GinPolMedProject ©
01 (69) 2025 : 001-006 • RESEARCH ARTICLE

Genetic insights into type 2 diabetes mellitus: Evaluating the role of TLR4 (Asp299Gly) polymorphism in an Iraqi women

Ahmed J. Mohammed[1,2,*], Akeel Abo Alard[3], Balsam G. Hassan[4]

[1]Department of Clinical Laboratory Sciences, College of Pharmacy, University of Kufa, Kufa, Iraq

[2]Department of Clinical Laboratory Sciences, College of Pharmacy, The Islamic University, Najaf 54001, Iraq

[3]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Kufa, Kufa, Iraq

[4]Department of Pharmacy, Al-Zahrawi University College, Karbala, Iraq

SUMMARY

AUTHORS' CONTRIBUTION: (A) Study Design · **(B)** Data Collection. **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** Funds Collection

Background: Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder with significant genetic contributions, including Toll-Like Receptor 4 (TLR4) gene polymorphisms such as Asp299Gly, which may influence disease susceptibility and progression.

Objectives: This study aims to evaluate the association of TLR4 (Asp299Gly) gene polymorphism with T2DM and its impact on clinical parameters in an Iraqi women.

Methods: A case-control study was conducted with 200 female participants (100 women with Type 2 Diabetes Mellitus (T2DM) and 100 healthy controls) aged 35–60 years. Clinical and biochemical parameters, including fasting plasma glucose (FPG), HbA1c, insulin levels, HOMA IR, QUICKI and lipid profiles, were measured. Genotyping for TLR4 (Asp299Gly) gene polymorphism was performed using PCR and restriction enzyme analysis.

Results: The AG genotype showed an Odds Ratio (OR) of 1.33 (P=0.36), while the GG genotype had an OR of 4.51 (P=0.18). The G allele frequency was higher in T2DM patients (20.5%) than controls (15%), with an OR of 1.53 (P=0.1). Clinical parameters did not differ significantly among TLR4 genotypes, except for HDL levels, which were higher in the GG genotype (P=0.04).

Conclusion: There was no association between the Toll-like receptor 4 (TLR4) Asp299Gly polymorphism and type 2 diabetes mellitus (T2DM) in the Iraqi women.

Keywords: Type 2 Diabetes Mellitus; Toll-Like Receptor 4 (TLR4); Asp299Gly polymorphism; Genetic predisposition; Iraqi Women

Address for correspondence:

Asst. Prof. Dr. Ahmed J. Mohammed,
Department of Clinical Laboratory Sciences, College of Pharmacy,
University of Kufa, Kufa, Iraq

Word count: 2799 **Tables:** 04 **Figures:** 00 **References:** 39

Received: 25.01.2025, Manuscript No. gpmp-25-159981; **Editor assigned:** 27.01.2025, PreQC No. P-159981; **Reviewed:** 11.02.2025, QC No. Q-159981; **Revised:** 28.02.2025, Manuscript No. R-159981; **Published:** 31.03.2025

INTRODUCTION

Diabetes Mellitus (DM) is a chronic disorder marked by persistent hyperglycemia due to impai insulin secretion, action, or both. It affects fat, carbohydrate, and protein metabolism [1]. Globally, it remains a major public health challenge, with 537 million adults affected as of 2021, projected to rise to 783 million by 2045. DM accounts for 10% of global health spending, emphasizing its economic burden [2]. Over 2 million Iraqis have diabetes, with a T2DM prevalence of 13.9% [3].

Generally, there is different types of Diabetes mellitus includes primarily Type 1 Diabetes Mellitus (T1DM), Type 2 Diabetes Mellitus (T2DM), Gestational Diabetes Mellitus (GDM), along with other rarer forms [4]. Normally, T1DM classfied as autoimmune disorder and represent 5–10% of accounting cases. In T1DM, the T-cell act on destruction of pancreatic β cells and this in turn cause sudden symptoms like polyuria, polydipsia, and weight loss [5]. On the other hand, about 90% of diabetes cases related to T2DM. This type arises from β-cells dysfunction and insulin resistance, pominatingly linked to obesity, genetic pisposition, sedentary lifestyle and inflammation. Usually, T2DM associated with other chronic complications such as atherosclerosis, nephropathy, neuropathy and retinopathy [6].

Usually, T2DM development and progression are intricately connected to genetic factors, with heritability estimates ranging between 20% and 80% [7]. Disease susceptibility significantly influenced by specific genetic polymorphisms, with distinguished examples involving KCNJ11 [8], PPARG [9], TLR4 [1] and TCF7L2 [10].

The insulin resistance which is mediated by Inflammation also has a genetic component as variations in TLR4 (such as Asp299Gly) have the ability to modulate the body's inflammatory response [11]. Also, Toll-like receptors, especially TLR4, are critical for interaction between oxidative stress, glucolipotoxicity and inflammation, this in turn exacerbate insulin resistance [12]. A valuable insight in pathophysiology of T2DM can be providing by these genetic factors. Additionally, these genetic factors highlight the valuable and importance of understanding individual variability in disease risk [13].

Recently, there is increasingly used of genetic insights to early pict the risk of T2DM and thus guide early interventions [14]. Additionally, using tools such as genome-wide association studies (GWAS) could help greatly in identify individuals with a genetic susceptibility, and thus in turn can allowing for personalized prevention

strategies such as targeted lifestyle changes and tailo therapy to assist in prevent or delay the onset of disease [15]. Moreover, genetic profiling act on enhances the ability to monitor and manage diabetes risk comprehensively, in combined with traditional diagnostic measures such as fasting plasma glucose tests and HbA1c [16].

In regard to pathogenesis of Type 2 Diabetes Mellitus, the chronic low-grade inflammation plays a critical role, with the TLR4-NF-κB signaling pathway acting as a major mediator [17]. Activation of TLR4 acting on initiates inflammatory cascades and thus in turn leads to exacerbate β-cell dysfunction and insulin resistance. The complex relationship between TLR4 gene polymorphisms such as Asp299Gly and an individual's at risk to T2DM highlighted through several genetic studies [18]. It was found that TLR4 gene polymorphisms usually associated with alte metabolic pathways and immune response, and thus potentially serving as markers for early diagnosis and risk stratification in individuals susceptibility to T2DM [19]. Therefore, understanding these genetic polymorphisms supply an opportunity to improve diagnostic accuracy and enhance pict disease onset in susceptible population [20].

This study aims to investigate the relationship between the TLR4 gene (Asp299Gly) polymorphism and Type 2 Diabetes Mellitus in Iraqi womn. Through identifying genetic variations associated with T2DM susceptibility and its clinical characteristics, this study seeks to contributed in understanding of disease pathophysiology and improving the potential for region-specific diagnostic strategies. The findings of this study may represent a basis for developing more accurate tools which can be used for early diagnosis and for risk assessment of T2DM in the Iraqi women.

SUBJECTS AND METHODS

This case-control study was carried out at Diabetes Center of Al-Sadder Teaching Hospital, Najaf governorate, Iraq, over an eight months peroid (January to August 2024) . This study involved two hund females participants, including 100 women (patients group) with type 2 diabetes mellitus, between 35 to 60 years old. These patients were selected randomly from those visiting the Diabet Center for clinical routine examination and glucose level monitoring. In addition, 100 healthy women (control group), aged between 35 and 60 years were randomly chosen and involved in the study.

Inclusion criteria: This study involved females with type 2 diabetes mellitus, identified by a fasting plasma glucose level of 126 mg/dL or higher, along with typical diabetic patient symptoms. Moreover, only subjects who had been diagnosed with type 2 diabetes by an endocrinologist were enrolled in the study.

Exclusion criteria: The study excluded people aged 34 or younger, patients with type 1 diabetes mellitus or who treated with insulin. Furthermore patients with heart problems, like cardiomyopathy, heart failure, and congenital heart disease. Additionally, individuals with immune diseases, cancer, severe hepatic or kidney disorders, pregnant women, and those taking glucocorticoids were not qualified for participate.

Materials

The kits and chemicals used in the study provided from Global sources, such as, Diamond safe stain from (USA) Promega company, agarose powder from (Spain) Condalab, and Taq Plus PCR smart mix kit korean orgin from SolGent company. Additionally, kits for cholesterol, DNA extraction, glucose, HbA1c, HDL, human insulin, and triglycerides were sourced from Linear (Spain), Favorgen (Taiwan), i-sens (South Korea), Tosoh (Japan), and Linear (Spain) respectively. Other materials included primers from Alpha DNA (USA), proteinase K from Peakendness (China), restriction enzymes from Promega (USA), Safe-Green Opti-DNA Marker from ABM (Canada), and Tris BE buffer (10X) from Promega (USA).

Methodology

After excluding non-respondent patients or those who met the above-mentioned exclusion criteria, all study participants including women with Type 2 Diabetes Mellitus and healthy female controls provided signed informed consent. The subjects were then refer for blood sampling, as well as phenotypic and genotypic analysis, at the Laboratory of the Clinical Laboratory Sciences Department, College of Pharmacy, Kufa University.

Phenotypic analysis: Data on age, body mass index, smoking history, family history of T2DM, and chronic medication use were collected for each participant. A blood sample was obtained from all subjects and divided into two portions. One portion was used to test fasting blood sugar, serum lipid profile, and serum insulin levels, while the other was used for genetic analysis of the TLR-4 gene. HbA1c levels were measu using the Finecare™ HbA1c (Hemoglobin A1c) Rapid Quantitative Test. Serum measurements were taken immediately after sample collection.

Genotyping measurements: DNA was extracted from the blood samples of all T2DM patients and control group using the Favor Prep™ Blood Genomic DNA kit (Favorgen). The concentration and purity of the DNA samples were assessed using a Nanodrop device. A Polymerase Chain Reaction (PCR) was performed to amplify a specific genomic region using a T-professional thermocycler, manufactu by Biometra (Germany). Primers, provided by Alpha-DNA as lyophilized powder, were used in this study, and the primer sequence was F (forward): 5′-GAT TAG CAT ACT TAG ACT ACT ACC TCC ATG-3′ and R (reverse): 5′-GAT CAA CTT CTG AAAAA GCA TTC CCAC-3′. The 2x Taq Plus PCR Smart Mix kit from SolGent (South Korea) was utilized for the reaction.

The PCR conditions were optimized to ensure efficient DNA amplification. The process began with an initial denaturation cycle at 95 ℃ for 5 minutes, followed by 30 cycles of denaturation at 95 ℃ for 30 seconds, annealing at 60 ℃ for 30 seconds, and extension at 72 ℃ for 30 seconds. The final synthesis step was performed at 72 ℃ for 10 minutes. After amplification, the amplicon was digested with the NcoI restriction enzyme (10 U/µl) for the Asp299Gly variant, incubated at 37 ℃ for 1 hour. This enzyme, obtained from Promega Corporation, facilitated the cutting process for further analysis. The digested product was then separated by 2% agarose gel electrophoresis (Condalab, Canada), stained with

Diamond Nucleic Acid Dye for visualization under UV light.

Ethical consideration

The study design was approved by the Scientific Committee for Research Ethics at the Faculty of Pharmacy, Kufa University (Approval No: 211#16-12-2022).

Patient consent

The study protocols were communicated to all participants, and a verbal and written informed consent was obtained from those who agreed to participate before sample collection.

Statistical analysis

Continuous variables were expressed as mean ± Standard Deviation (SD). Differences in means between the T2DM and healthy groups were analyzed using Student's t-test in SPSS version 26 and Microsoft Office Excel 2019. The Hardy-Weinberg equilibrium was evaluated using the goodness-of-fit chi-square test. Odds ratios were determined through logistic regression analysis. A p-value of less than 0.05 was deemed statistically significant.

RESULTS

The comparison of serum lipid profiles between T2DM patients and the control group revealed significant differences ($p \leq 0.0001$) in all measu parameters (**Tab. 1.**). T2DM patients exhibited markedly higher levels of triglycerides (249.04 ± 24.38 mg/dl), cholesterol (221.42 ± 38.54 mg/dl), LDL (127.68 ± 27.37 mg/dl), and VLDL (48.28 ± 6.26 mg/dl) alongside lower levels of HDL (35.27 ± 6.83 mg/dl), compa to the control group (triglycerides: 127.52 ± 29.59 mg/dl, cholesterol: 163.58 ± 27.19 mg/dl, LDL: 82.19 ± 27.95 mg/dl, HDL: 55.34 ± 10.67 mg/dl, VLDL: 25.31 ± 5.92 mg/dl).

The comparison between the T2DM patients and the control group was conducted to assess key metabolic markers associated with diabetes, **Tab. 2.** The results revealed significant differences in Fasting Plasma Glucose (FPG), HbA1c, plasma insulin levels, insulin resistance (HOMA-IR), and QUICKI index. The T2DM group had markedly higher FPG (233.25 ± 31.44 mg/dl), HbA1c (9.17 ± 1.53%), plasma insulin levels (23.28 ± 6.89 mIU/L), and HOMA-IR (9.59 ± 5.27) compa to the control group, which showed lower values (80.06 ± 16.43 mg/dl, 5.38 ± 0.52%, 13.36 ± 4.27 mIU/L, and 2.64 ± 0.93, respectively). The QUICKI index was significantly lower in the T2DM group (0.28 ± 0.013) than in controls (0.33 ± 0.02), all with p-values < 0.0001, indicating a strong association between these markers and type 2 diabetes.

The genotype and allele frequencies of the TLR4 (Asp299Gly) polymorphism and its association with T2DM risk are presented in **Tab. 3.**, which reveals no association between the TLR4 (Asp299Gly) polymorphism and T2DM. The AG genotype (33% in T2DM vs. 28% in controls) showed an OR of 1.33 (95% CI: 0.72–2.44; P=0.36), while the GG genotype (4% in T2DM vs. 1% in controls) had an OR of 4.51 (95% CI: 0.49–41.4; P=0.18). For alleles, the G allele frequency was higher in T2DM (20.5%) compa to controls (15%), with an OR of 1.53 (95% CI: 0.91–2.56; P=0.1).

The study investigated the impact of Toll-like 4 receptors gene (Asp299Gly) polymorphism on various clinical parameters in type 2 diabetes patients. The results are summarized in the **Tab. 4.** The results from the table reveal no significant differences in clinical characteristics such as BMI, FBS, HbA1c, insulin levels, cholesterol,

Tab. 1. Comparison of serum lipid profile between Type 2 Diabetes Mellitus (T2DM) patients and healthy controls.

Serum Lipid Profile	Patients (*n* = 100)	Control (*n* = 100)	P
Triglyceride (mg/dl)			
Mean ± SD	249.04 ± 24.38	127.52 ± 29.59	0.0001
Cholesterol (mg/dl)			
Mean ± SD	221.42 ± 38.54	163.58 ± 27.19	0.0001
HDL (mg/dl)			
Mean ± SD	35.27 ± 6.83	55.34 ± 10.67	0.0001
VLDL (mg/dl)			
Mean ± SD	48.28 ± 6.26	25.31 ± 5.92	0.0001
LDL (mg/dl)			
Mean ± SD	127.68 ± 27.37	82.19 ± 27.95	0.0001

Tab. 2. Comparison of fasting plasma glucose, HbA1c, insulin level and insulin resistance between T2DM and control group.

Descriptive Statistics	Patients (*n* = 100)	Control (*n* = 100)	P
FPG (mg/dl)			
Mean ± SD	233.25 ± 31.44	80.06 ± 16.43	0.0001
HbA1c			
Mean ± SD	9.17 ± 1.53	5.38 ± 0.52	0.0001
Plasma insulin level mIU/L			
Mean ± SD	23.28 ± 6.89	13.36 ± 4.27	0.0001
HOMA-IR index			
Mean ± SD	9.59 ± 5.27	2.64 ± 0.93	0.0001
QUICKI			
Mean ± SD	0.28 ± 0.013	0.33 ± 0.02	0.0001

Tab. 3. Association of toll-like receptor 4 (Asp299Gly) gene polymorphism with Type 2 diabetes mellitus: genotype and allele distribution.

(rs4986790)	T2DM, n (%)	Control, n (%)	OR (95% CI)	P- value
Genotypes				
AA	63(63%)	71 (71%)	Reference	
AG	33(33%)	28 (28%)	1.33(0.72-2.44)	0.36
GG	4(4%)	1 (1%)	4.5079(0.49-41.4)	0.18
Total (N)	**100**	**100**		
Alleles				
A	**159(79.5%)**	**170(85%)**		
G	**43(20.5%)**	**30(15%)**	1.53(0.91-2.56)	**0.1**
Total (2N)	**200**	**200**		

Tab. 4. The relation between toll-like 4 receptors gene (Asp299Gly) (A>G) gene polymorphism genotypes and the investigated parameters in patients group under the codominant model.

Clinical Characteristic	Genotype M ± ST.DE.			P V
	AA (n=63)	AG (n=31)	GG (n=6)	
BMI	32.82 ± 7.9	34.14 ± 8.3	33.31 ± 8.1	0.75
FBS (mg/dl)	244.08 ± 31.33	237.64 ± 31.16	232.27 ± 32.38	0.49
HbA1c	9.42 ± 1.72	9.03 ± 1.25	9.18 ± 1.55	0.52
Insulin(*μ*IU/mL)	22.07 ± 6.94	23.17 ± 7.25	22.61 ± 6.43	0.77
Cholesterol (mg/dl)	227.25 ± 41.21	222.07 ± 40.06	211.43 ± 40.25	0.60
HDL (mg/dl)	34.60 ± 6.82	38.18 ± 7.11	38.52 ± 5.14	0.04
Triglycerides(mg/dl)	255.05 ± 23.06	246.51 ± 24.62	243.72 ± 25.14	0.18
VLDL (mg/dl)	49.62 ± 5.62	47.84 ± 6.94	46.58 ± 6.09	0.26
LDL (mg/dl)	134.17 ± 27.05	133.22 ± 21.57	121.27 ± 26.37	0.49
HOMA IR	9.16 ± 5.92	10.36 ± 5.43	9.66 ± 5.64	0.63
QUICKI	0.258 ± 0.018	0.255 ± 0.014	0.257 ± 0.012	0.71

triglycerides, VLDL, LDL, HOMA-IR, and QUICKI between the genotypes (AA, AG, and GG) of the Toll-like 4 receptor (Asp299Gly) gene polymorphism. The p-values for these parameters were all above the 0.05 threshold, indicating that genotype does not influence these clinical markers in the studied patients.

However, a notable finding was observed for HDL levels, where the GG genotype showed significantly higher HDL levels (38.52 ± 5.14 mg/dl) compa to the AA and AG genotypes (34.60 ± 6.82 mg/dl and 38.18 ± 7.11 mg/dl, respectively) with a p-value of 0.04. Despite this, other lipid parameters, including triglycerides, VLDL, and LDL, did not exhibit any significant differences between the genotypes.

DISCUSSION

Genome-wide association studies (GWAS) have identified over 60 SNPs associated with T2DM risk, aiding high-risk individual identification [21].

Toll-like receptor 4 (TLR4) detects Gram-negative bacteria and is linked to insulin resistance (IR) and type 2 diabetes mellitus (T2DM) by upregulating proinflammatory pathways in adipocytes, skeletal muscles, and the liver. TLR4 is overexpressed in pancreatic islet cells under high glucose levels and obesity, contributing to β-cell dysfunction. Lipopolysaccharide (LPS) inhibits insulin gene expression in β-cells *via* TLR4 and NF-κB signaling, with effects observed at endotoxemia-level LPS concentrations, suggesting microbiota-related metabolic disturbances [22,23].

Numerous molecular studies have investigated SNPs in the TLR4 gene, though only a few have focused on various types of diabetes. In Iraq, molecular screening efforts have targeted conditions such as hepatitis C virus (HCV) [18], glaucoma, leukemia, cancer and cardiovascular diseases (CVD), [1,10]. However, no studies on T2DM have been conducted in the Iraqi women.

The findings of this study contribute to the growing body of evidence investigating the association between Toll-Like Receptor 4 (TLR4) Asp299Gly polymorphism and type 2 diabetes mellitus (T2DM). Our results showed a higher frequency of the G allele and GG genotype in T2DM patients compa to controls, though the association did not reach statistical significance. While previous studies have shown conflicting evidence on the role of this polymorphism in T2DM, our findings align with some reports suggesting a potential, albeit weak, genetic pisposition linked to TLR4 variations. Studies like Yin et al.'s meta-analysis reported no significant association between TLR4 polymorphisms and an increased risk T2DM. However, Belforte et al. found that this polymorphism was associated with a uced risk of metabolic disorders, including T2DM and metabolic syndrome [24].

To evaluate the relationship between the TLR4 Asp299Gly polymorphism and the risk of T2DM, a comprehensive analysis of nineteen studies comprising 7150 patients and 9993 healthy controls was conducted. Among these, 8 studies were focused on Asian population while, eleven studies deals with Caucasian population. The findings of these studies revealed that there is no significant association between the TLR4 Asp299Gly polymorphism and T2DM likelihood. The lack of association opens the door to suggest that this specific polymorphism could be not having a direct role that can influence on susceptibility to T2DM across different ethnic groups [25].

In regard to TLR4 rs4986790 SNP, Buraczynska et al. [26] highlighted a GG genotype prevalence of 0.46% in

T2DM patients and 0.13% in control group. However, Singh et al. reported a prevalence of 0.8% [27] and 0.52% [28] in T2DM patients. Moreover, in Indian and Romanian populations, the prevalence of the GG genotype among T2DM cases and controls was reported as 3.01%/3.94% [29], 1.24%/0.76% [30], and 4.54%/5%, respectively [31].

Additionally, some studies found a GG genotype prevalence of 0.87% [32] and 1% [33] in control groups. In our study, the prevalence of the GG genotype was 4% in T2DM cases compa to 1% in controls.

Our lipid profile analysis revealed significant differences between T2DM patients and controls, with T2DM patients exhibiting higher triglycerides, total cholesterol, and Very-Low-Density Lipoprotein (VLDL) levels while having lower High-Density Lipoprotein (HDL) levels. These findings are consistent with previous studies, which have reported a similar pattern of dyslipidemia in T2DM patients [32]. This indicates that T2DM patients typically present with dyslipidemia characterized by atherogenic lipid profiles, a major risk factor for cardiovascular diseases. In our study, the GG genotype was associated with slightly higher HDL levels compa to AA and AG genotypes, a finding that resonates with Taís Silveira Assmann's meta-analysis [34], which suggested protective roles of TLR4 polymorphisms in some ethnic groups. However, there is no significant differences were found in other lipid profile results among genotypes, thus suggesting a limited effect of the rs4986790 polymorphism on the metabolism of lipid in our cohort.

Our analysis of glycemic parameters and insulin resistance indices also highlighted significant differences between T2DM patients and controls. In T2DM patients, elevated values of fasting plasma glucose, fasting plasma insulin levels, HbA1c, and HOMA-IR emphasize the characteristic insulin resistance and poor glycemic control detected in these patients. As well as, the QUICKI index, which was significantly lower in the patients group, represents further supports to the presence of insulin resistance. These findings are compatible with the well-established fact of pathophysiology for Type 2 Diabetes Mellitus, where the resistance to insulin in peripheral tissues, in combined with dysfunction in beta-cell, results in increase blood glucose level and hyperinsulinemia [35]. The findings of this study revealed no significant genotypic differences in glycemic parameters, which contrast with other studies like Al-Fatlawi et al., who reported that TLR4 rs4986790 SNP was associated with uced T2DM risk and improve glycemic outcomes. This discrepancy in findings could be attributed to differences in study populations, sample size, and environmental factors influencing gene expression [36].

On the other hand, in terms of the broader implications of TLR4 gene polymorphisms in inflammatory and metabolic pathways, this study added a lot to the mixed results from previous studies. The TLR4's role as inflammatory mediator and its link to insulin resistance and metabolic dysfunction has been documented in animal studies [37]. Similarly, recent studies have hypothesized that TLR4 expression may intersect with AMP-Activated Protein Kinase (AMPK) signaling, suggesting a potential anti-inflammatory mechanism. While this study didn't directly assess these pathways, the absence of considerable differences in biochemical parameters between genotypes suggests that the TLR4 gene Asp299Gly polymorphism alone may not substantially affects these inflammatory or metabolic processes in our cohort [38]. This aligns seamlessly with meta-analysis of Yin et al.'s [39], which found there is no robust association between TLR4 polymorphisms and increased T2DM susceptibility. However, further studies involving larger, multi-ethnic cohorts and functional analyses are needed to clarify the exact role of TLR4 polymorphisms in T2DM pathogenesis.

CONCLUSION

This study highlights that there was no association between the Toll-like receptor 4 (TLR4) Asp299Gly polymorphism and type 2 diabetes mellitus (T2DM) in the Iraqi population. While the GG genotype of TLR4 showed a trend toward higher HDL levels, other metabolic and clinical markers such as BMI, fasting blood sugar, HbA1c, and insulin resistance were not significantly influenced by the genotypes. The G allele frequency was higher among T2DM patients than controls, suggesting a potential genetic pisposition. These findings contribute to understanding the genetic basis of T2DM and may aid in developing region-specific diagnostic and pictive tools for T2DM risk. Further studies are warranted to validate these results and explore the mechanistic role of TLR4 polymorphisms in T2DM pathophysiology.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Ahmed J. Mohammed: Visualization, Investigation, Methodology, acquisition, and analysis of data. Akeel Abo Alard: Conceptualization and design of the study, Data curation., interpretation of data. Balsem G. Hassan: Writing - original draft – review & editing.

DECLARATION OF COMPETING INTEREST

The authors did not report any conflict of interest in competing financial or personal relations.

FUNDING

The source of this research comes from myself.

REFERENCES

1. **Jasim, Muntadher Noaman, Mohammed AJ.** The impact of toll-like receptor 4 gene polymorphism on therapeutic response to metformin at the crossroad type two diabetes mellitus patients. *GinPolMedProject.* 2024;2:001–5.
2. **Siam NH, Snigdha NN, Tabasumma N, et al.** Diabetes Mellitus and Cardiovascular Disease: Exploring Epidemiology, Pathophysiology, and Treatment Strategies. *Rev Cardiovasc Med*. 2024;25:436.
3. **Abusaib M, Ahmed M, Nwayyir HA, et al.** Iraqi experts consensus on the management of type 2 diabetes/prediabetes in adults. *Clin Med Insights Endocrinol Diabetes*. 2020;13:1179551420942232.
4. **Baynes HW.** Classification, pathophysiology, diagnosis and management of diabetes mellitus. *J diabetes metab*. 2015;6:1–9.
5. **Addissouky TA, Ali MMA, El Sayed IET, et al.** Type 1 diabetes mellitus: retrospect and prospect. *Bull Natl Res Cent*. 2024;48:42.
6. **Oguntibeju OO.** Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. *Int J Physiol Pathophysiol Pharmacol*. 2019;11:45.
7. **Albajy MA, Mihailescu DF.** Mechanisms of development of type 2 diabetes*. Int J Adv Res Biol Sci*. 2023;10:162-179.
8. **Moazzam-Jazi M, Najd-Hassan-Bonab L, Masjoudi S, et al**. Risk of type 2 diabetes and KCNJ11 gene polymorphisms: a nested case–control study and meta-analysis. *Sci Rep*. 2022;12:20709.
9. **Al-Shali KZ, House AA, Hanley AJG, et al.** Genetic variation in PPARG encoding peroxisome proliferator-activated receptor γ associated with carotid atherosclerosis. *Stroke*. 2004;35:2036-2040.
10. **Hassan BG, Ridha MM, Mohammed AJ.** The association of TCF7L2 Gene (rs12255372) single nucleotide polymorphism with type two diabetes mellitus in Al Najaf Governorate. *J Pharm Sci Res.* 2018;10:2163-2165.
11. **Titi-Lartey O, Mohammed I, Amoaku WM.** Toll-like Receptor Signalling Pathways and the Pathogenesis of Retinal Diseases. *Front Ophthalmol*. 2022;2:850394.
12. **Bagnati M**. Identification of Novel Mechanisms of Glucolipotoxicity In Type 2 Diabetes. *Queen Mary University of London*; 2015.
13. **Szabo M, Máté B, Csép K, et al.** Genetic approaches to the study of gene variants and their impact on the pathophysiology of type 2 diabetes. *Biochem Genet*. 2018;56:22-55.
14. **Goodarzi MO, Rotter JI.** Genetics insights in the relationship between type 2 diabetes and coronary heart disease. *Circ Res*. 2020;126:1526-1548.
15. **Strianese O, Rizzo F, Ciccarelli M, et al.** Precision and personalized medicine: how genomic approach improves the management of cardiovascular and neurodegenerative disease. *Genes (Basel)*. 2020;11:747.
16. **Zhang J, Zhang Z, Zhang K, et al.** Early detection of type 2 diabetes risk: limitations of current diagnostic criteria. *Front Endocrinol (Lausanne).* 2023;14:1260623.
17. **Yuan H, Xu G, Liu J, et al.** Astragalus mongholicus Polysaccharides Alleviate Insulin Resistance through Modulation of PI3K/AKT, TLR4/NF-kB Signaling Pathway and Microbiota in Rats with Type 2 Diabetes Mellitus. *J Tradit Complement Med.* 2024.
18. **He W, Rebello O, Savino R, et al.** TLR4 triggered complex inflammation in human pancreatic islets. *Biochim Biophys Acta (BBA)-Molecular Basis Dis*. 2019;1865:86–97.
19. **Azarova I, Klyosova E, Polonikov A.** The link between type 2 diabetes mellitus and the polymorphisms of glutathione-metabolizing genes suggests a new hypothesis explaining disease initiation and progression. *Life.* 2021;11:886.
20. **Weischenfeldt J, Symmons O, Spitz F, et al.** Phenotypic impact of genomic structural variation: insights from and for human disease. *Nat Rev Genet.* 2013;14:125–138.
21. **Al-Fadhel SZ, Al-Ghuraibawi NHA, Mohammed Ali DM, et al.** Serum cytokine dependent hematopoietic cell linker (CLNK) as a predictor for the duration of illness in type 2 diabetes mellitus*. J Diabetes Metab Disord*. 2020;19:959-966.
22. **Assmann TS, Lemos NE, de Almeida Brondani L, et al.** Association between Asp299Gly and Thr399Ile Polymorphisms in Toll-Like Receptor 4 Gene and Type 2 Diabetes Mellitus: Case-Control Study and Meta-Analysis. *J Diabetes Metab.*2014; 5: 427.
23. **Amyot J, Semache M, Ferdaoussi M, et al.** Lipopolysaccharides impair insulin gene expression in isolated islets of Langerhans *via* Toll-Like Receptor-4 and NF-κB signalling. *PLoS One*. 2012;7:e36200.
24. **S Belforte F, Coluccio Leskow F, Poskus E, et al.** Toll-like receptor 4 D299G polymorphism in metabolic disorders: a meta-analysis. *Mol Biol Rep*. 2013;40:3015-3020.
25. **Fan J, Liang R.** Quantitative assessment of TLR4 gene polymorphisms and T2DM risk: A meta-analysis. *Mol Genet Genomic Med*. 2020;8:e1466.
26. **Buraczynska M, Baranowicz-Gaszczyk I, Tarach J, et al.** Toll-like receptor 4 gene polymorphism and early onset of diabetic retinopathy in patients with type 2 diabetes. *Hum Immunol*. 2009;70:121-124.
27. **Semlali A, Al Mutairi M, Oqla Alanazi I, et al**. Toll-like receptor 4 polymorphisms in Saudi population with cardiovascular diseases. *Mol Genet genomic Med*. 2019;7:e852.
28. **Kim YS, Hwang YJ, Kim SY, et al.** Rarity of TLR4 Asp299Gly and Thr399Ile polymorphisms in the Korean population. *Yonsei Med J.* 2008;49:58–62.
29. **Beijk MAM, Boekholdt SM, Rittersma SZH, et al.** Toll-like receptor 4 gene polymorphisms show no association with the risk of clinical or angiographic restenosis after percutaneous coronary intervention. *Pharmacogenet Genomics.* 2010;20:544-552.
30. **Wang XH, Ma AG, Han XX, et al.** Relationship between Toll-like receptor 4 and type-2 diabetes mellitus complicated by tuberculosis. *Int J Tuberc Lung Dis*. 2017;21:910-915.
31. **Jiang ZS, Wang SX, Jia HX, et al.** Association of toll-like receptor 4 polymorphisms with type 2 diabetes mellitus. *Inflammation.* 2013;36:251-257.
32. **Illig T, Bongardt F, Schopfer A, et al.** The endotoxin receptor TLR4 polymorphism is not associated with diabetes or components of the metabolic syndrome. *Diabetes*. 2003;52:2861-2864.
33. **Bagarolli RA, Saad MJA, Saad STO.** Toll-like receptor 4 and inducible nitric oxide synthase gene polymorphisms are associated with Type 2 diabetes. *J Diabetes Complications*. 2010;24:192-198.
34. **Silva MJA, Santana DS, de Oliveira LG, et al.** The relationship between 896A/G (rs4986790) polymorphism of TLR4 and infectious diseases: A meta-analysis. *Front Genet.* 2022;13:1045725.
35. **Lee SH, Park SY, Choi CS.** Insulin resistance: from mechanisms to therapeutic strategies. *Diabetes Metab J*. 2022;46:15-37.
36. **Hydrie MZI.** Risk factors for Type 2 Diabetes Mellitus: Metabolic Syndrome, Insulin Resistance and Primary Prevention. 2012.
37. **Chen Z, Xue Q, Cao L, et al.** Toll-like receptor 4 mediated oxidized low-density lipoprotein-induced foam cell formation in vascular smooth muscle cells *via* Src and Sirt1/3 pathway. *Mediators Inflamm*. 2021;2021:6639252.
38. **Al-Fatlawi RB, Mohammad BI, Al-Aubaidy HA, et al.** Association of Toll-Like Receptor (TLR-4) Gene Polymorphisms in Diabetic Kidney Disease: Iraqi Cohort Study. *Ann Rom Soc Cell Biol.* 2021;6497–506.
39. **Yin YW, Sun QQ, Hu AM, et al.** Toll-like receptor 4 gene Asp299Gly polymorphism in myocardial infarction: a meta-analysis of 15,148 subjects. *Hum Immunol*. 2014;75:163-169.