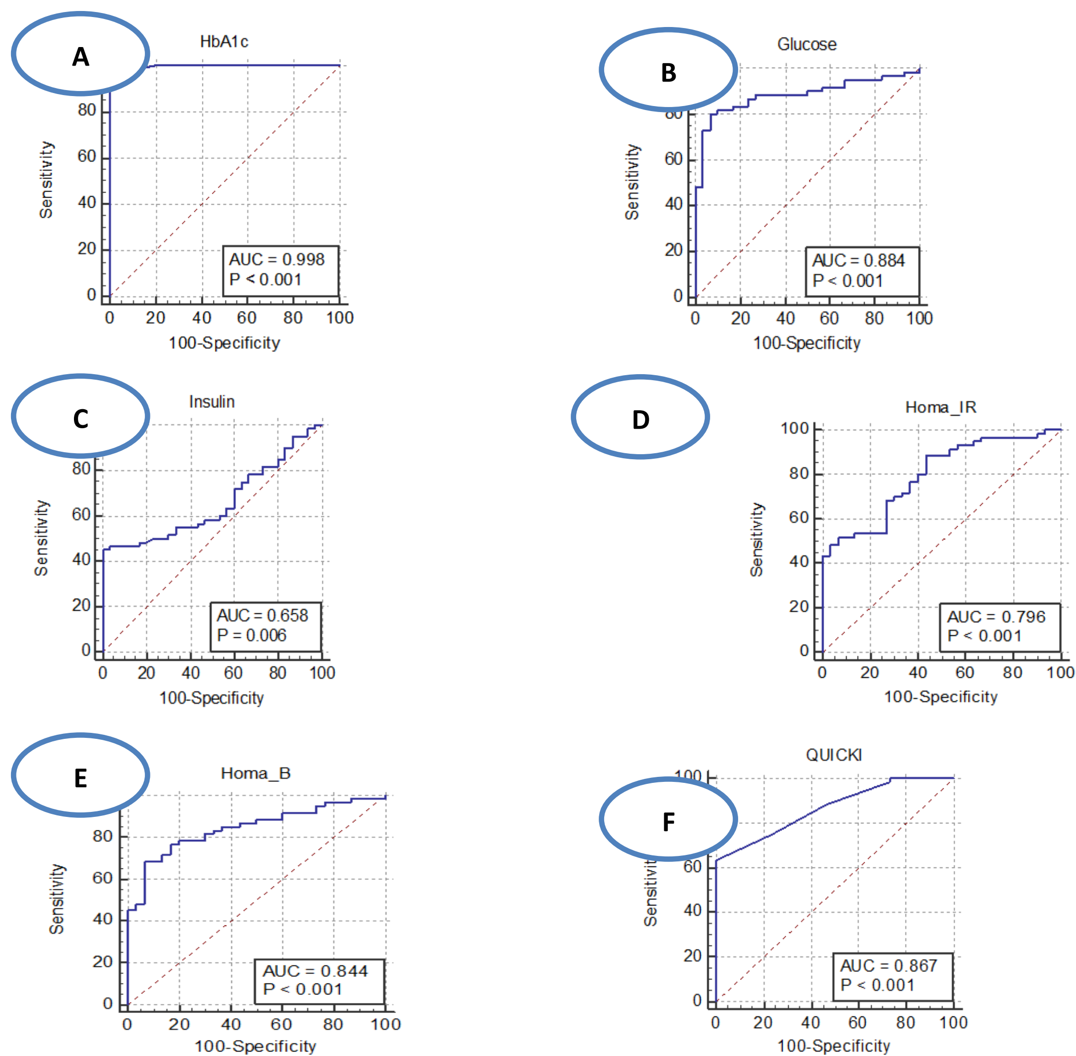


Fig. 4.2: The AUC value of HbA1c; Glucose; Insulin; HOMA-IR; HOMA-B and QUICKI in studied group.

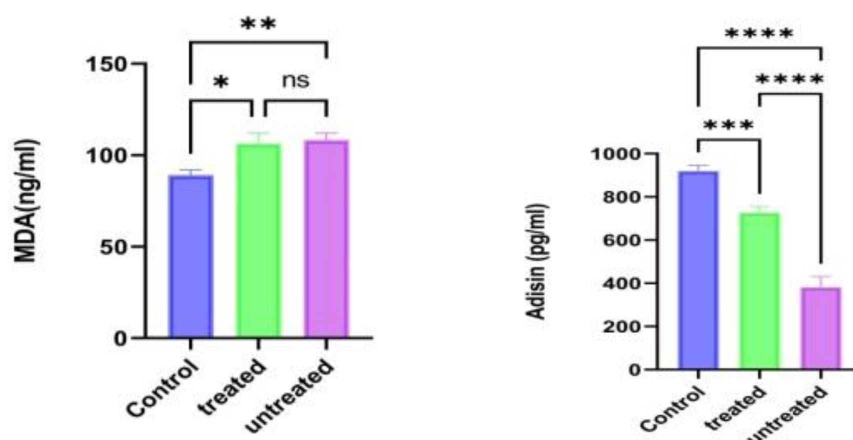
Oxidative stress, antioxidant and Aflatoxin m1 parameters

According to the oxidative stress and antioxidant parameters, Table 4.4 and figure 4.7 demonstrated that the serum level of malondialdehyde (MDA) increased significantly in treated 106.4±5.63 and untreated group 108.4±3.62 of T2DM compared to control group 89.23±2.75, with decreased non-significantly in treated group when compared to untreated group. Level of adipsin decreased dramatically in untreated group 382.5±20.66 and 727.3±19.72 treated group compered to control group 919.8±16.77, p value= 0.002 with significant effect among all three groups. Serum level of Carnosine decreased significantly in both treated 22.89±1.77 and untreated group 21.84±0.89 when compared to control group 31.38±3.78, however non-significant decrease appear between the two groups of T2DM. Serum level of glutathione peroxidase (GPX) increased non-significantly in treated 4.94±0.29 group when compared with control group 4.33±0.14 and increased significantly in untreated group 7.39±0.34 compared to control group. In addition, increased significantly in untreated group compared to treated group. Level of Catalase decreased significantly in treated 1.03±0.06 and untreated group 0.96±0.04 of T2DM when compared with control group1.26±0.04, but decreased non-significantly in untreated group compared to treated group. AUC value of these parameters showed active and strong biomarkers for T2DM patients. The AUC value of MDA was 0.72, adipsin was 0.89, carnosine 0.78, GPX was 0.77, catalase was 0.78 and p values for all parameters were 0.001, respectively.

As far as we know this is the first study in Kurdistan region that investigate the effect of T2DM on aflatoxin m1 level of this parameter in serum of untreated group was increased non-significantly 0.90±0.05 compared to control group 0.80±0.18 and treated group 0.69±0.05, serum aflatoxin m1 level non-significantly in untreated group compared to treated group. Present study showed the AUC value of aflatoxin m1 which did not consider as a biomarker for T2DM with significant change as AUC=0.63 and p value=0.023 table 2.

Table2: Oxidative stress, antioxidant and aflatoxin m1 parameters in studied groups.

Parameters	Control	Treated	Untreated	p. value
	Mean±SE			
MDA ng/ml	89.23±2.75	106.4±5.63	108.4±3.62	0.0026
Adipsin pg/ml	919.8±16.77	727.3±19.72	382.5±20.66	0.0001
Carnosine pg/ml	31.38±3.78	22.89±1.77	21.84±0.89	0.0152
GPX ng/ml	4.33±0.14	4.94±0.29	7.39±0.34	0.0001
Catalase ng/ml	1.26±0.04	1.03±0.06	0.96±0.04	0.0008
Aflatoxin m1 ng/ml	0.80±0.18	0.69±0.05	0.90±0.05	ns



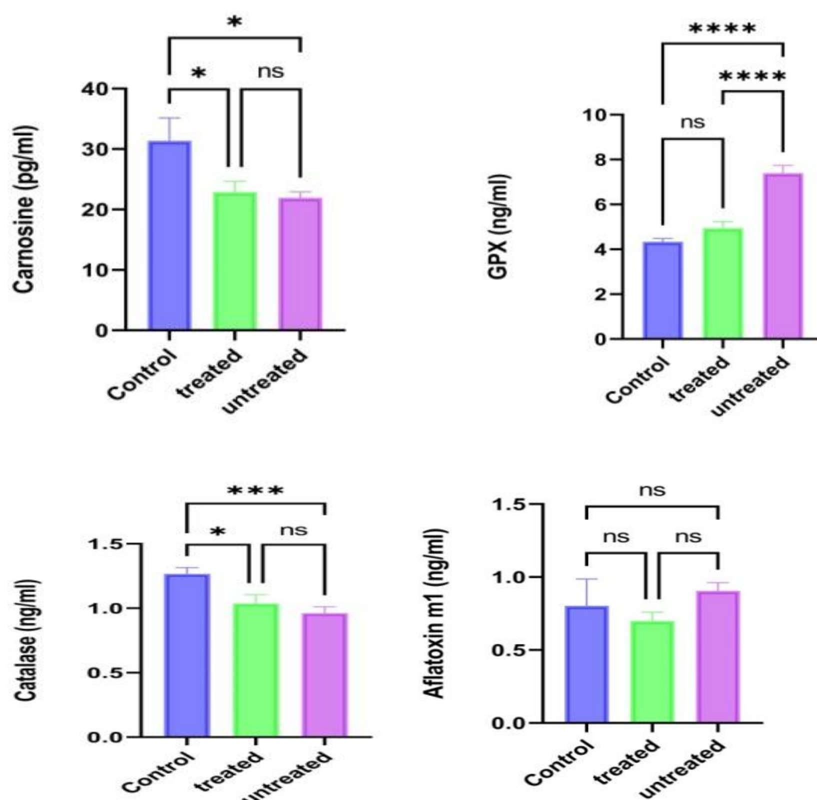


Fig.4.3: Comparison of oxidative stress and antioxidant MDA, Adipsin, Carnosine, GPX, Catalase and Aflatoxin m1 in studied group.

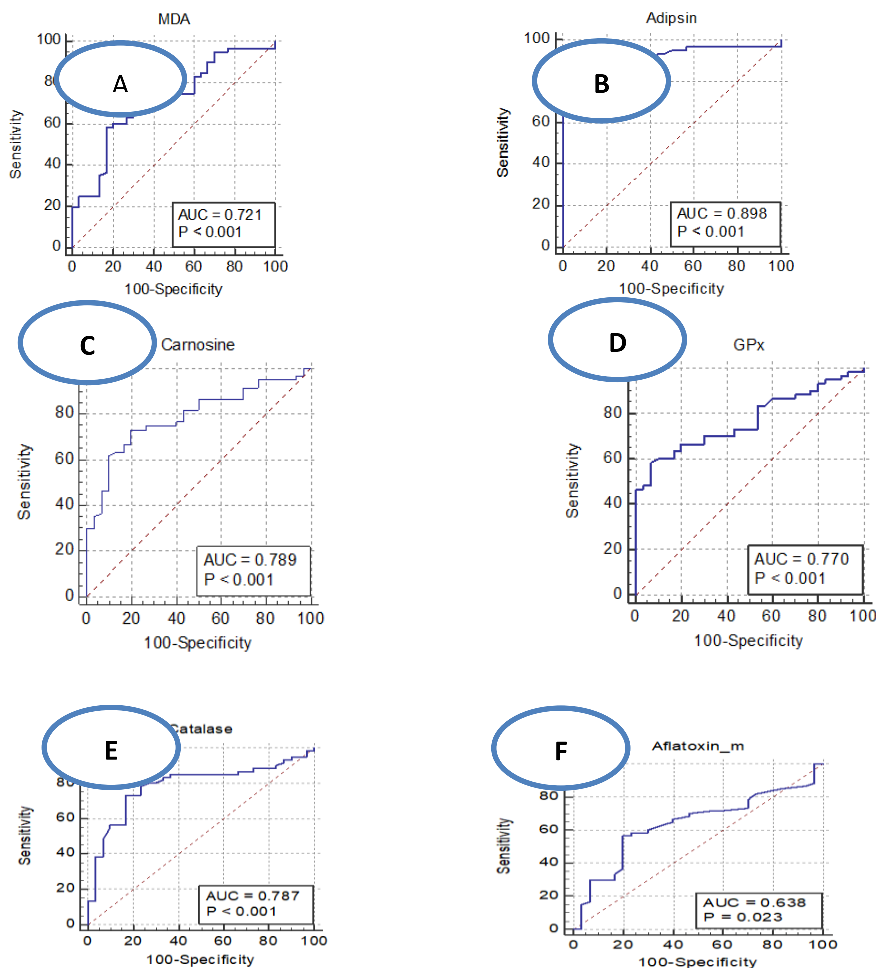


Fig. 4.4: The AUC value of MDA; Adipsin; Carnosine; GPX, Catalase and aflatoxin m1 in studied group.

Transcription Factor 7 Like 2 (TCF7L2 C/T) genotype

The amplification of the TCF7L2 C/T gene was carried out, and the amplicons were gel electrophoresed the CC (432/211bp) genotype implies normal homozygous, the CT (432/211/272bp) genotype indicates heterozygous, while TT (432/272bp) implies mutant homozygous figure 1.

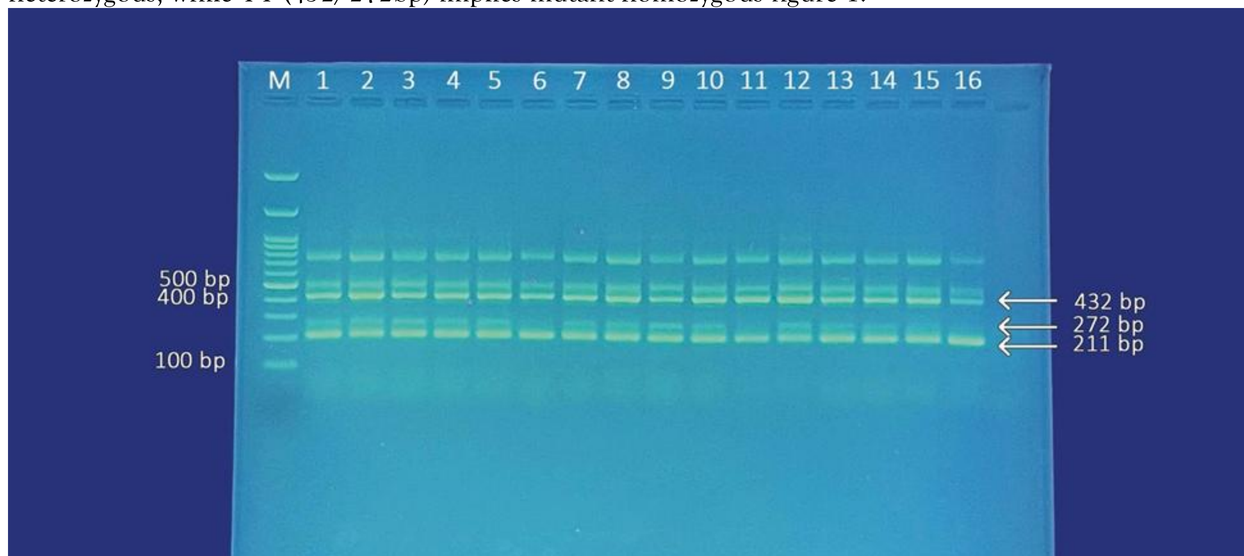


Figure1: Agarose gel electrophoresis illustrating PCR products for the TCF7L2 gene rs7903146 (C > T) SNPs. Ethidium bromide used to make the DNA visible under UV light with PCR products. M stands for DNA Marker (Ladder) 100bps, CC: normal homozygous, CT: heterozygous genotype, TT: homozygous genotype (mutant type).

Associations of TCF7L2 (C/T) genotypes distributions and alleles frequencies in T2DM

Table 4.12 showed the distribution of TCF7L2 rs7903146 variant in T2DM patient and control group. Among T2DM group the frequencies of individuals of CC, CT and TT genotypes were 11(18.33%), 47(78.78.33%) and 2(3.33%) in T2DM group and 5(16.6%), 22(73.33%) and 3(10%) in control group, respectively. The analysis of TCF7L2 genotype CC and CT showed a 1.12 and 1.31times risk factor for T2DM with p= 1. 00 and 0.606. While about TT statistically not significant and not risk for T2DM disease.

The frequencies of major allele C and minor allele T in T2DM groups were 69(57.5%) and 51(42.5%), in control group were 32(53.3%) and 28(46.6%) respectively. The rs7903146 variant was also associated with a higher risk of T2DM in the recessive model (CC+CT) vs TT 58(96.6%) in patient group and 27(90%) in control group with RR=3.22, p=0.328 this correlation was non-significant, respectively. According to the researchers information, this is the first study in the Kurdistan region that investigate the effect or the role of transcription factor 7 like 2 (TCF7L2 C/T) gene polymorphism SNP on the clinical management of diabetic medication treated and untreated T2DM Kurdish male and female patients table 3.

Table3: The genotypes and allele frequencies of TCF7L2 C/T in T2DM and control group.

TCF7L2 (C/T) (rs7903146)	Patients N%	Control N%	Relative Risk (RR)	Etiology or Preventive Fraction	Exact Fishers Probability (P)	95% Confidence Intervals (CI)
Genotype						
CC	11(18.33%)	5(16.6%)	1.12	0.02	1.00	0.36-3.53
CT	47(78.33%)	22(73.33%)	1.31	0.18	0.606	0.48-3.58
TT	2(3.33%)	3(10%)	0.31	0.06	0.328	0.05-1.92
(CC+CT) vs TT	58(96.6%)	27(90%)	3.22	0.66	0.328	0.52-19.98

(TT+CT) vs CC	49(81.6%)	25(83.33%)	0.89	0.09	1.00	0.28-2.80
Allele						
C allele	69(57.5%)	32(53.33%)	1.18	0.08	0.635	0.64-2.20
T allele	51(42.5%)	28(46.6%)	0.84	0.07	0.635	0.45-1.57

The genotype frequencies of TCF7L2 among the categories of T2DM were assessed by the HWE calculation. The differences in frequency of CC, CT and TT between observed and expected in T2DM group were 11(18.33%), 19.84(33.06%); 47 (78.33%), 29.33 (48.88%) and 2(3.33%), 10.84(18.06%). In control group the observed and expected frequency were 5(16.66%), 8.53(28.44%); 22 (73.33%), 14.93 (49.78%) and 3(10%), 6.53(21.78%) respectively. The variance between the observed and expected values of genotype frequencies was statistically non-significant, indicating that the distribution of this cohort was under HWE table 4.

Table4: Hardy-Weinberg equilibrium (HWE) test for the genotypes and allele distributions of TCF7L2 C/T in the healthy person and T2DM patients.

Case Categories		TCF7L2 gene at position--- C/T (rs7903146)					
		Genotypes			HWE p. value	Alleles	
		CC	CT	TT		C	T
Patients	Observed	11 (18.33%)	47 (78.33)	2 (3.33%)	0.0001	69 (57.5%)	51 (42.5%)
	Expected	19.84 (33.06%)	29.33 (48.88%)	10.84 (18.06%)		NA	
Control	Observed	5 (16.66%)	22 (73.33)	3 (10%)	0.0095	32 (53.33%)	28 (46.66%)
	Expected	8.53 (28.44%)	14.93 (49.78%)	6.53 (21.78%)		NA	
NA: Not applicable							

DESCUSSION

The levels of HbA1c and glucose were considerably raised in patients in the current investigation, which aligns with the findings of (Nazari et al. 2021). The concentration of FBS is directly proportional to the HbA1c level; elevated FBS results from impaired destruction of pancreatic beta cells. An increase in HbA1c occurs when blood glucose levels rise, and inadequate glucose regulation in the body leads to significant elevations in both parameters. Consequently, HbA1c levels assess glucose levels over a three-month period and are regarded as the optimal indicator for diagnosing and monitoring T2DM (Hashim 2024).

HbA1c evaluates long-term glucose management and predicts the likelihood of microvascular problems in individuals with diabetes. The HbA1c level in the blood sample indicates the average lifespan of red blood cells and functions as a metric for long-term glycaemic control. The extended consequences of diabetes mellitus may adversely affect different organs, resulting in diverse physiological alterations in the body contingent upon the severity and duration of the condition (Sulfikar et al. 2023). Glucose concentrations are dictated by the rate of glucose influx and efflux in the circulation. Normal glucose metabolism transpires when sufficient insulin is released to offset the level of physiological insulin resistance and carbohydrate consumption. Glucose metabolism exhibits a diurnal cycle, marked by elevated glucose levels in the early morning and diminished values at night (Coetzee 2023).

Type 2 diabetes mellitus (T2DM) is characterised by insulin resistance and concomitant beta-cell dysfunction. Initially, there is a compensatory augmentation in insulin secretion that sustains glucose levels within the normal range. As the disease advances, beta cells undergo alterations, resulting in inadequate insulin secretion to sustain glucose homeostasis, hence causing hyperglycemia (Goyal et al. 2023). Insulin resistance (IR) refers to a reduction in the metabolic response of target cells to insulin or, at a systemic level, a diminished capacity of insulin to lower blood glucose levels. Insulin resistance may

arise from reduced insulin secretion, the presence of insulin antagonists in the plasma, and a lessened insulin response in target tissues (Czech 2017).

The activity of insulin is modulated by many hormones, including growth factors and insulin-like growth factor 1 (IGF-1) during the fed state, and glucagon, glucocorticoids, and catecholamines during fasting. Therefore, the immoderate synthesis of these hormones may induce IR. The equilibrium between insulin and glucagon is crucial as it dictates the extent of phosphorylation of downstream enzymes in the regulatory signalling pathways (Galicia-Garcia et al. 2020).

The function of HOMA-IR in non-diabetic individuals remains ambiguous (Lee et al. 2023). Increased fat accumulation in the pancreas hinders its responsiveness to elevated blood sugar levels and reduces insulin production. HOMA-IR is the most effective metric for identifying insulin resistance in individuals with T2DM (Tanase et al. 2020). Insulin resistance and β-cell dysfunction contribute to increased blood glucose levels, exacerbating the pathophysiology of type 2 diabetes mellitus (T2DM). Alterations in β-cell function transpire during the initial phases of diabetes onset (the prediabetic stage) and progressively deteriorate as the disease advances. Therefore, it is essential to clarify the pathogenic pathways behind β-cell malfunction to mitigate problems associated with T2D, particularly those involving inflammation and oxidative stress (Dludla et al. 2023).

Traditionally, β-cell dysfunction has been ascribed to the reduction of β-cell mass resulting from β-cell exhaustion during sustained elevations in glucose metabolism and insulin secretion, with β-cell apoptosis induced by glucotoxicity and lipotoxicity. It is proposed that the dysfunction of β-cells may stem from intricate mechanisms and connections (Christensen and Gannon 2019). One potential mechanism is the dedifferentiation of β-cells, characterised by the loss of transcription factors that define β-cells. The loss of identity of a β-cell may occur due to glucotoxicity. Another mechanism is the transdifferentiation of β-cells, a process that converts one terminally differentiated cell type into another; the function of β-cells can also be compromised by the activation of forbidden genes (Młynarska et al. 2025). β-Cell functionality was assessed utilising parameters derived from a 75 g oral glucose tolerance test (OGTT), including the insulinogenic index (IGI), disposition index (DI), oral disposition index (DIO), and homeostasis model assessment of β-cell function (HOMA-B). The duration of diabetes or HbA1c AUC shown a negative correlation with log(HOMA-B) (Kim et al. 2024).

The quantitative insulin-sensitivity check index (QUICKI) is an innovative mathematical transformation of fasting blood glucose and insulin concentrations. In obese and diabetic individuals, QUICKI has a markedly superior linear correlate with glucose clamp assessments of insulin sensitivity compared to minimal-model estimations, aligning with the findings of the current investigation (Naiemian et al. 2020). In the current investigation, all glycaemic markers significantly increased in individuals with T2DM compared to the control group, with the exception of HOMA-B and QUICKI, a finding consistent with certain studies (Abbas et al. 2020, Ali 2025).

Oxidative stress is a situation arising from an imbalance between the generation of reactive oxygen species (ROS) and the capacity of the body's antioxidant defence mechanism to neutralise them. Reactive oxygen species, encompassing free radicals and other highly reactive molecules, are inherent consequences of standard cellular metabolism. Oxidative stress arises when the creation of free radicals surpasses the body's antioxidant capacity to neutralise them (Forman and Zhang 2021). Oxidative stress in diabetes results from a complex interaction of multiple factors, including the accumulation of glycolytic intermediates, activation of the polyol pathway, formation of advanced glycation end products (AGEs), activation of Protein Kinase C (PKC), and stimulation of the hexosamine pathway (Oguntibeju 2019).

Oxidative stress has a crucial role in the pathogenesis of insulin resistance and diabetes mellitus. It can diminish peripheral insulin sensitivity by at least five principal biological mechanisms, including β-cell dysfunction, inflammatory responses, GLUT-4 downregulation and/or mislocalization, mitochondrial dysfunction, and disruption of normal insulin signalling pathways (Yaribeygi et al. 2020).

Banik et al. (2021), The present study concurs that serum MDA levels were significantly elevated in the patient group relative to the control subjects. However, it contradicts the current study regarding GPX, as the meta-analysis indicated no significant difference in GPX levels between the patient and control groups (Banik and Ghosh 2021). Plasma MDA, a measure of lipid peroxidation, was markedly raised in the patient cohort (Najafi et al. 2021).

Adipsin, also known as complement factor D, is an adipokine and was the first adipokine to be identified. Adipsin regulates adipose tissue homeostasis and enhances insulin production in response to glucose. Furthermore, it facilitates the formation of C3a (an active version of component 3, C3) by regulating the alternative complement pathway, resulting in heightened insulin release from the pancreas (Tafere et al.

2020). Moreover, adipsin enhances glucose absorption and promotes triglyceride production in adipocytes (Milek et al. 2022). Numerous adipokines are released by adipose tissue, and many participate in energy homeostasis and inflammation. The dysregulation of adipokine production compromises organ function and contributes to the onset of metabolic disorders, including insulin resistance (Gu et al. 2022). In current study adipsin decreased significantly in treated and untreated group T2DM compared to control group and elevated in treated group compared to untreated group, This result aligns with several studies indicating that serum adipsin levels significantly decreased in newly diagnosed diabetic patients, as well as in both the metformin-treated group and the sitagliptin/metformin-treated group, in comparison to the control group. Conversely, serum adipsin levels were significantly elevated in both the metformin-treated and sitagliptin/metformin-treated patients when compared to the newly diagnosed diabetic patients (Milek et al. 2022, Mohammed et al. 2024). Another study revealed that serum adipsin levels were highest in individuals with normal glucose tolerance and lower in those with newly diagnosed diabetes (Wang et al. 2019).

Maity et al. (2024), discovered that plasma adipsin exhibits no significant connection with insulin release in individuals with diabetes. Among the risk variables for Type 2 Diabetes (T2D), adipsin levels were solely correlated with age, and the positive association between plasma adipsin and age in those without T2D was absent in those with T2D (Sujay Krishna et al. 2024).

The elevation of oxidative stress and inflammatory mediators in hyperglycemia may be the primary contributor to the progression of type 2 diabetes mellitus (T2DM) and its associated complications, in addition to other cardiovascular disease (CVD) risk factors linked to diabetes, which manifest as metabolic disorders and insulin resistance characteristic of type 2 diabetes, including increased serum levels of fasting glucose, HbA1c, triglycerides, very-low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) (Shaheed and Ali 2023). Carnosine exhibits significant biological activities by influencing age-related diseases, including cardiovascular disease, diabetes mellitus, cancer, and neurological disorders, and is crucial for enhancing functional capacity in ischaemic conditions (Huang et al. 2020). Previous studies have demonstrated that carnosine normalises plasma glucose levels and reduces insulin levels following oral glucose intake. Additionally, carnosine has been shown to lower fasting glucose levels, decrease serum triglycerides, enhance lipid metabolism, and improve glycaemic control by reducing HbA1c, insulin resistance, and increasing insulin secretion (Kilis-Pstrusinska 2020, Peng et al. 2020). Carnosine has demonstrated the ability to mitigate hyperglycemia in diabetic individuals and in animal models of diabetes (Matthews et al. 2021). Beta cells augmented with carnosine exhibited protection against oxidative and glucolipotoxic stress, leading to enhanced insulin production relative to cells lacking carnosine supplementation. Furthermore, the use of carnosine can induce the production of insulin and glucagon within 60 to 90 minutes, likely mediated by its histidine concentration. This may expedite the normalisation of postprandial blood glucose levels and aid in the decrease of HbA1c. Carnosine, in addition to its effects on beta cells, safeguards myotube cells against glucolipotoxic stress, allowing these cells to sustain normal glucose absorption in response to insulin stimulation. Collectively, these findings indicate that carnosine may maintain beta cell functionality and facilitate adequate glucose absorption (Qiu et al. 2025).

Glutathione peroxidase (GPx), an enzyme crucial for safeguarding the organism from oxidative damage caused by free radicals, serves as a primary signal of current oxidative stress within the body. Elevated levels of reactive oxygen species (ROS) in diabetic patients are hypothesised to result, in part, from diminished activity of glutathione antioxidant enzymes, potentially due to mechanisms including protein glycation, which may impair their function. In fact, de Vega et al. (2016), indicate that diminished GPx activity in diabetic patients may contribute to diabetic neuropathy (de Vega et al. 2016). GPX1 is essential for sustaining redox homeostasis and safeguarding pancreatic β-cells from oxidative stress, especially in hyperglycaemic environments. Its overexpression may avert β-cell death and enhance insulin production, whereas GPX1 deficiency exacerbates glucose intolerance (Begum and Lakshmanan 2024).

Catalase (CAT) is another crucial antioxidant enzyme that decomposes hydrogen peroxide into oxygen and water. CAT deficiency correlates with an elevated risk of T2D, as it impairs β-cell functionality due to CAT's role in safeguarding β-cells from ROS damage. Reduced CAT activity in T2D patients is consistently associated with the onset of T2D, and hyperglycemia results in the downregulation of CAT expression (Darenskaya et al. 2021).

A prior study identified a robust association between CAT and T2D risk in individuals exhibiting elevated HOMA-IR and total cholesterol (TC) levels; diminished CAT activity correlates with both factors due to the cumulative toxic effects of glucotoxicity, which induces insulin resistance, and lipotoxicity resulting

from increased cholesterol levels. They influence and modify insulin secretion and the transcription of the insulin gene. Consequently, these processes have been proposed as a mechanism in the development of T2D (Promyos et al. 2023).

Conversely, certain investigations corroborate the current research, indicating a substantial correlation between CAT and GPX1 concentrations in both the control and study groups, with the highest catalase concentration observed in the control group and the lowest in T2DM patients. Significantly, the highest levels of GPX1 were observed among people with type 2 diabetes. The control group exhibited the lowest levels concurrently (Promyos et al. 2023, Cecerska-Heryć et al. 2025). Other studies contradict the current research, indicating reduced GPx activity in individuals with DM2 compared to the control group, and no significant correlation was found between the GPx1 polymorphism and T2DM (Dworzański et al. 2020, Habibullah et al. 2024).

Aflatoxin exposure has previously been associated with type 2 diabetes mellitus. It may occur by the ingestion of food, milk, and dairy products (Mohamed et al. 2022). AFB1 is metabolised into several hydroxylated derivatives, including aflatoxin M1 (AFM1), which is the most carcinogenic among these metabolites. Aflatoxin M1 can be identified in dairy products derived from animals that have ingested feed infected with AFB1. The International Agency for Research on Cancer (IARC) has designated AFB1 as a group 1 carcinogen and AFM1 as a category 2B carcinogen for humans (Ofori-Attah et al. 2024).

In all animal species, the organs most adversely impacted by aflatoxins are the liver and kidneys (Qing et al. 2022). Aflatoxins undergo metabolism in the liver, yielding extremely reactive chemical intermediates that induce free radical generation, lipid peroxidation, and cellular damage (Eftekhari et al. 2018). Aflatoxins and their metabolites affect several segments of the nephron, resulting in nephrotoxicity prior to urinary excretion. Aflatoxin-induced toxicities have been linked to reductions in kidney weight, thickening of the glomerular basal membrane, diminished glomerular filtration rate, decreased urine output, and apoptosis in animal studies (Owumi et al. 2023). AFB1 exposure can influence lipid metabolism and induce hepatocyte apoptosis and damage via the phosphatidylinositol 3 kinase/protein kinase B (PI3K/Akt) signalling pathway (Wang et al. 2024). Research indicates that elevated dietary levels of AFB1 can impair normal hepatic lipid metabolism and elevate blood indicators of liver damage (Liu et al. 2023). Exposure to AFB1 can impair glycerophospholipid metabolism and fatty acid breakdown, particularly when coupled with aflatoxin M1 (AFM1); this disruption is more pronounced, resulting in severe metabolic problems. The study's results indicate for the first time that the lipid metabolic imbalance induced by AFB1 and AFM1 is a primary contributor to enterotoxicity, offering new insights into the harmful processes of AFB1 and AFM1 in both animals and humans (Yang et al. 2023). AFB1 exposure can induce oxidative stress and mitochondrial impairment, exacerbating lipid metabolic diseases (Feng et al. 2023).

Moreover, AFB1 diminishes plasma glucose levels, disrupts cellular glucose metabolism, and impedes glycogen formation. It can also influence carbohydrate metabolism by diminishing liver glycogen and expediting glucose-6-phosphate oxidation, hence impacting growth markers (Barany et al. 2021). Nonetheless, varying outcomes have been noted regarding dietary AFB's elevation of blood glucose levels (Al-Rubaiy et al. 2018, Khalafalla et al. 2022).

The notable correlation between various serum biomarkers and AFM1 exposure in study participants indicates that AFM1 is a toxic metabolite of aflatoxins (AFs), contributing to dyslipidaemia, hyperglycemia, oxidative stress, and inflammatory responses, which may ultimately induce diabetes mellitus and related metabolic disorders (Akash et al. 2021).

The TCF7L2 gene encodes a transcription factor that is crucial in the Wnt/β-catenin signalling system, influencing cell proliferation, polarisation, embryogenesis, and tissue homeostasis. The Wnt pathway governs the proliferation of pancreatic β-cells and influences insulin production. Alterations in this route have been found to modify insulin action and resistance, hence promoting the onset of T2DM (Wunsch et al. 2019).

rs7903146 (C/T) is a prevalent variant in the transcription factor 7-like 2 (TCF7L2) gene, with the T-allele recognised as the risk allele for type 2 diabetes (Hansen et al. 2025). This variant presents one of the most significant single-gene population attributable hazards for type 2 diabetes and is linked to compromised β-cell activity, inadequate regulation of α-cell secretion by glucose, and diminished insulin production in individuals both with and without diabetes (Zeini et al. 2024).

A prior study indicated that persons with the CT genotype of the rs7903146 SNP exhibit an elevated risk of Type 2 Diabetes Mellitus (P-value 0.0019), and the rs7903146 T allele is likely a potential risk factor linked to the pathogenesis of T2DM (Mustafa and Younus 2021). Likewise, another study did not identify

a statistically significant difference in genotype or allele frequencies between patients and controls. Upon calculating the odds ratio, the genotypic distributions of the TCF7L2 rs7903146(C/T) polymorphism indicated that heterozygous (CT) individuals are at an increased risk for type 2 diabetes mellitus (T2DM). Furthermore, the TT genotype was identified solely within the patient cohort (Bahaaeldin et al. 2020).
The link of TCF7L2 rs7903146T with T2DM risk may also extend to T2DM consequences, particularly the risk of peripheral neuropathy and myocardial infarction. A clinically applicable risk predictive model for T2DM should incorporate both genetic and non-genetic risk factors to significantly enhance its efficacy in the early identification of patients predisposed to T2DM, hence facilitating timely interventions. This indicates that rs7903146T may possess significant clinical predictive applications not only for T2DM risk but also for T2DM-related comorbidities, which is beneficial for disease monitoring and early intervention (Chaudhary et al. 2024).
A prior study indicated that the mean nocturnal glucose level was elevated in people possessing the rs7903146-T variant in TCF7L2. Under typical physiological conditions, plasma glucose originates from endogenous glucose synthesis in the liver through glycogenolysis during the fasting state, influenced by glucagon. In the fed state, plasma glucose originates from nutrients. At this stage, both gluconeogenesis and glycogenolysis are inhibited by insulin (van der Kroef et al. 2016). TCF7L2 is significant in the regulation of low-density lipoprotein, very low-density lipoprotein, triglycerides, gluconeogenesis, and the modulation of hepatic insulin sensitivity (Palizban et al. 2017), The CT genotype exhibited a highly significant correlation with BMI, WHR, fasting and postprandial glucose, total cholesterol, low-density lipoprotein, serum creatinine, as well as systolic and diastolic blood pressures (Verma et al. 2021). Mustafa et al.,(2021), showed that The prevalence of the CT genotype was significantly elevated in the cases compared to the controls, suggesting that the CT genotype is associated with an increased risk of diabetes. The T allele had a significantly elevated frequency in the patients relative to the controls. The findings indicated that the T allele may serve as a risk factor for heightened susceptibility to T2DM occurrence within the Iraqi Kurdish population (Mustafa and Younus 2021).
In terms of CT genotype this study was in line with present study, Bahaaeldin et al. (2020) study indicated that The strong relationship between the TCF7L2 (C/T) polymorphism and T2DM was not substantiated; however, it indicated a potential high risk of T2DM development in patients with the TT genotype. Additional research with a larger sample size is required to elucidate the link (Bahaaeldin et al. 2020). Furthermore, a prior study indicated that the homozygous TT genotype presents nearly a two-fold risk relative to the wild-type CC genotype. A comparable risk of 1.96 times was linked to heterozygote CT in relation to CC (Kumar et al. 2024). Abd et al., (2023), The T allele of the rs7903146 polymorphism in TCF7L2 is associated with an increased risk of developing Type 2 Diabetes Mellitus in the Iraqi population (Abd and Al-Jumaili 2023).

Conclusion

From the data analyzed by diverse bio statistical methodologies, we can derive the patients with T2DM, glycemic indices considerable increases, except HOMA-β and QUICKI demonstrated significant decreases, and elevated serum levels of these glycemic parameters elucidate their crucial role in the pathophysiology of T2DM. Aflatoxin M1 exhibited a non-significant increase in patients with Type 2 Diabetes Mellitus and is not regarded as a biomarker for the condition. The serum level of oxidative stress and GPX elevated but other antioxidant lowered in the T2DM group relative to the control group. TCF7L2 gene polymorphism was detected in genotype frequencies in Erbil T2DM patients. The heterozygous type of SNP rs7903146 (C > T) was the most frequent genotype. Gene polymorphisms of TCF7L2 (C > T) was considered as a risk factor for T2DM in Kurdish population.

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