Transcription Factor 7 Like 2 Gene Polymorphism in Diabetic Patients

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Abstract:

Introduction: Diabetes mellitus (DM) results from insulin resistance, inadequate secretion, and absolute insulin deficiency. DM is associated with macrovascular and microvascular complications. Microvascular complications like diabetic retinopathy, nephropathy, and peripheral neuropathy. The incidence and progression of DM may be influenced by genetic variation. Together with other genes, the transcription factor 7-like 2 (TCF7L2) gene may have had a role in the development of DM. TCF7L2 is an essential constituent of the Wnt signaling pathway and regulates beta cells' insulin release in the pancreas.

Patients and Methods: Genotyping of single-nucleotide polymorphism (SNP) rs7903146 of the TCF7L2 gene (using PCR-RFLP) was studied on 54 diabetic patients, 18 prediabetic patients, and 18 apparently healthy individuals as a control group.

Results: genotype distributions of TCF7L2 (rs7903146 C/T) SNP in the control group were (CC: 77.8% and CT: 22.2%), respectively, while in the prediabetic group, they were (CC: 55.6% and CT: 44.4%). In the diabetic group, the genotype frequencies were (CC: 27.8%; CT:57.4% and TT:14.8%) respectively. The result of allele distributions of the TCF7L2 (rs7903146 C/T) SNP in the control group was (C allele: 88.9% and T allele: 11.1%), respectively. The prediabetic group was (C allele: 77.8% and T allele: 22.2%) respectively. The diabetic group's frequencies were (C allele: 56.5% and T allele: 43.5%), respectively. The C allele of TCF7L2 (rs7903146 C/T) SNP was predominant in healthy controls.

Conclusion: The T allele of TCF7L2 (rs7903146 C/T) SNP was associated with the susceptibility to the development of DM.

Keywords: Single nucleotide; Genotype; Phenotype.

Introduction:

Diabetes mellitus (DM) results from insulin resistance, inadequate secretion, and absolute insulin deficiency. Microvascular complications like diabetic retinopathy, nephropathy, and peripheral neuropathy. The incidence and progression of diabetes mellitus may be influenced by genetic variation **(4).**

The transcription factor 7-like 2 (TCF7L2) gene was mapped on chromosome 10q25.3. TCF7L2 is a chief constituent of the Wnt-signaling pathway and essential for regulating insulin release by pancreatic beta cells and maintaining glucose homeostasis. TCF7L2 is widely expressed in mature pancreatic β-cells and peripheral and omental adipocytes **(7).**

Variation in the TCF7L2 gene involving two SNPs: rs12255372 (G/T) and rs7903146 (C/T). The (rs7903146 C/T) SNP is more related to T2DM, which is mediated by lowered insulin secretion linked to or not with a defective insulin processing, reduced influences of glucagon-like peptide-1 (GLP-1), increased hepatic glucose production, and insulin resistance **(16).**

According to certain research, there is an association between DM and the activin receptor-like kinase 1 (ALK1)/Smad1 pathway, specifically the (rs7903146 C/T) SNP of the TCF7L2 gene. Additionally, AGEs helped transfer TCF7L2 from the cytoplasm to the nucleus by enhancing its expression through transforming growth factor-β (TGF-β). TCF7L2 was then combined with the ALK1 promoter to boost its expression. ALK1 caused glomerular sclerosis by supporting the effects of TGF-β and encouraging the phosphorylation of cellular Smad1 **(20).**

Patients and Methods:

The study was made on 54 T2DM cases (their age ranged from 30 to 70 years old), 18 prediabetic patients, and 18 apparently healthy subjects as a control group. Patients were recruited from the Diabetic Outpatient Clinic of the Internal Medicine Department, Assiut University Hospital. The study duration was from October 2020 to April 2021.

IRB local approval number 17200364

Exclusion criteria:

- T2DM patients on insulin therapy.
- T1DM patients.
- Secondary DM.

Patients had complete medical history, including their age, place of residence, duration of diabetes mellitus, family history, and clinical examination, including blood pressure, weight, and height, for the purpose of calculating BMI, fundus, neurological examination, and laboratory tests:

I) Routine laboratory investigations:

a. Complete blood count (CBC) (Done using ADVIA 2120i, Siemens Healthineers, USA).

b. Fasting serum glucose, HbA1c, fasting serum insulin, and calculation of the Homeostasis Model Assessment for Insulin Resistance

$$\text{HOMA} - \text{IR} = \frac{\text{fasting insulin (mU/L)} \times \text{fasting glucose(mmol/l)}}{22.5}$$

c. Kidney function tests, Urine analysis, albumin/creatinine ratio (ACR), and eGFR.

Estimated GFR using the Cockcroft-Gault equation was calculated as follows [7]:

$$\text{eGFR} = \frac{(\text{weight in kg}) \times (0.85 \text{ if female})}{\text{Serum creatinine (mg/dl)} \times 72} \times ((140 - \text{age}) \times \quad)$$

d. Liver function tests and Lipid profile (Total Cholesterol - Triglycerides - (HDL-c) - (LDL-c)).

(All investigations were done using ADVIA 1800 Chemistry Auto-Analyzers, Siemens Healthineers, USA).

II) Special laboratory investigations: Detection of the rs7903146 SNP of the TCF7L2 gene

By PCR-RFLP:

A- **Isolation of Genomic DNA:** Blood samples were aspirated on EDTA, and DNA was isolated by GeneJET DNA Purification Kit (Thermo Fisher Scientific, Catalog number: K0721, Baltics).

B- **Genotyping of SNP rs7903146 of the TCF7L2 gene:** TCF7L2 (rs7903146 C/T) SNP was genotyped by amplifying 180 bp intron 3 regions of the TCF7L2 gene by using Dream Taq Green PCR Kit, Thermo Fisher Scientific, Catalog number: K1081, Baltics.

Forward Primer: 5 ` ACA ATT AGA GAG CTA AGC ACT TTT TAG GTA 3'.

Reverse Primer: 5' GTG AAG TGC CCA AGC TTC TC 3 `.

The amplification reaction system had a final volume of 25µL, containing 2.5µL 10 X PCR loading buffer, 2.0µLdNTP mix, 1.0 µL forward primer, 1.0 µL reverse primer, 2.0µL Taq DNA polymerase, 2.0µL genomic DNA template, 2.0µL MgCl2 solution, and 12.5µL sterilized double-distilled water.

Touchdown thermal cycling was applied to avoid non-specific amplification. The thermal cycler conditions comprised initial denaturation at 95°C (3 min) and 10 cycles of [denaturation at 95°C (30 sec), annealing at 65°C with 1°C lowering per cycle (30 sec), and extension at 72°C (45 sec), followed by 24 cycles when the annealing temperature became 60°C.

After amplification, the 188-bp PCR products were checked in 1% agarose gel electrophoresis, and then digested with the RsaI restriction enzyme (Thermo Fisher Scientific, Catalog number: ER1121, Baltics) by incubation at 37°C overnight. The digestion reaction was 20µL, comprising 2.0 µL 10X buffer, 1 µL (10 U/µL) RsaI restriction enzyme for rs7903146, derived from Rhodopseudomonas sphaeroides, 10 µL PCR products, and 7.0 µL double-distilled water. DNA fragments were analyzed using 3% agarose gel electrophoresis.

The RsaI enzyme cuts the restriction site 5'GT▼AC3' at the C allele of the SNP into two fragments, 159 and 29 bp. Still, the T allele (mutant) is not digested, leaving the intact PCR product. So the digestion of Homozygous wild type (C/C) yields 2 fragments, 159 bp and 29 bp. In contrast, digestion of the Homozygous mutant type (T/T) remains the original size (188 bp), whereas digestion of the Heterozygous (C/T) rs7903146 produces 3 fragments with 188 bp, 159 bp, and 29 bp.

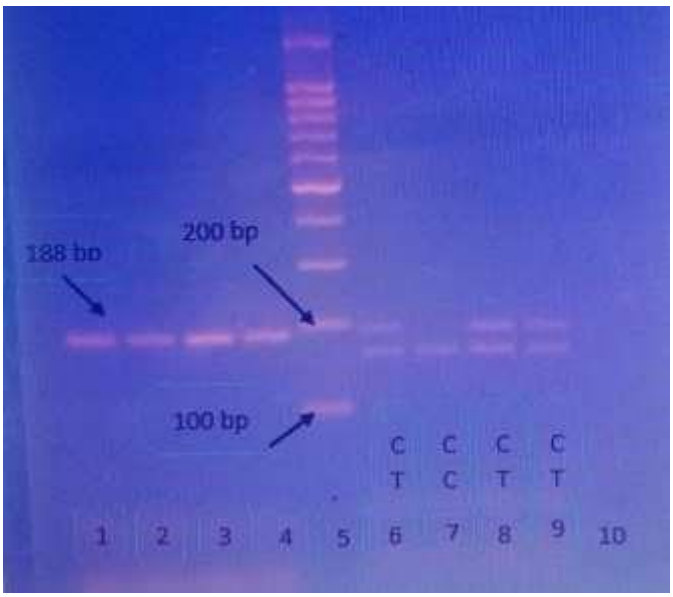

Figure (1): Electrophoresis image of PCR products. Lanes 1,2,3, and 4 are PCR products before RsaI digestion. Lane 5 is a 100 bp ladder. Lanes 6,7,8, and 9 are PCR products after digestion with RsaI. Lane 10 is the negative control.

Statistical Methods:

Data was analyzed using SPSS (IBM, Armonk, New York). The Shapiro test was applied to explore the normal distribution of data. Based on this test, it was found that each of age, BMI, uric acid, eGFR, cholesterol, HDL-c, and LDL-c were normally distributed quantitative data, while the other quantitative data in the current study were non-normally distributed data.

Non-normally and normally distributed data were compared using Mann-Whitney U and Student t tests. Chi^2 test was implemented on Nominal data.

Results:

• **Demographic data:**

Table I: Demographic data among studied groups

	Control group (n= 18)	**Prediabetic group (n= 18)**	**Diabetic group (n= 54)**	**P value**
Age (years) Range Mean ± SD	 28-50 33.50 ± 5.19	 30-55 43.72 ± 8.33	 30-70 53.70 ± 7.97	 P1 < 0.001** P2 < 0.001** P3 < 0.001**
Sex Male Female	 9 (50%) 9 (50%)	 10 (55.6%) 8 (44.4%)	 25 (46.3%) 29 (53.7%)	 P1 = 0.50 P2 = 0.49 P3 = 0.34
BMI (kg/m^2) Range Mean ± SD	 22.74 -25.00 24.01 ± 1.27	 25.40-39.50 32.50 ± 3.79	 23.40-39 31.68 ± 3.98	 P1 < 0.001** P2 < 0.001** P3 = 0.41
Smoking Yes No	 2 (11.1%) 16 (88.9%)	 12 (66.7%) 6 (33.3%)	 45 (83.3%) 9 (16.7%)	 P1 < 0.001** P2 < 0.001** P3 = 0.12
Diabetes Family history Yes No	 9 (50%) 9 (50%)	 16 (88.9%) 2 (11.1%)	 48 (88.9%) 6 (11.1%)	 P1 = 0.01* P2 < 0.001** P3 = 0.68
SBP (mmHg) Range Mean ± SD	 110-130 120.56 ± 4.16	 110-140 126.67 ± 10.85	 100-165 130.65 ± 12.59	 P1 = 0.12 P2 < 0.001** P3 = 0.19
DBP (mmHg) Range Mean ± SD	 70-90 80 ± 3.43	 80-100 85.56 ± 7.84	 70-100 84.73 ± 9.13	 P1 = 0.06 P2 = 0.03* P3 = 0.70

Data expressed as frequency (percentage), mean (SD), and range. *: significant ($p< 0.05$). **: highly significant. **BMI**: body mass index; **SBP**: systolic blood pressure; **DBP**: diastolic blood pressure.

Table II: Kidney function tests among studied groups

	Control group (n= 18)	**Prediabetic group (n= 18)**	**Diabetic group (n= 54)**	**P value**
S. urea (mmol/l) Range Mean ± SE	 2.3-5.40 3.97 ± 0.21	 3.7-6.0 4.62 ± 0.29	 2.4-34 6.72 ± 0.88	 P1 = 0.10 P2 = 0.12 P3 = 0.88
S. Creatinine (µmol/l) Range Mean ± SE	 52-101 83.10 ± 2.92	 58-118 83.38 ± 3.46	 51-624 115.47 ± 15.56	 P1= 0.71 P2 = 0.86 P3 = 0.91
S. uric acid (mg/dl) Range Mean ± SD	 3.30-6.20 4.83 ± 1.13	 3.40-7.30 5.26 ± 0.97	 2.40-9.10 5.49 ± 1.38	 P1 = 0.31 P2 = 0.06 P3 = 0.50
eGFR (ml/min) Range Mean ± SD	 99-125 114.80 ± 9.28	 90.50-125 109.48 ± 17.35	 16-135 81.60 ± 27.37	 P1 = 0.53 P2 < 0.001** P3 < 0.001**
Albumin/creatinine ratio (mg Albumin/gm creatinine) Range Mean ± SE	 2.90-20 13.22 ± 1.19	 4.30-29.30 13.07 ± 1.8	 2.70-4200 393.17 ± 111.56	 P1 = 0.99 P2 = 0.03* P3 = 0.03*

Data is expressed as mean (SD or SE) and range. *: significant (p<0.05); ** highly significant. e**GFR**: estimated glomerular filtration rate.

Table III: Glycemic profile and advanced glycated end products among studied groups

	Control group (n= 18)	**Prediabetic group (n= 18)**	**Diabetic group (n= 54)**	**P value**
Fasting serum glucose (mmol/l) Range Mean ± SD	 3.70-5.30 4.62 ± 0.48	 5.7-6.9 6.03 ± 0.58	 4.60-25.90 11.17 ± 5.11	P1 = 0.29 P2 < 0.001** P3 < 0.001**
Glycosylated hemoglobin (HbA1c) (%) Range Mean ± SD	 4.80-5.60 5.18 ± 0.29	 5.70-6.40 6.13 ± 0.21	 5.30-13.10 8.77 ± 2.01	P1 = 0.07 P2 < 0.001** P3 < 0.001**
Fasting serum Insulin (mU/L) Range Mean ± SE	 4.04-10.24 7.57 ± 0.46	 2.08-132.78 28.99 ± 7.41	 3.40-204.10 31.98 ± 4.57	P1 < 0.001** P2 < 0.001** P3 = 0.50
HOMA-IR Range Mean ± SE	 0.94-1.87 1.51 ± 0.07	 0.97-34 7.90 ± 2.01	 0.92-108.80 16.48 ± 2.82	P1 < 0.001** P2 < 0.001** P3 = 0.01*

Data expressed as range and mean (SD) or (SE). *: significant (p<0.05); **: highly significant. **HbA1C**: Glycosylated hemoglobin; **HOMA-IR**: hemostasis model assessment-insulin resistance.

Table IV: Genotyping results of TCF7L2 (rs7903146 C/T) SNP among studied groups

	Control group (n= 18)	**Prediabetic group (n= 18)**	**Diabetic group (n= 54)**	**P value**
Genotype				
CC	14 (77.8%)	10 (55.6%)	15 (27.8%)	P1 = 0.14
CT	4 (22.2%)	8 (44.4%)	31 (57.4%)	P2 < 0.001**
TT	0	0	8 (14.8%)	P3 = 0.04*
*Risk allele carrier genotypes (CT+TT)	4 (22.2%)	8 (44.4%)	39 (72.2%)	P1 = 0.06
*Non-risk allele carrier genotype (CC)	14 (77.8%)	10 (55.6%)	15 (27.8%)	P2 < 0.001** P3 = 0.01*
Allele				
C	32/36(88.9%)	28/36 (77.8%)	61/108 (56.5%)	P1 = 0.20
T	4/36(11.1%)	8/36 (22.2%)	47/108 (43.5%)	P2< 0.001** P3 = 0.02*

Data expressed as frequency (percentage). *P*-value was significant if < 0.05. * significant; ** highly significant**. TCF7L2:** transcription factor 7like 2.

Table (V): Association of Genotype and Allele distribution TCF7L2 (rs7903146 C/T) SNP with risk of occurrence of prediabetes.

	Control group (n= 18)	**Prediabetic group (n= 18)**	**P value**	**OR (95%CI)**
Genotype				
CC	14 (77.8%)	10 (55.6%)		Reference
CT	4 (22.2%)	8 (44.4%)	< 0.001**	2.8 (1.658- 11.923)
TT	0	0	-	-
Allele				
C	32/36 (88.9%)	28/36 (77.8%)		Reference
T	4/36 (11.1%)	8/36 (22.2%)	0.03*	2.28 (1.621-8.412)

Data expressed as frequency (percentage). *: significant (p<0.05); **: highly significant.

The risk of occurrence of prediabetes was higher in subjects carrying the CT genotype (OR=2.8, P<0.001).

The risk of prediabetes was prevalent among subjects carrying the T allele with an odds ratio of 2.28 (95% CI 1.621-8.412, P value 0.03) (Table V).

Table (VI): Association of Genotype and Allele distribution of TCF7L2 (rs7903146 C/T) SNP with risk of occurrence of DM

	Control group (n= 18)	**Diabetic group (n= 54)**	**P value**	**OR (95%CI)**
Genotype				
CC	14 (77.8%)	15 (27.8%)		Reference
CT	4 (22.2%)	31 (57.4%)	0.003**	7.02 (2.02-25.77)
TT	0	8 (14.8%)	0.99	1.17 (0.23-3.45)
*Risk allele carrier genotypes (CT+TT)	4 (22.2%)	39 (72.2%)	<0.001**	9.1 (2.58 -32.11)
*Non-risk allele carrier genotype (CC)	14 (77.8%)	15 (27.8%)		Reference
Allele				
C	32/36 (88.9%)	61/108 (56.5%)		Reference
T	4/36 (11.1%)	47/108 (43.5%)	<0.001**	6.164 (2.038-18.645)

Data expressed as frequency (percentage). *: significant (p<0.05); ** highly significant.

The risk of occurrence of DM was higher in subjects carrying the CT genotype (OR=7.02, p<0.003) than in the TT genotype (OR=1.17, p=0.99). Also, the risk of occurrence of DM was higher in subjects carrying risk allele carrier genotypes (CT+TT) (OR=9.1, p<0.001).

The risk of occurrence of DM was higher in subjects carrying the T allele 164 (OR=6.164, p<0.001) (Table VI).

Table (VII): Genotype and allele distribution of TCF7L2 (rs7903146 C/T) SNP among the diabetic group according to the onset of diabetes

	Diabetic group (n = 54)		**P value**	**OR (95% CI)**
	Early onset < 45 years (n= 38)	**Late onset ≥ 45 years (n= 16)**		
Genotype				
CC	6 (15.8%)	9 (56.2%)		Reference
CT	25 (65.8%)	6 (37.5%)	0.01*	6.25 (1.59-24.45)
TT	7 (18.4%)	1 (6.3%)	0.02*	10.5 (1.015- 36.12)
*Risk allele carrier genotypes (CT+TT)	32 (84.2%)	7 (43.8%)	< 0.001**	6.857 (1.836-25.606)
*Non-risk allele carrier genotype (CC)	6 (15.8%)	9 (56.2%)		Reference
Allele				
C	37/76 (48.7%)	24/32 (75%)		Reference
T	39/76 (51.3%)	8/32 (25%)	0.03*	3.162 (1.263-7.918)

Data expressed as frequency (percentage). *: significant (p<0.05); ** highly significant.

The result showed that the CT and TT genotypes and risk allele carrier genotypes (CT+TT) had significantly higher frequency in diabetic group with early onset of DM (<45 years) compared to diabetic group with late onset of DM (≥ 45 years), non risk allele carrier genotype (CC) had significantly higher frequency in diabetic group with late onset of DM (≥ 45 years) compared to diabetic group with early onset of DM (<45 years) (P=0.01, 0.02 and <0.001 respectively). Also, the C allele was considerably prevalent in the diabetic group with late onset of DM (≥ 45 years) compared to the diabetic group with early onset of DM (<45 years). Also, the T allele was substantially higher in the diabetic group with early onset of DM (<45 years) compared to the diabetic group with late onset of DM (≥ 45 years) (P=0.03).

The risk of occurrence of DM early (< 45years) was higher in subjects carrying the TT genotype (OR=10.5, P=0.02) than in subjects carrying the CT genotype (OR=6.25, P=0.01). Also, the risk of occurrence of DM early (< 45years) was higher in subjects carrying risk allele carrier genotypes (CT+TT) (OR=6.857, P<0.001).

The risk of occurrence of DM early (< 45years) was higher in subjects carrying the T allele with an odds ratio of 3.162 (OR=3.162, P=0.03) (Table VII).

Table (VIII): Association between different genotypes of TCF7L2 (rs7903146) SNP and laboratory parameters in diabetic patients.

	Non-risk allele carrier genotype (CC) (n= 10)	**Risk allele Carrier genotypes (CT + TT) (n= 8)**	***P* value**
S. urea (mmol/l)			
Range	3.7-5.7	4.0-6.0	0.75
Mean ± SE	4.37 ± 0.33	4.92 ± 0.51	
S. creatinine (µmol/l)			
Range	65-105	58-118	0.36
Mean ± SE	80.6 ± 3.73	86.8 ± 6.32	
S. uric acid (mg/dl)			
Range	3.4-6.4	4-7.3	0.72
Mean ± SD	5.25 ± 0.94	5.28 ± 1.08	
eGFR (ml/min)			
Range	90.5-115	96.3-125	0.11
Mean ± SE	110.8 ± 10.25	107.8 ± 7.57	
Albumin/Creatinine ratio (mg Albumin/gm Creatinine)			
Range	4.3-25	6-29.3	0.26
Mean ± SE	12.45 ± 2.17	13.85 ± 3.16	
Fasting serum Glucose (mmol/l)			
Range	5.9-6.3	5.7-6.9	0.78
Mean ± SD	5.97 ± 0.61	6.01 ± 0.57	
Glycosylated hemoglobin HbA1C (%)			
Range	5.7-6.4	5.9-6.4	0.49
Mean ± SD	6.16 ± 0.23	6.11 ± 0.18	
Fasting serum Insulin (mU/L)			
Range	2.08-36.5	8-132.78	0.02*
Mean ± SE	15.67 ± 3.01	45.63 ± 14.6	
HOMA-IR			
Range	0.97-9.41	2.2-34	0.01*
Mean ± SE	4.19 ± 0.82	12.54 ± 3.9	

Data expressed as range and mean (SD) or (SE). *: significant (P<0.05); ** highly significant.

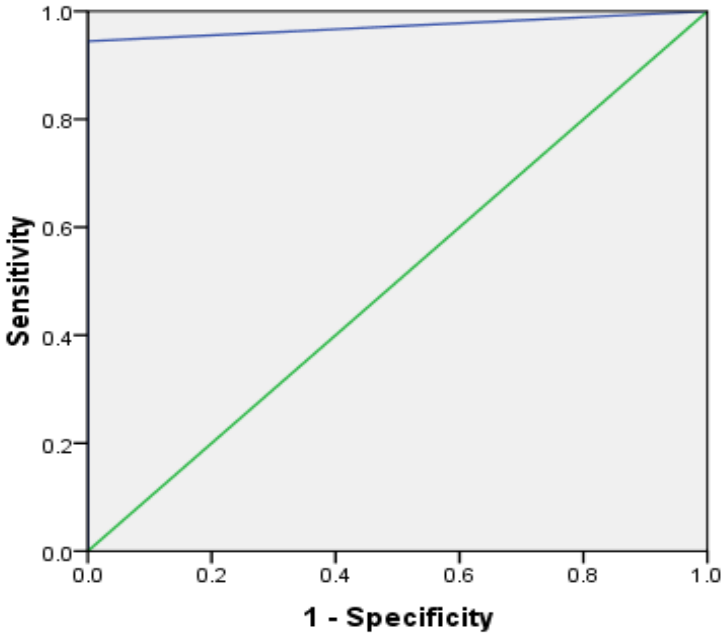

Figure (2): ROC curve of HOMA-IR as a predictor of prediabetes.

Fasting serum insulin and HOMA-IR were significantly elevated in risk allele carrier genotypes (CT+TT) compared to non-risk allele carrier genotype (CC) (P= 0.02 and 0.01, respectively) (Table VIII).

Table (IX): Diagnostic performance of HOMA-IR in the prediction of prediabetes

	HOMA-IR
Cut-off point	> 1.5
Area under the curve	0.97
Accuracy	97.2%
Sensitivity	94.4%
Specificity	100%
Positive predictive value	100%
Negative predictive value	94.7%
P value	< 0.001**

*: significant (P<0.05); ** highly significant

Receiver Operating Characteristic (ROC) was applied to assess the sensitivity of HOMA-IR for predicting prediabetes compared to healthy controls and to identify the ideal diagnostic cut-off value. The ROC curve was constructed to compare their diagnostic performance, where the higher area under the curve (AUC) indicates a better diagnostic test.

HOMA-IR: At a cut-off value > 1.5, HOMA-IR was 97.2 % accurate, 94.4 % sensitive, and 100 % specific for the diagnosis of prediabetes, with an area under the curve of 0.97, a positive predictive value of 100 % and a negative predictive value of 94.7 % (P<0.001) (Table IX and Figure 2).

Discussion

Diabetes Mellitus, a common endocrine disorder, results from insulin resistance and inadequate secretion or absolute deficiency of insulin**.** DM is associated with macrovascular and microvascular complications. Microvascular complications like diabetic retinopathy, nephropathy, and peripheral neuropathy **(14).**

We aimed to study the distribution of TCF7L2 (rs7903146 C/T) SNP in controls, prediabetic and diabetic patients, and its association with biochemical parameters. To measure the level of AGEs in controls, prediabetic, and diabetic patients and their correlation with biochemical parameters and demographic data.

In this study, the mean age in diabetic and prediabetic groups was statistically significantly higher than in controls. Also, the diabetic group had statistically significantly higher mean age compared to the prediabetic group. This was consistent with **Kurniawan and Kusrini et al. (2020),** who reported that 15.6 % of the diabetic group were less than 40 years old and 44.6% were more than 40 years old **(9)**. **Sagawah et al. (2021)** reported that in individuals aged 40-59, the ability of pancreatic beta cells to produce insulin begins to decline according to the aging process, decreased physical activity, and increased sedentary time **(16)**.

In the present study, 46.3 % of patients were male, while females represented 53.7 %. **Rahim et al. (2023)** reported that females showed an insignificantly higher disease prevalence rate than males **(15)**. This difference was attributed to females being less physically active than males, consuming foods containing chocolate, sugar, and fast-food snacks. This little physical activity causes the body not to use a lot of carbohydrates or glucose and triggers metabolic diseases such as diabetes (**9)**.

The mean value of BMI revealed a significant elevation in diabetic and prediabetic groups compared to the control group, with no significant difference in the other studied groups. Our findings were similar to **Sagawah et al.'s (2021) study,** which showed that the relative risk for T2DM in adults increased markedly with increasing BMI over 30 kg/m2 **(16)**.

In this work, the frequency of smoking was considerably higher in diabetic and prediabetic groups than in controls. This was aligned with **Rahim et al.'s (2023)** study, as they reported that the incidence of smoking was higher in DM patients. Because tobacco exposure and hyperglycemia interact to worsen kidney degeneration through mechanisms of atherosclerosis, oxidative stress, hyperlipidemia, and prolonged sympathetic activity, smoking was correlated with an increased risk of developing DM**.**

In this work, the percentage of family history of DM was statistically dominant among prediabetics and diabetics compared to the control group. Additionally, the percentage of family history was significantly elevated in diabetic cases with nephropathy than in those without it. This difference was attributed to genetic risk factors (multi-SNP genetic risk factor (**15).**

SBP and DBP were significantly higher in the diabetic group than in controls, which was aligned with **Aboelkhair et al. (2021),** who stated that blood pressure was significantly elevated in T2DM patients compared to healthy individuals (1). Because they have several common causes, such as a sedentary lifestyle with high calorie intake, obesity, inflammation, oxidative stress, and insulin resistance, diabetes and hypertension frequently coexist. **(19)**.

eGFR was statistically significantly lower in the diabetic group than in the control and prediabetic groups. Our results were consistent with those of **Rabia Ali et al. (2021),** who reported that higher ACR values and lower eGFR were found in diabetic patients with nephropathy than those without it. This is explained by thickening of the GBM and diffuse or nodular mesangial expansion. These structural changes correlated with the level of ACR and GFR in T2DM **(18)**.

Lipid profile revealed that serum triglycerides and LDL-C were considerably

elevated in diabetic and prediabetic groups compared to controls, with no significance in other groups. Dyslipidemia has been attributed to insulin resistance. Increased hepatic secretion of very low-density lipoprotein and delayed elimination of TG-rich lipoproteins, mainly resulting from elevated substrate levels of TG synthesis, were the causes of elevated triglyceride levels in diabetics and prediabetics **(1)**.

Insulin is essential for glucose uptake by cells; any disruption in the transduction of the insulin signal is linked to hyperglycemia. The majority of metabolic diseases, including obesity, dyslipidemia, metabolic syndrome, hypertension, atherosclerosis, nonalcoholic fatty liver disease (NAFLD), T2DM, and some forms of T1DM, share insulin resistance. **(10)**. Any abnormalities in the expression or function of these agents may hinder proper insulin signaling, resulting in IR in peripheral tissues, because insulin signal transduction is a complex process that involves several enzymes and modulatory proteins. A complex condition known as insulin resistance occurs when cells that depend on insulin cannot react appropriately to normal amounts of insulin in the blood **(17)**.

We found that fasting insulin and HOMA IR were significantly raised in diabetic and prediabetic groups compared to controls, which was similar to **Sagawah et al. (2021),** who described that fasting insulin and HOMA IR were significantly higher in individuals with T2DM than those free of DM. HOMA-IR was a key indicator of IR, the primary cause of T2DM. IR was characterized by increased insulin production by the pancreatic β-cells to compensate for the hyperglycemia, which resulted in hyperinsulinemia. This compensated phase of insulin resistance involves upregulation of β-cell function, higher insulin level, and maintained blood glucose concentration. If compensatory insulin secretion failed, fasting glucose or postprandial glucose concentrations increased, and T2DM occurred.

The higher your HOMA-IR score, the more problematic your IR. The main causes of IR and high HOMA-IR were overeating and being inactive. Lifestyle changes could improve IR and prevent T2DM (**6)**. We evaluated the diagnostic performance of HOMA-IR for the prediction of prediabetes. Our results showed that HOMA-IR was more sensitive (94.4%) with an AUC of 0.97. Our results agreed with **Lin et al. (2021)** as they reported that the AUC of the HOMA-IR ROC curve was 0.81 and had high predictive values for prediabetes (10).

The TCF7L2 gene was positioned on chromosome 10q25.3. A crucial part of the Wnt-signaling system, TCF7L2, controls pancreatic beta cells' insulin release and preserves glucose homeostasis. Controlling the growth of endothelial cells and the proliferation of smooth muscle cells also contributes to vascular remodeling. TCF7L2 is extensively expressed in peripheral and omental adipocytes and mature pancreatic beta cells (2). T2DM is more likely to be caused by SNP rs7903146 C/T, which is mediated by lower insulin secretion linked to or unrelated to abnormalities in insulin processing, diminished effects of GLP-1, triggering hepatic glucose synthesis, and IR **(8).**

We found that the non-risk allele carrier genotype (CC) frequency was considerably higher in control group (77.8%) than prediabetic and diabetic groups (55.6% and 27.8% respectively), on the other hand, CT and TT genotypes frequencies were significantly higher in prediabetic (44.4% and 0 respectively) and diabetic groups (57.4% and 14.8% respectively) compared to control group (22.2% and 0 respectively). Risk allele carrier genotypes (CT+TT) frequency was considerably higher in prediabetic and diabetic groups compared to the control group.

Likewise, **Bahaaeldin et al. (2020)** reported that in T2DM cases, 39.6% had the CC genotype, 57.1% had the CT genotype, and 4.3% had the TT genotype (5). Conversely, 46.7% of the controls were the CC genotype, 53.3% were the CT genotype, and no one in the control group had the TT genotype (5). A study by Sagawah et al. (2021) (16) reported that the CC genotype

was more frequent in controls than in T2DM cases and that CT and TT genotypes were higher in patients with T2DM than in controls. Similarly, **Mustafa and Younus et al. (2021)** informed that the percentage of the CC genotype in the control group was substantially more than that of the diabetic group, and the CT genotype exhibited a very high frequency among DM patients when compared to controls, and the TT genotype was rare in their population (13).

In this study, subjects with one risk allele (CT) have a 7.02-fold risk of T2DM compared to those without any risk allele (homozygous non-carrier, CC). Subjects carrying both risk alleles (homozygous carrier, TT) have about 1.17-fold risk of developing T2DM compared to those without any risk allele (homozygous non-carrier, CC). These results were higher than those in **Sagawah et al. (2021)**, which had an OR of 4.00, explained by genetic variation among different ethnicities and sample size among studies.

Our study showed that the C allele was significantly frequent in the control group (88.9%) compared to the prediabetic and diabetic groups (77.8% and 56.5% respectively). The T allele was significantly more frequent in prediabetic and diabetic groups (22.2% and 43.5% respectively) compared to controls (11.1%). The risk of T2DM was higher in the risk allele (T) than the non-risk allele (C) (OR=6.164).

The same was reported by **Sagawah et al. (2021)**; they found that T allele frequency was higher in patients with T2DM than in controls, and the T allele increased the risk of T2DM compared to the C allele (OR=2.07). **Mustafa and Younus et al. (2021)** (13)found that T allele frequency was significantly higher in T2DM patients than in controls. **Madhu et al. (2022)** reported that the T allele is still significantly higher in the T2DM group than in the normal glucose tolerance group (11).

We found that risk allele carrier genotypes (CT+TT) of the rs7903146 SNP of the TCF7L2 gene showed significantly higher A/C ratio, AGEs, and HOMA-IR levels, and significantly lower eGFR level, compared with non-risk allele carrier genotype (CC). Our observations were in alignment with **Madhu et al. (2022) and Maghraby et al. (2022)**, as they detected that risk allele carrier genotypes (CT+TT) of rs7903146 showed significantly higher AGEs, ACR, and HOMA-IR, which was a marker of higher insulin resistance compared to the CC genotype (12).

Our study showed that the non-risk allele carrier (CC) genotype had a significantly higher frequency in late onset DM cases (≥ 45 years), CT, TT, and risk allele carrier (CT+TT) genotypes had a significantly higher frequency in early onset DM cases (< 45 years). The risk of occurrence of DM early (< 45years) was higher in the TT genotype (OR=10.5, P=0.02) than in CT (OR=6.25, P=0.01). Our result was the same as that by **Akhormeh et al. (2018),** who reported that the frequency of the TT genotype was higher in patients with early onset of diabetes than those with late onset, and the TT genotype was at a higher risk for the occurrence of diabetes earlier in life (OR=5.4) **(3)**.

TCF7L2 (SNP rs7903146 C/T) SNP is linked to diminished insulin secretion, related or not to insulin processing disturbance, reduced effects of GLP-1, elevated hepatic glucose release, and insulin resistance **(16).**

Financial Support:

No financial support.

Conflicts of Interest:

There are no conflicts of interest.

Ethical Considerations:

The Institutional Review Board (IRB) of the Faculty of Medicine at Assiut University approved this work before it was carried out (IRB no: 17200364). Every participant also gives their informed consent. Additionally, each participant was given a code number for analysis purposes, ensuring participant confidentiality. There were no incentives for the participants in the study.

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