Iraqi Journal of Biotechnology, 2024, Vol. 23, No. 3, 90-99

Genetic Polymorphism of IL-6 and TLR-4 Genes Correlated with *E.coli* Infection in UTI and Diabetic Patients

[1]Rabiha Q. Thajeel, [1]Abdulameer M. Ghareeb, [2]Qays A. M. Al-Khafaji

[1]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad
[2]Consultant Immunologist, Kamal Al-Samarraee Hospital for Infertility and IVF

Received: October 5, 2023 / Accepted: December 20, 2023 / Published: December 30, 2024

Abstract: Urinary tract infections (UTIs) and diabetes have a complex relationship that works both ways. UTIs are more common in people with diabetes mellitus (DM). The aim of the study a potential role of TLR-4 in the dynamics between diabetes and UTIs . Blood samples were collected from 100 patients with UTI and 100 patients with DM patients groups in addition to 100 apparently in individual (control group). The IL-6 and TLR-4 genes were amplified upon DNA extraction and submitted for sequencing. In addition, bacterial DNA has been extracted and the specified DNA sequence of the PapA gene was amplified. The results of rs1800797 showed higher frequency of the AA and AG genotypes within the patients than control group. The GG genotype showed a higher frequency in control group than patients group. The genotype frequencies of the SNP showed a none-significant higher frequency of the GG in control group than patient group. The genotype CG showed a non-significant higher frequency in patients (27.27) than in control (10). The genotype GG showed a significant (P-Value=0.0065) higher frequency in control (80) than in patient (19.09) with a high protection value (odd=0.0278). The genotype AA showed a none-significant (P-Value= 0.5383) higher frequency in control groups (90) than in control (72.72) with high-risk ratio (odds=0.444). The genotype AG showed a non-significant higher frequency in patients (18.18) than in control (10) (odd= 2.25, P-Value= 0.5383 and C.I.= 0.1701 to 29.7687). 25% of samples were positive for *E. coli*, underscoring a notable prevalence of this bacterium within the group under investigation. Urinary tract infections (UTIs) and diabetes have a complex relationship that works both ways. UTIs are more common in people with diabetes mellitus (DM). Blood samples were collected from 100 patients with UTI and 100 patients with DM in addition to 100 apparently healthy control group. The findings revealed that the expression levels of IL-6, TLR-4, higher in individuals with both diabetes and UTI than in those with diabetes. There was a Investigations into the relationship between IL-6 (TLR-4) inflammation and immune system regulation have shown that IL-6 can control the immune response by either upregulating or downregulating

Keywords: UTI, DM, IL-6, TLR-4 rs1800797 SNP.

Corresponding author: (Email: ameermgh@ige.uobaghdad.edu.iq).

Introduction

Diabetes and urinary tract infections (UTIs) are common health problems that often overlap. When individuals have both conditions, they can experience exacerbated symptoms (1). UTIs originate when bacteria penetrate the urinary system through the urethra, leading to an infection. Given their shorter urethra,

women are at a heightened risk of UTIs. Yet, in men, factors like urinary system anomalies or catheter use can trigger UTIs. Diabetes, on the other hand, is a long-standing metabolic ailment marked by elevated blood sugar levels due to either inadequate insulin output or its ineffective utilization by the body (2). Diabetes persists over time. Gestational diabetes, occurring solely during pregnancy, typically resolves after childbirth. However, this condition amplifies the likelihood of women developing type 2 diabetes in subsequent years (3).

Diabetes is characterized by chronic low-grade inflammation, notably influenced by the cytokine IL-6, which plays a vital role in immune responses (4). In the context of UTIs, IL-6 acts as an agent that exacerbates inflammation by rallying immune cells and boosting the release of other inflammatory markers. Studies indicate a significant interaction between IL-6, diabetes, and UTIs, with diabetic individuals suffering from UTIs displaying elevated IL-6 levels compared to non-diabetics (5). In the event of a UTI, the inflammatory cascade within the urinary tract can be intensified by IL-6, complicating the local inflammatory scenario. Moreover, IL-6 can modulate the production of antimicrobial compounds crucial for urinary tract defense. TLR-4, a key component of the innate immunity, aids in detecting harmful pathogens. It's also implicated in the subtle inflammation often associated with diabetes and is vital for spotting bacterial intruders in UTIs. A significant portion of TLR-4 is present on immune cells like macrophages (6).

The Pap operon in *E. coli* holds significance in the onset of UTIs. This review offers an in-depth analysis of the role, regulation, and implications of the Pap operon in *E. coli* (7). Comprising genes such as papBA and papG, the Pap operon is essential for the production of P fimbriae, structures that facilitate *E. coli's* adherence to urinary tract cells (8). UTIs, primarily caused by *E. coli*, are widespread globally (9). The presence and function of the Pap operon, especially its role in generating P fimbriae, are crucial for *E. coli's* colonization in the urinary system. However, there exists genetic variability within the Pap operon across *E. coli* strains, affecting the formation and functionality of P fimbriae (10). This genetic diversity bears significance for UTI prognosis and offers insights for therapeutic and diagnostic strategies (11).

Research suggests a potential role of TLR-4 in the dynamics between diabetes and UTIs (12). Diabetic individuals reportedly exhibit increased TLR-4 levels, thereby heightening their susceptibility to UTIs (13). An amplified inflammatory reaction in the urinary tract, driven by heightened TLR-4 activity, can further predispose these individuals to UTIs (14).

Materials and methods

Subjects

This study included 100 patients suffering from UTI and Diabetic mellitus patients group who were attending to the Specialist Center for Deaf Diseases and Diabetes Glands for Therapy. This study also included 100 healthy subjects Control group. 3 µl of blood were collected from both patients and control groups in EDTA tubes then 100ml of the blood had mixed with EDTA then transferred into a new sterile test tube and 200 µl of DNA/

RNA shield was added to the blood and kept in the freezer until use.

Molecular detection

DNA extraction and amplification of TLR-4 and IL-6

Genomic DNA was extracted from all the included samples the commercial kit Quick-gDNA Blood MiniPrep (Promega, USA) and used specific primer listed in table (1). The mix was conducted using 20µl of the addtaq master mix with 1.5µl of forward primers and 1.5µl of reverse primer, 3µl of genomic DNA. Finally, the reaction mix was completed to 40µl with nucleases free water. The sequence of forward primer is CAAATCTGCTCTAGAGGGCCTG, and the reverse primer is; ACCCTTTCAATAGTCACACTCAC. The reaction conditions were as follow; Initial denaturation for 2 min at 95 °C. Followed by 40 cycles of (Denaturation for 30 sec at 95 °C, Annealing, 30 sec at 60 °C then Extension for 30 sec at 72 °C) the final step was extension for 5 min at 72 °C.

Table (1): The studies designed primers.

Primer	Sequence (5′→3′ direction)	Refinance and Product Primer
TLR-4		
Forward	CAAATCTGCTCTAGAGGGCCTG	Designee by researcher
Reverse	ACCCTTTCAATAGTCACACTCACC	
IL-6 gene		
Forward	CCTCAATGACGACCTAAGCTG	Designee by researcher
Reverse	TAAATCTTTGTTGGAGGGTGAGGG	
PapA gene		
Forward	GCAACAGCAACGCTGGTTGCATCAT	Designee by researcher
Reverse	AGAGAGAGCCACTCTTATACGGACA	

DNA extraction and amplification of PapA gene

For PCR amplification of the PapA gene, the mix was conducted using 12.5 Master mix promega(Promega GoTaq™ Green MasterMix) with 1µl of forward primers and 1µl of reverse primer, 3µl of genomic DNA. Finally, the reaction mix was completed to 25µl with nucleases free water. The sequence primer was used was listed in (Table 1) .Program initiated the reaction with a denaturation step at 95°C for 3 minutes and 30 seconds. This was followed by 40 cycles, each consisting of a denaturation phase at 95°C for 40 seconds, an annealing phase at 60°C for 40 seconds, and an extension phase at 72°C for 40 seconds. Concluding the cycles, a final extension was performed at 72°C for 5 minutes.

DNA sequencing

The amplified products were sent to Macrogen Company for DNA sequencing to reveal the nucleotides sequencing by Sanger sequencing. Then results was analyzed using NCBI database.

Results and discussion

The eluted DNA from all the included samples have been subjected to gel electrophoresis. The results showed clear single band for each sample. The results of gel electrophoresis for 10samples are shown in (Figure1).

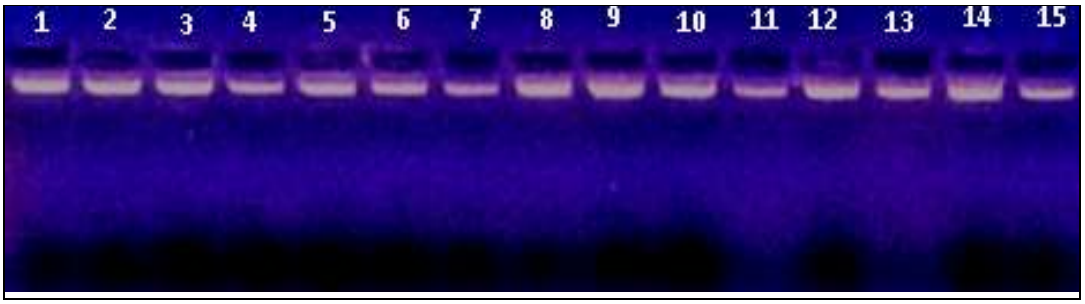

Figure (1): Agarose gel electrophoresis of the extracted genomic DNA from blood samples of the UTI and diabetic patients and control groups using 1.5% agrose at 8 V/cm for1 hr lanes-15: External genomic DNA.

After the amplification process of the IL-6B gene by PCR, the amplified products were run through gel electrophoresis. The results in the (Figure 2) shown a single band at 2500bp which prove the succession of the amplification process.

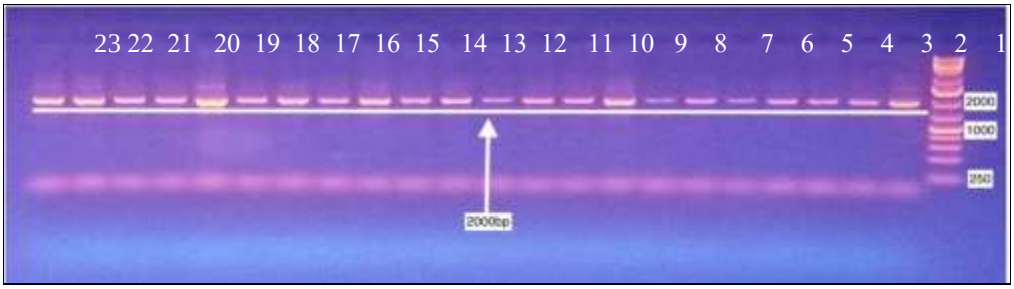

Figure (2): Agarose gel electrophoresis of PCR products of ILB6 segment using 1.5%-agars for 1hr at 8V/cm.lanc 23: 100bp DNA lader, lanes 2-23 :PCR products.

The amplification of TLR-4 also has been followed by gel electrophoresis. The results are shown in (Figure 3) demonstrated a single band of DNA at 2000bp size for 22 samples.

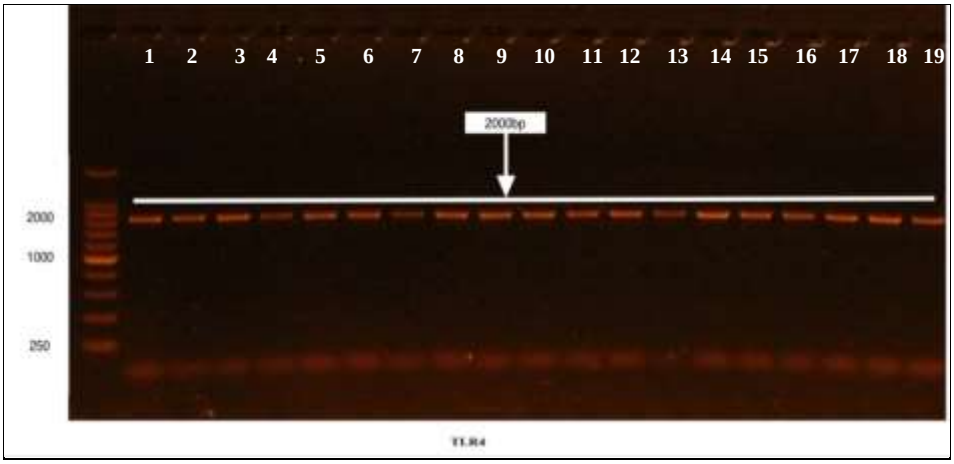

Figure (3): Agarose gel electrophoresis of PCR products of TLR segment using 1.5%-agars for 1hr at 8V/cm. lane 1: 100bpDNA lader, lanes 2-20: PCR products.

The PCR resulted in the successful amplification of the target DNA sequence. The presence of a single band, when compared to a DNA

ladder, corresponds to the expected size of 337 base pairs (bp) as shown in (Figure 4). No other extraneous bands were observed, suggesting the PCR was specific for the intended target without any non-specific amplification. The absence of multiple or smeared bands indicates that there was no amplification of off-target sequences or primer-dimer formations, respectively. In summary, the PCR protocol effectively amplified the desired DNA fragment of 337bp in length.

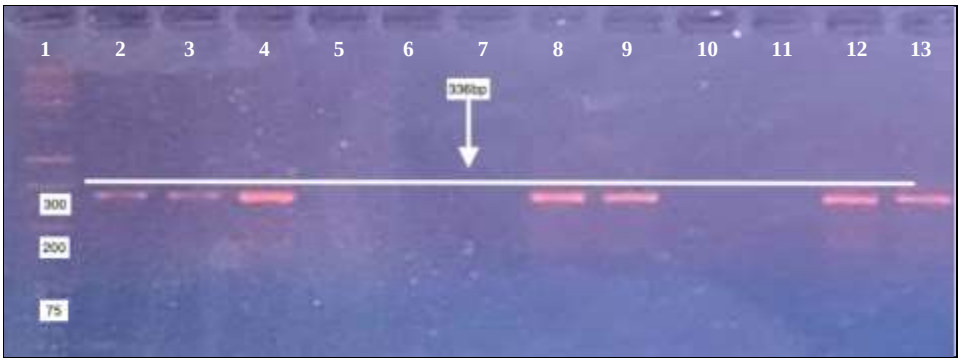

Figure (4): Agarose gel electrophoresis of PCR products of PapA gene segment using 1.5%agars for 1hr at 8V/cm.lanc 1: 100bpDNA lader, lanes 2,3,4,8,9,12,13 :PCR products.

The obtained sequences of the IL-6 are analyzed by using Benchling software. The analysis showed three SNPs within the obtained sequences (Rs1800795, Rs1800796 and Rs1800797) as shown in figures (5 as example) for the SNPs (Rs1800795, Rs1800796 and Rs1800797, respectively). Two peaks within the corresponding nucleotide means heterozygous genotype.

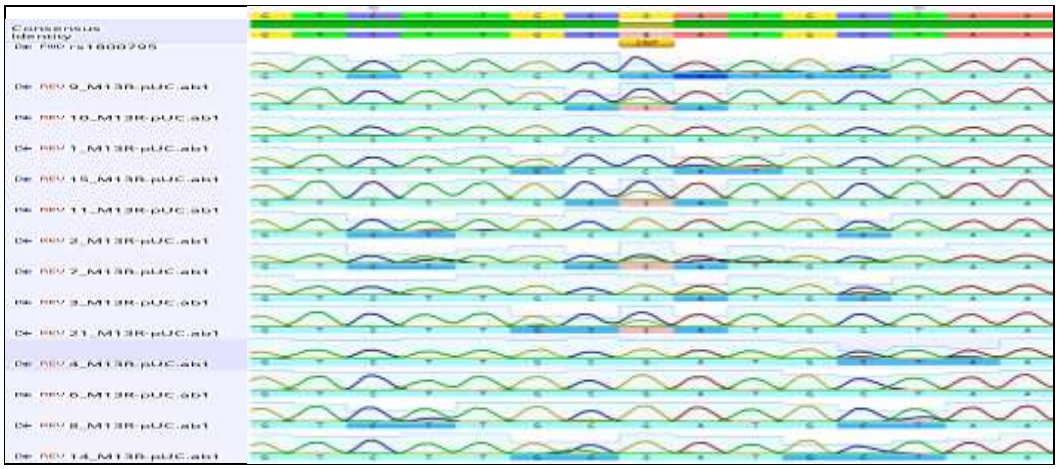

Figure (5): Multiple sequence alignment to compare the sequences peaks for Rs1800795 polymorphism within the gene IL-6B.

For the sequences obtained by the amplified products of the TLR-4 gene have showed two SNPs (Rs1800790 and Rs1800791). Two peaks within the corresponding nucleotide means heterozygous genotype.

After the analysis of obtained sequences, the results of the SNPs frequencies have been tested for Odd, P-value, and confidence intervals by

using Winipepi software. The results of the genotype frequencies and statistical analysis of the SNP rs1800797 are shown in (Table 2). The results showed higher frequency of the AA and AG genotypes within the patients than control group (odd= 2, P-Value= 0.597 and C.I.= 0.1527 to 26.1886). the GG genotype showed a higher frequency in control groups than patients (odd= 0.4375, P-Value= 0.4126 and C.I.= 0.0606 to 3.1605).

Table (2): Odd ratios, P-value, and C.I. corresponding to the genotype's frequencies of the SNP rs1800797.

rs1800797	Patients	Control	P-value	Odd ratio	C.I
AA	2 (18.18)	1 (10)	0.597	2	0.1527 to 26.1886
AG	2 (18.18)	1(10)	0.597	2	0.1527 to 26.1886
GG	7 (63.63)	8 (80)	0.4126	0.4375	0.0606 to 3.1605

The results of the genotype frequencies of the SNP Rs1800796 are shown in (Table 3). The results showed a none-significant higher frequency of the GG in control groups than patients (odd= 0.75, P-Value= 0.7576 and C.I.= 0.1207 to 4.6622). The genotype GC showed a none-significant higher frequency 36.36 in patients than control 20 (odd= 2.2857, P-Value= 0.4126 and C.I.= 0.3164 to 16.5122). The only one group in control (odd= 0.2754, P-Value= 0.4456 and C.I.= 0.0100 to 7.5712).

Table (3): Odd ratios, P-value, and C.I. corresponding to the genotypes frequencies of the SNP rs1800796.

rs1800796	Patients	Control	P-value	Odd ratio	C.I
GG	7 (63.63)	7 (70)	0.7576	0.75	0.1207 to 4.6622
GC	4 (36.36)	2 (20)	0.4126	2.2857	0.3164 to 16.5122
CC	0 (0)	1 (10)	0.4456	0.2754	0.0100 to 7.5712

The results of rs1800795 genotypes frequencies are shown in (Table 4), the results of genotype CC showed a significant (P-Value= 0.0352) higher frequency in patients (54.54) than in control (10) with high-risk ratio (odds=13.5). the genotype CG showed a non-significant higher frequency in patients (27.27) than in control (10) (odd= 3.8571, P-Value= 0.284 and C.I.= 0.3265 to 45.5721). The genotype GG showed a significant (P-Value=0.0065) higher frequency in control (80) than in patient (19.09) with a high protection value (odd=0.0278).

Table (4): Odd ratios, P-value, and C.I. corresponding to the genotypes frequencies of the SNP rs1800795.

rs1800795	Patients	Control	P-value	Odd ratio	C.I
CC	6 (54.54)	1 (10)	0.0352	13.5	1.1973 to 152.2181
CG	3 (27.27)	1 (10)	0.284	3.8571	0.3265 to 45.5721
GG	1 9.09)	8 (80)	0.0065	0.0278	0.0021 to 0.3675

The results of rs4986790 genotypes frequencies are shown in (Table 5), the results of genotype AA showed a none-significant (P-Value= 0.5383) higher frequency in control samples (90) than in control (72.72) with high-risk ratio (odds=0.444). the genotype AG showed a non-significant higher frequency in patients (18.18) than in control (10) (odd= 2.25, P-Value= 0.5383 and C.I.= 0.1701 to 29.7687). None of the samples of control and patients showed GG genotype.

Table (5): Odd ratios, P-value, and C.I. corresponding to the genotypes frequencies of the SNP rs4986790.

rs4986790	Patients	Control	P-value	Odd ratio	C.I
AA	8 (72.72)	9 (90)	0.5383	0.4444	0.0336 to 5.8802
AG	2 (18.18)	1 (10)	0.5383	2.25	0.1701 to 29.7687
GG	0 (0)	0 (0)	1	1	0.0181 to 55.2710

Similar to the findings shown with rs4986791, which are given in (Table 6), the frequency of the genotype CC was found to be greater in the control groups (90) than in the control (72.72), with a high-risk ratio (odds=0.444). Patients had a non-significantly greater frequency of the genotype CT (18.18) compared to controls (10), as measured by odds ratio (odds= 2.25) and confidence interval (CI) (0.1701-29.7687). Neither the healthy controls nor the sick samples had the TT genotype.

Table (6): Odd ratios, P-value, and C.I. corresponding to the genotype's frequencies of the SNP rs 4986791.

rs4986791	Patients	Control	P-value	Odd ratio	C.I
CC	8 (72.72)	9 (90)	0.5383	0.4444	0.0336 to 5.8802
CT	2 (18.18)	1 (10)	0.5383	2.25	0.1701 to 29.7687
TT	0 (0)	0 (0)	1	1	0.0181 to 55.2710

In this a total of 100 patients samples were collected. From these, 25 groups (25%) tested positive for *E. coli*, indicating a significant presence of this bacterial strain within study cohort. Furthermore, of these *E. coli* positive samples, 16 (64%) were found to carry the PapA gene, as summarized in (Figure 6).

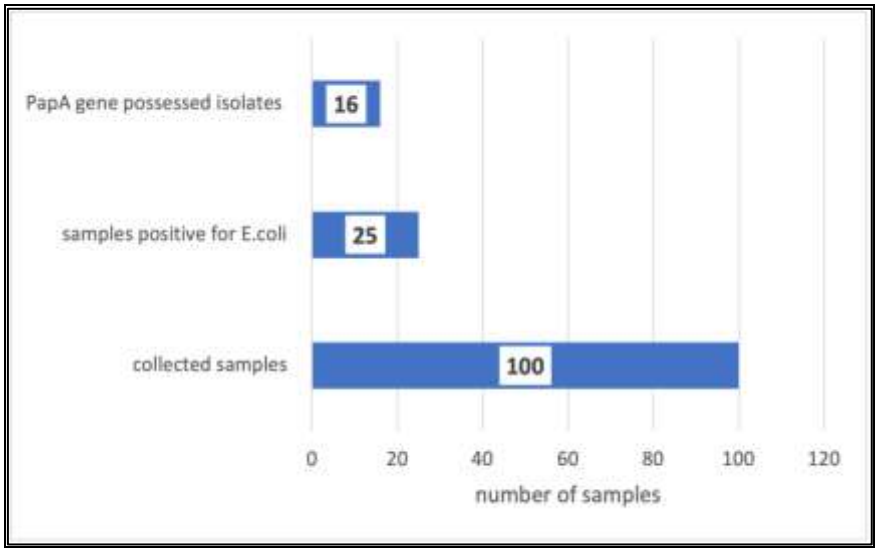

Figure (6): Summarization of positive samples for *E.coli* and isolates that possess the PapA gene.

Urinary tract infections (UTIs) exhibit a higher frequency, greater intensity, and poorer outcomes in individuals with type 2 diabetes. These infections often demonstrate antibiotic resistance. Type 2 diabetes mellitus encompasses conditions marked by varying insulin resistance levels, reduced insulin production, and increased glucose creation (15). Due to

compromised immune functions, diabetics are more infection-prone. This susceptibility often results in hospitalizations, thereby escalating the morbidity and mortality linked to infections. UTIs in diabetes patients are notably more severe, resistant to treatments, and come with dire consequences. They frequently experience challenging UTI symptoms such as asymptomatic bacteriuria (ASB), renal abscesses, and renal papillary necrosis compared to non-diabetics. Not just community-acquired UTIs, but also catheter-related UTIs and post-kidney transplant recurrent UTIs are associated risks for those with type 2 diabetes. Such individuals often grapple with UTIs caused by antibiotic-resistant pathogens, like those resistant to fluoroquinolones, carbapenems, vancomycin, or positive for extended-spectrum beta-lactamase-producing Enterobacteriaceae (16). For diabetics, UTIs exacerbate blood sugar management challenges, demanding enhanced glucose monitoring, diminishing life quality, and incurring significant financial strain (17). Local research indicates that Escherichia coli is the predominant UTI causative agent, responsible for 50-90% of cases (18).

Adipose tissue (AT) imbalances in immunity and hormone functions, along with cytokine secretion, contribute to a persistent, mild inflammatory state. IL-6, a versatile cytokine termed a "metabolic hormone," plays roles in immune mechanisms and glucose, protein, and lipid metabolism. Elevated IL-6 levels in plasma and adipose tissue mark this chronic inflammation. IL-6 gene promoter's rs1800795 is a studied link with these inflammatory markers, though the polymorphism itself isn't the sole determinant of IL-6 blood concentration. The functional promoter polymorphisms rs1800795 (174 G/C) and rs1800796 (572 G/C) govern IL-6 gene expression and correlate with IL-6 transcription activity and its blood concentrations (19).

The study observed a notably higher prevalence of the CC genotype of rs1800796 in patients compared to controls, suggesting a heightened risk of the condition. This differed from prior studies which didn't discern significant variations in SNP frequencies between lower UTIs and controls. Yet, in a study on acute pyelonephritis in adults, the CC genotype correlated with bacteremia emergence, while the rare GG genotype was tied to increased b-defensin 2 productions. The rs1800796 results aligned with prior research on IL6-DAL gene interactions and their impacts on metabolic characteristics, though no genotype differences for this SNP related to serum FBG levels were discerned. Contrarily, earlier findings indicated a higher prevalence of rs1800796 GG genotype in diabetics. Additionally, SNP rs1800797 results supported a study (20) that saw no notable genotype differences between diabetics and the control group for this SNP.

This investigation did not unveil significant genotype differences for rs4986791 and rs4986790 between the patient and control groups. These observations are consistent with prior research by Huang, Xu, and Wan (2020), which found no meaningful variations between these groups for either targeted SNP.

Conclusion

The results of this study offer insights, into the factors that contribute to the vulnerability of diabetic patients to urinary tract infections (UTIs). This understanding opens up possibilities for efficient treatment methods improving the overall outcomes for this susceptible

group. Ongoing research in this field is crucial to comprehend the genetic mechanisms that influence UTI risk and to devise personalized approaches, for managing the disease in diabetic patients.

References

1. Aggarwal, N. and Lotfollahzadeh, S. (2022). Recurrent Urinary Tract Infections. StatPearls, 4-6.
2. Naseer, R.; Obaidullah, S. and Haider, Z. (2018). Urinary Tract Infection in Diabetics. Journal of Pakistan Medical Association, , 30(1): 2–5.
3. Plows, J.F.; Stanley, J.L.; Baker, P.N.; Reynolds, C.M. and Vickers, M.H. (2018). The Pathophysiology of Gestational Diabetes Mellitus. International Journal of Molecular Sciences, 19(11): 2-21.
4. AL-jubouri, S. S. M., & Shami, A. M. Study Gene Expression of *OXA-48*and *CTX-M-1* Genes Cephalo-sporin resistance in *Escherichia coli* isolated from urinary tract infections in Baghdad hospitals. Bionatura, 8:1-9.
5. Kistner, T.M.; Pedersen, B.K. and Lieberman, D.E. (2022). Interleukin 6 as an energy allocator in muscle tissue. Nature Metabolism, 4(2): 170–179.
6. Khanam, A.; Hithamani, G. Naveen, J.; Pradeep, S.R.; Barman, S. and Srinivasan, K. (2023). Management of Invasive Infections in Diabetes Mellitus: A Comprehensive Review. Biologics3(1): 40–71.
7. Sameer, A.S. and Nissar, S. (2021). Toll-Like Receptors (TLRs): Structure, Functions, Signaling, and Role of Their Polymorphisms in Colorectal Cancer Susceptibility. BioMed Research International, 42(3): 791–798.
8. Salih, E.G.M.; Nader, M.I. and Rasheed, M.N. (2015). Rapid detection of uropathogenic escherichia coli virulence factors in iraqi patients by multiplex polymerase chain reaction. World Journal of Pharmaceutical Research, 4(12): 507-515.
9. Ghafer, A.; Ghareeb, A.M. and Abdul Hussain, M.A. (2020). Association the allelic variation and SNP rs12917707 genotyping with UMOD serum level among Iraqi patients infected with uropathogenic *Escherichia coli*. Centro de Biotecnologia y Biomedicina, Clinical Biotec Universidad Catolica del Oriente (UCO), Univesidad Yachay Tech, 8(1): 1–12.
10. AL-Jubouri, S. S., & Shami, A. M. (2022). Molecular Detection of Cephalosporin Resistance Genes in Escherichia coli Isolated from Urinary Tract Infections in Baghdad Hospitals. Iraqi Journal of Biotechnology, *21*(2), 145-152.
11. Khalaf, M.A.; Al-Saadi, B.Q.H. and Mohammed, H.Q. (2022). Evaluation of TLR-3, TLR4, IL-7, and IL37 Immunological Markers in β-Thalassemia Major Iraqi Patients. Iraqi Journal of Biotechnology, 21(1): 115-123.
12. Al-Dragy, W.A. and Baqer, A.A. (2018). Detection of Escherichia coli O157:H7 in Human patients Stool and Food by Using Multiplex PCR Assays Targeting the rfbE and the eaeA Genes compared with Detection by Biochemical Test and Serological Assay. Al-Nahrain Journal of Science, 17(3): 124-131.
13. Butler, D.; Ambite, I.; Wan, M.L.Y.; Tran, T.H.; Wullt, B. and Svanborg, C. (2022). Immunomodulation therapy offers new molecular strategies to treat UTI. Nature Reviews Urology, 19(7): 419–37.
14. Hekmat, E.M.; Nader, M.I. and Al-Allaq, H.A.J. (2023). TOLL like Receptor Type Four Gene Polymorphism and Susceptibility to Urinary Tract Infections in Patients of Abu Gharaib Hospital. Acta Biomedica, 94 (2): 1-5.
15. Nitzan, O.; Elias, M.; Chazan, B. and Saliba, W. (2015). Urinary tract infections in patients with type 2 diabetes mellitus: review of prevalence, diagnosis, and management. Diabetes, Metabolic Syndrome and Obesity, , 8(129): 129-136.
16. Salari, N.; Karami, M.M.; Bokaee, S.; Chaleshgar, M.; Shohaimi, S. Akbari, H., *et al*. (2022). The prevalence of urinary tract infections in type 2 diabetic patients: a systematic review and meta-analysis. European Journal of Medical Research, 27(1): 20.
17. Khanna, D.; Khanna, S.; Khanna, P.; Kahar, P. and Patel, B. M. (2022). Obesity: a chronic low-grade inflammation and its markers. Cureus, 14(2): 2-11.
18. Ghanemi, A. and St-Amand, J. (2018). Interleukin-6 as a “metabolic hormone.” Cytokine, 112: 132–136.
19. Amr, K.; El-Awady, R. and Raslan, H. (2016). Assessment of the −174G/C (rs1800795) and −572G/C (rs1800796) Interleukin 6 Gene Polymorphisms in Egyptian Patients with Rheumatoid

Arthritis. Open Access Medical Sciences, 4(4): 574.

20. Hussein, A.; Saad, K.; Askar, E.; Zahran, A.M.; Farghaly, H.; Metwalley, K., *et al*. (2018). Functional variants in intercellular adhesion molecule-1 and toll-like receptor-4 genes are more frequent in children with febrile urinary tract infection with renal parenchymal involvement. Acta Paediatrica, International Journal of Paediatrics, 107(2): 339-46.
21. Rasoulinejad, S.; Akbari, A. and Nasiri, K. (2021). Interaction of miR-146a-5p with oxidative stress and inflammation in complications of type 2 diabetes mellitus in male rats: Anti-oxidant and anti-inflammatory protection strategies in type 2 diabetic retinopathy. Iranian Journal of Basic Medical Sciences, 24 (8): 1078.
22. Ali, F. A. (2018). Distribution of CTX-M gene among Escherichia coli strains isolated from different clinical samples in Erbil City. Iraqi Journal of Biotechnology, 17(1).
23. Aboudounya, M. and Heads, R. (2021). COVID-19 and Toll-Like Receptor 4 (TLR4): SARS-CoV-2 May Bind and Activate TLR4 to Increase ACE2 Expression, Facilitating Entry and Causing Hyper inflammation. Mediators of Inflammation, 18: 1-14.