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Association of Serum IL-6 Levels and TLR4 Gene Polymorphisms (rs4986790 Asp299Gly and rs4986791 Thr399Ile) with Susceptibility to *Escherichia coli*-Induced Urinary Tract Infection.

Hayder A. Ali

[1] Department of Pathological Analyses , College of Science , University of Sumer- Thi-Qar, Iraq .

Corresponding Author Email: haedr.abaas@uos.edu.iq

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Abstract

Background: Urinary tract infection UTI is one of the most common bacterial infection , predominantly caused by E.Coli this case control study aimed to evaluate demographic characteristic ,hematological parameters ,serum interleukin IL-6 and associated of TLR4 gene polymorphism (rs4986790 and rs4986791) with susceptibility to UTI **Aim**:To evaluated demographic characteristics inflammatory and hematological parameters ,serum ;IL-6 levels and the association of TLR4 gene polymorphism (rs 4986790 and rs 4986791)**. Methods:**A total of 66 UTI patients and 55 healthy control were enrolled demographic data ,complete blood count , C–reactive protein CRP , urine analysis finding and serum IL-6 level were measured .TLR4 polymorphism were determining using PCR-RFLP , and Statistical analysis including odds ratios , 95% confidences intervals and effect size estimation **.Results:** No significant differences were found between patients and control healthy age or sex distribution ($p > 0.05$). UTI patients exhibited significantly elevate white blood cell counts, urinary red blood, neutrophil percentages, CRP levels, cells, and pus cells compared to controls ($p < 0.001$). Hemoglobin levels were lower in patients; however, this difference was not statistically significant ($p = 0.08$). serum IL-6 were markedly increased in UTI patients (47.9 ± 17.6 pg/mL) .compared with control (10.4 ± 4.8 pg/mL), indicated strong statistical significant $p < 0.001$ and a large effect size (Cohen's $d = 2.65$). Genotype and allele distributions of TLR4 rs4986790 (Asp299Gly) and rs4986791 (Thr399Ile) did not demonstrate statistically significant associations with UTI susceptibility ($p > 0.05$) **.Conclusion:** UTI patients showed significant systemic and local inflammatory responses compared to healthy controls. TLR4 polymorphisms (rs4986790 and rs4986791) were not significantly associated with UTI susceptibility. These results highlight the diagnostic value of inflammatory markers, particularly IL-6, and suggest further studies on host genetic factors.

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1. Introduction

Urinary tract infections (UTIs) are, include are a common bacterial that courses inflammation of pyelonephritis and cystitis ailment in all ages specific children with about from (1.8% to 7.5%) [1]. In world present 150 million patients in Every year suffer from urinary tract infections, which are the most results from bacterial infection [2] .70-90% from infection in UTI sickness mostly caused by

Escherichia coli . however it can also be caused by Enterobactea , Proteus , Klebsiella, and Enterococcus, and species, among other common pathogenic bacteria [3].infection of Urinary tract infections (UTIs) can causes potentially severe kidney with impairment and renal scarring [4] .Additionally, it is caused by a number of diseases and is considered a severe public health concern. The economic burden of these illnesses is seriously threatened by the developed antibiotic resistance [5] .

Escherichia coli (E. coli) is one of the most common germ of bacteria that cause infections of the urinary tract system . This bacterium can ascend from the gastrointestinal tract and develop an infection in urinary tract . the risk factors associated with the infection and incidence of E. coli are essential for treatment of UTIs and the effective prevention [6].This dissertation examines the epidemiology of Urinary tract infections that caused by E. coli, focusing on factors like (gender, age, opiate usage and underlying medical conditions . we hope to shed light on the risk factors associated , infection rates and distribution patterns, urinary tract with E. coli infections [7].

Infection-induced inflammation of the (urethritis), (cystitis), and (pyelonephritis) are among the clinical signs of UTIs, which are identified by the high levels of bacteria in the urine with accompanying symptoms. Frequent urination, dysuria (burning feeling and pain during urination), lower abdominal discomfort and/or suprapubic pain, and murky, unpleasant-smelling urine are all signs of cystitis. In addition to flank discomfort and fever, pyelonephritis symptoms include bacteriuria and pyuria (white blood cells (WBCs) in the urine), which may also contain various symptoms of cystitis. To cause urinary tract irritation tract mucosa, the pathogens employed a wide range of molecular pathways. Certain bacteria in the mucosa can activate dendritic cells and macrophages, while other bacteria can cause the release of inflammatory mediators from epithelial cells. The release of these inflammatory mediators is responsible for a number of infection-related symptoms and indicators [8].

IL-6 is one of interukines that role plays important in inflammation and a small polypeptide , it consists of two disulfide bonds and 184 amino acid residues. gene of IL-6 encoding is located on chromosome (7p and includes 5 exons and 4 introns) [9] that produced by (macrophages, ,B lymphocytes and T lymphocytes) including microglia, as well as mast cells , mesangial cells , keratinocytes, dendritic cells. Fibroblasts and IL-6 expression is mainly activated by interleukin 1 b and tumor necrosis factor-alpha (TNFa); on the other hand , there are ways to activation its synthesis such as prostaglandins , Toll-like receptor activation (TLRs), adipokines, and other cytokines [10].

Toll like receptor 4(TLR4) is role player important in innate immunity, and considered as a type of recognition receptor (PRR) for indicate gram-negative bacteria's [11].In humans, a genomic region of (TLR4 gene) which approximately 13.3 kb with 3 exons encodes a 224 amino acid protein [12]. In recent years, a research has emerged that the relationship between (single nucleotide polymorphisms SNP) of TLRs and urinary tract infections [13].TLR4 gene is highly polymorphic, 3 primers were used, 1primer for the mutant and another for the wild type, and one common reverse primer. The aim of this study was to explore the prevalence of the IL-6 serum levels and TLR4 polymorphisms (rs4986790 and rs4986791) with Correlated with E. coli Infection in UTI .

2. Material and methods

2.1 Study deigns

The 66 sample patients had diagnosed UTI with a urine culture indicating presence of bacteria (Gram negative) addition to 55 sample(control healthy). All patients were enrolled from Al-Zahraa General Hospital, Wasit province through out the period of August 2025 to February 2026. 5 ml of venous blood were collected, 2 ml transferred into a gel tube to separate serum for ELISA , CRP, and 3 ml into EDTA-containing tubes for the study of CBC test and genetic polymorphisms. The samples were preserved at -20°C until DNA extraction and test of urine Analysis (RBC and pus present) .

2.2 Determination of serum IL-6 Concentration

Estimation of serum IL-6 levels using kit (Biospes IL-6 ELISA Kit – (Biospes, Chongqing, China) .

2.3 Genotyping of TLR4

For TLR4 (rs4986790 Asp299Gly and rs4986791 Thr399Ile) genotyping, (3) ml of peripheral venous blood were collected and placed into sterile tubes containing ethylene diaminetetraacetic acid (EDTA) for DNA extraction by the salting out method (2) ml of peripheral venous blood were collected into gel tube to obtain serum for analysis of serum of IL-6 concentration. Both extracted DNA and serum samples were preserved at -20 ˚C until molecular and ELISA analysis.

2.4 DNA extraction:

PCR products were separated by gel electrophoresis using 2% agarose gel containing 5% ethidium bromide twice: once to confirm the presence of PCR product and again to determine the genotypes after restriction fragment length polymorphism (RFLP). PCR volume was 25 µl for both genes as follow: 12.5 µl master mix (New England BioLabs, USA), 1 µl forward primer, 1 µl reverse primer, 2 µl of extracted DNA, and 8.5 µl H2O. The amplification conditions for both genes were; initial denaturation at 94 °C for 30 seconds, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 58.5 °C for 1 minute, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 7 minutes For detection of the TLR4 genotype (SNP rs4986790) and (rs4986791) 2 µl of restriction enzyme Nco I (Thermo Fisher Scientific Inc., Waltham, MA) was added to 8 µl of PCR product, 2.5 µl of NE Buffer, and 12.5 µl of H2O, Then incubation at 37 °C for 1 hour. Genotyping was classified as follows AG genotype (249) bp, 223 bp, and 26 bp, AA genotype 249base pairs (bp), and GG genotype (223 bp, and 26 bp). For detection of the TLR4 genotype (SNP rs4986791), 2 µl of restriction enzyme Hinf

I (New England BioLabs) in a condition similar to SNP rs4986790. Genotyping was classified as follows CT genotype (406 bp, 291 bp, and 115 bp), CC genotype 406 base pairs (bp), and TT genotype (291 bp, and 115 bp) showed in Table (1 , 2) . PCR products were separated by horizontal gel electrophoresis (Cleaver Scientific, UK) using 2% agarose gel containing 5% ethidium bromide. Bands are visualized by the UV Gel .

Table (1): PCR-RFLP Primers and Conditions for TLR-4 Polymorphisms (rs4986790 and rs4986791)

SNP	Amino Acid Change	Primers (5′→3′)	PCR Product (bp)	Restriction Enzyme	Fragment Sizes (bp
rs4986790	Asp 299 Gly	**F:**GATTAGCATACTTAGACTACTACCTCCATG **R:**GATCAACTTCTGAAAAAGCATTCCCAC	249	Ncol	AA:249(uncut) AG:249 + 223 +26 GG:223+26
rs4986791	Thr 399lle	**F:**TCAAAGCAGTTCTTTCCTGTTG **R:**TTCAGCAGTTCTGCCAGTG	406	Hinfl	CC:406 (uncut) CT:406 +291+115 TT:291+115

Table (2) : Steps of PCR-RFLP Primers

Step	Temperature	Time	Cycles
initial denaturation	94	5 min	1
denaturation	94	30 s	30-35
annealing	55-62	30s	30-35
extension	72	30s-1 min	30-35
final extension	72	5 min	1

*Depends on primer Tm (rs4986790 ≈ 58 °C, rs4986791 ≈ 60 °C)

Data Processing and Analysis

The mean ± standard deviation (SD) for normally distributed data or the median with interquartile range (IQR) for non-normally distributed data were the results of tests for the normality of continuous variables. The independent samples t-test for parametric data and the Mann–Whitney U test for non-parametric data were used to compare UTI patients (n = 66) with healthy controls (n = 55). The Chi-square (χ^2) test or, when applicable, Fisher's exact test were used to evaluate group differences. Numbers and percentages were used to represent categorical variables, such as sex, age groups, results from urine analyses, and genetic distributions , to determine the degree of correlation between laboratory parameters, alleles, genotypes and UTI risk, odds ratios (OR) with 95% confidence intervals (CI) were computed. For continuous variables, effect sizes were calculated using Cohen's d, and where appropriate, the correlation coefficient (r).

4. Results :

4.1 The distribution of demographic characteristics between UTI patients and healthy controls in UTI

The distribution of demographic characteristics between UTI patients (n = 66) and healthy controls (n = 55) is shown in Table (3) and Figure (1,2)**:** . Regarding sex distribution, females constituted the majority of participants in both groups. Among UTI patients, (43) 65.15% were females and (23) 34.85% were males, while in the control healthy group, (35) 63.70% were females and (20) 36.30% were males. There was no statistically significantly different between the two groups concerning sex distribution (χ^2 = 0.03) (p = 0.86). Concerning age distribution, the highest proportion of UTI patients was observed in the 41–50 years age group 33.30%, followed by (31–40) years 31.80%. Similarly, in the control group, the (31–40) years category represented the highest proportion 34.55% , followed by 41–50 years 27.27% .Statistical analysis revealed no significant difference between patients and controls healthy across different age categories (χ^2 = 0.86) (p = 0.93).Overall, there were no significant differences between UTI patients and healthy controls in terms of age and sex distribution (p > 0.05), indicating that both groups were demographically comparable.

Table (3): correction between the Sex ,age Patients and Healthy Controls with Correlated with E. coli Infection in UTI

	UTI Patients N:66			**Healthy Controls N:55**			
Variables	**Groups**	**N**	**(%)**	**N**	**(%)**	**χ^2**	**P-value**
Sex	**Male**	**23**	**(34.85%)**	**20**	**(36.30%**	**0.03**	**0.86**
	Female	**43**	**(65.15%)**	**35**	**(63.70%)**		
		N	**(%)**	**N**	**(%)**		
Age (years)	**18 -20**	**5**	**(7.60%)**	**5**	**(9 %)**	**0.86**	**0.93**
	21-30	**10**	**(15.15%)**	**10**	**(18.18%)**		
	31- 40	**21**	**(31.80%)**	**19**	**(34.55%)**		
	41- 50	**22**	**(33.30%)**	**15**	**(27.27 %)**		

	>50	8	(12.15%)	6	(11 %)		
Total		66	(100%)	55	(100%)		

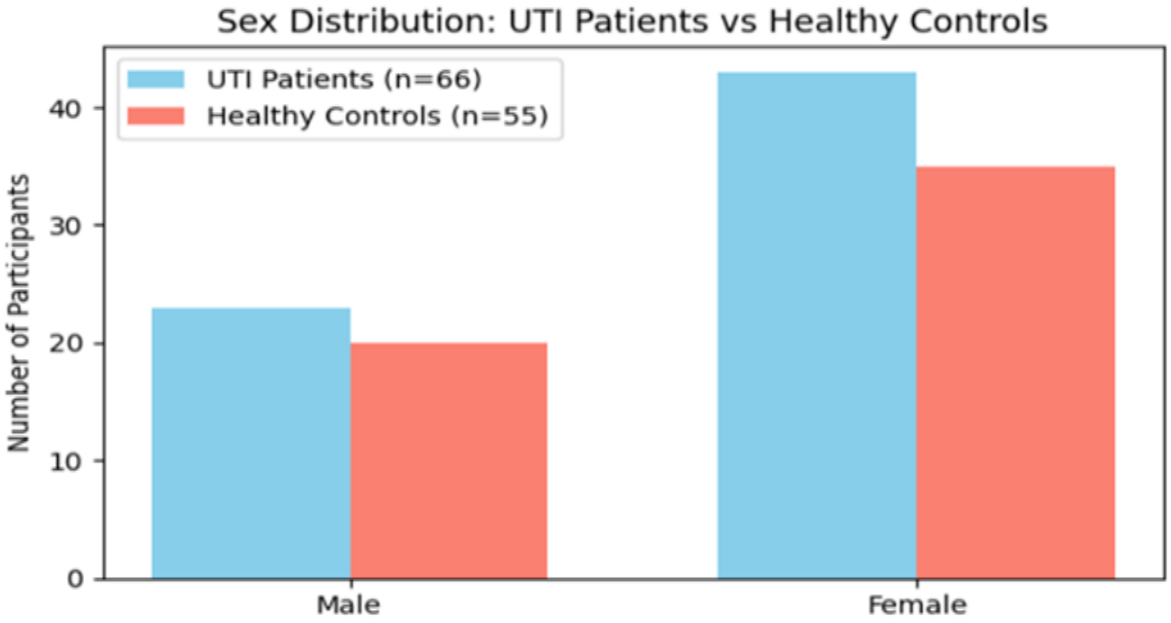

Figure (1): Relationship between the sex in Patients and Healthy Controls with Correlated with E. coli Infection in UTI

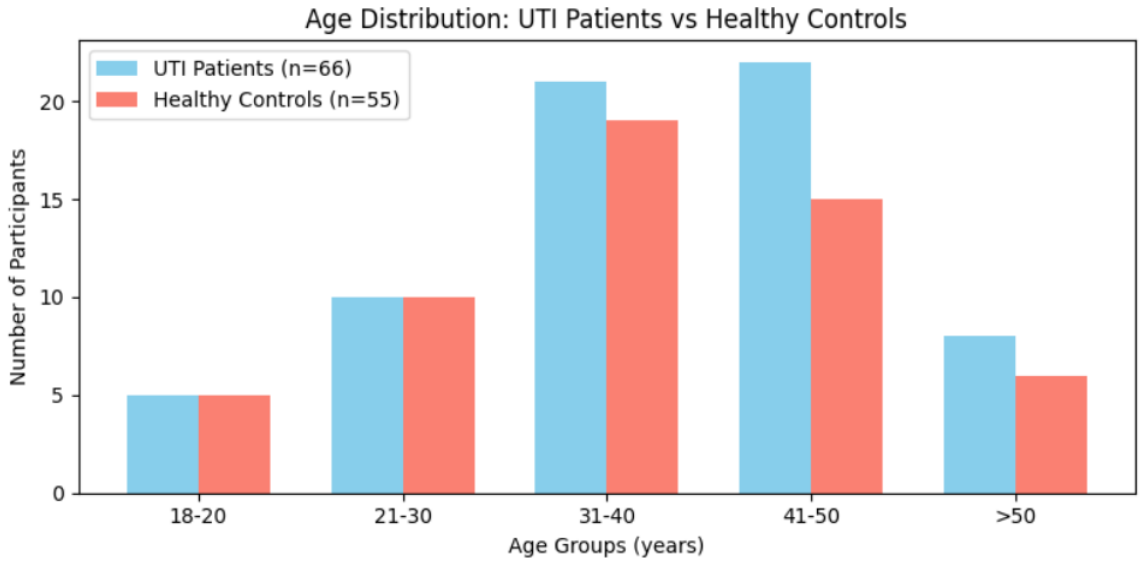

Figure (2): Relationship between the age groups in Patients and Healthy Controls with Correlated with E. coli Infection in UTI .

4.2 Hematology parameters Patients and Healthy Controls with Correlated with E. coli Infection in UTI

The results showed clear differences between UTI patients (n = 66) and healthy controls (n = 55) showed in Table (4). The mean WBC count was significantly higher in UTI patients (12.8 ± 3.4 $\times 10^3/\mu L$) compared to controls (7.1 ± 1.9 $\times 10^3/\mu L$), with a strong association (OR = 4.12, 95% CI: 2.01–8.45, $p < 0.001$). Neutrophil percentage was higher in UTI patients (74.6 ± 8.2%) compared to healthy controls (55.3 ± 6.7%), showed a significant (OR = 5.36, 95% CI: 2.48–11.58, $p < 0.001$). CRP levels were substantially higher in patients (18.5 ± 6.7 mg/dL) than in healthy controls (4.2 ± 2.1 mg/dL), with a strong statistical association (OR = 6.74, 95% CI: 3.05–

14.88, p < 0.001). Similarly, RBCs in urine were present in 41 (62.1%) of patients than with 6 (10.9%) of healthy controls (OR = 13.3, 95% CI: 4.9–36.1, p < 0.001). In contrast, hemoglobin levels were less in UTI patients (11.2 ± 1.5 g/dL) than in healthy controls (12.6 ± 1.3 g/dL), but this difference did not reach statistical different significance (OR = 0.58, 95% CI: 0.31–1.07, p = 0.08).Regarding urine examination, pus cells were detected in 61 (92.4%) of UTI patients compared to 7 (12.7%) of controls, demonstrating the strongest association (OR = 56.2, 95% CI: 18.9–165.4, p < 0.001).

Table (4) :Relationship between the hematology parameters Patients and Healthy Controls with Correlated with E. coli Infection in UTI

Parameter	UTI Patients N:66	Healthy Controls N:55	OR	95% CI	p-value
WBC(×10³/µL)	12.8 ± 3.4	7.1 ± 1.9	4.12	2.01–8.45	<0.001*
Neutrophils	74.6 ± 8.2	55.3 ± 6.7	5.36	2.48–11.58	<0.001*
Hemoglobin	11.2 ± 1.5	12.6 ± 1.3	0.58	0.31–1.07	0.08
CRP mg/dL	18.5 ± 6.7	4.2 ± 2.1	6.74	3.05–14.88	<0.001*
Urine Analysis					
RBC	41 (62.1%)	6 (10.9%)	13.3	4.9–36.1	<0.001*
Pus cells	61 (92.4%)	7 (12.7%)	56.2	18.9–165.4	

4.3 **Correction serum IL-6 between the Patients and Healthy Controls with with E. coli Infection in UTI**

The analysis of serum IL-6 levels showed a marked elevation in UTI patients compared with healthy controls showed in Table (5) . the mean serum IL-6 concentration in patients was (47.9 pg/mL) whereas in the control groups it was (10.4 pg/mL) .when expressed as mean ± SD, patients demonstrated levels of (47.9 ± 17.6 pg/mL) compared to (10.4 ± 4.8 pg/mL) in Healthy control . A statistically significantly difference (P < 0.001) was found between the two groups. The significant significance of the change was indicated by the big effect size (Cohen's d = 2.65). On the other hand , there was a statistically significant difference (P < 0.001) between the median serum IL-6 level in UTI patients (45 pg/mL; IQR: 34–58) and healthy controls (9 pg/mL; IQR: 7–13). The large effect size was further supported by the rank-biserial correlation (r = 0.71). These results show that UTI patients' serum IL-6 levels are much higher than those of healthy people.

Table (5) : Serum IL-6 Levels in UTI Patients and Healthy Controls with Correlated with E. coli Infection in UTI

Parameter Serum IL-6	UTI Patients (n=66)	Healthy Control(n=55)	P-value	Effect Size
Mean (pg/mL)	47.9	10.4	P <0.001	
Mean ± SD (pg/mL)	47.9 ± 17.6	10.4 ± 4.8	P <0.001	Cohen's d = 2.65
median (IQR)	45 (34–58)	9 (7–13)	P <0.001	r = 0.71

4.4 Correlation of TLR4 polymorphisms among patients with urinary tract infections (UTI) and healthy individuals

The distribution of TLR4 polymorphisms among patients with urinary tract infections and healthy individuals is illustrated in showed in Table (6) and Figure (3) . For the single nucleotide polymorphism (SNP) rs4986790 (Asp299Gly), the AA genotype emerged as the most prevalent genotype in both cohorts, being recorded in 68.2% of UTI patients and 76.4% of healthy controls, thereby serving as the reference genotype. The heterozygous AG genotype was identified in 27.3% of the patient group versus 21.8% of the control group. Although the odds ratio suggested a 1.44-fold elevated risk of UTI among individuals carrying the AG genotype (OR = 1.44; 95% CI: 0.63–3.30), this finding did not attain statistical different significance (P = 0.38). The GG genotype, which was relatively infrequent, was observed in 4.5% of patients and 1.8% of controls, and it also did not demonstrate a statistically different significant correlation with disease risk (OR = 2.61; 95% CI: 0.28–24.4; P = 0.39).The allelic analysis of rs4986790 revealed that the frequency of the G allele was 18.2% in the patient population and 12.7% in the healthy control group. The presence of the G allele was correlated with a 1.52-fold increased risk of UTI (OR = 1.52; 95% CI: 0.74–3.11), yet this association did not achieve statistical significance (P = 0.25). In relation to SNP rs4986791 (Thr399Ile), the CC genotype represented the predominant genotype in both patients (72.7%) and Healthy controls (80.0%) and was utilized as the reference genotype. The CT genotype was identified in (22.7%) of patients and (18.2%) of controls, with no statistically different significant association with susceptibility to urinary tract infections (OR = 1.38; 95% CI: 0.59–3.20; P = 0.46). The TT genotype was recorded in 4.5% of patients and 1.8% of controls, demonstrating no statistically significant difference (OR = 2.61; 95% CI: 0.28–24.4; P = 0.39). In the allelic comparison of rs4986791, the frequency of the T allele was 15.9% among patients and 10.9% among controls. Although the odds ratio indicated a modest increase in risk for UTI (OR = 1.54; 95% CI: 0.71–3.34), In summary, neither rs4986790 nor rs4986791 polymorphisms exhibited a statistically significant relationship with susceptibility to urinary tract infection within the examined population .

Table (6) :Polymorphism Genotyping of TLR4 SNPs in UTI Patients E. coli Infection and healthy Controls (PCR-RFLP Results with OR and P-value)

Genotype (N (%))	Allele	UTI Patients N :66	healthy Controls N:55	P value	OR (95% CI)
249 (SNP rs4986790)	AA	45 (68.2%)	42 (76.4%)	-	Reference

(Asp299Gly)	**AG**	**18 (27.3)%**	**12(21.8%)**	**0.38**	**1.44 (0.63–3.30)**
	GG	**3 (4.5 %)**	**1 (1.8%)**	**0.39**	**2.61 (0.28–24.4)**
Alleles (SNP rs4986790)	**G/A**	**24/132(18.2%)**	**14/110 (12.7%)**	**0.25**	**1.52 (0.74–3.11)**
406 (SNPrs4986791) Thr399lle	**CC**	**48 (72.7%)**	**44 (80.0%)**	**-**	**Reference**
	CT	**15 (22.7%)**	**10 (18.2%)**	**0.46**	**1.38 (0.59–3.20)**
	TT	**3 (4.5%)**	**1 (1.8%)**	**0.39**	**2.61 (0.28–24.4)**
Alleles (SNP rs4986791)	**T/C**	**21/132 (15.9%)**	**12/110 (10.9%)**	**-**	**1.54 (0.71–3.34)**

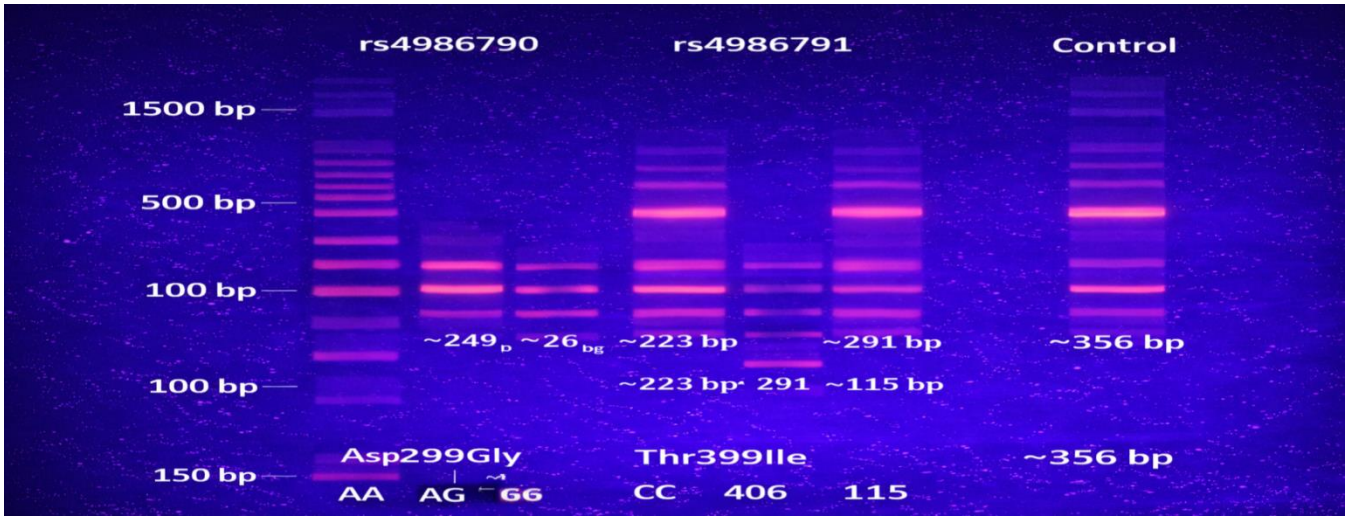

Figure (3): A garose gel electrophoresis showing the TLR4 Gene Polymorphisms (rs4986790 Asp299Gly and rs4986791 Thr399Ile) in patients and control healthy .

5. Disscusion :

This study aimed to investigate demographic characteristics hematological parameters serum IL-6 levels and TLR4 polymorphism in patients with urinary tract infection caused by infection E Coli , comparing them with healthy control . demographic characteristic ous analysis revealed that female predominate among both patients 65.15% and healthy controls 63.70% . this aligns with the well established epidemiology of UTIs where female are more susceptible due to a shorter urethra ,proximity to the anal region and hormonal influences [14]. No statistically significant differences were indicated in sex or age distribution between the groups indicate demographic comparability , The most affected age groups among UTI patients were 41–50 years 33.3% and 31–40 years 31.8% whereas in healthy controls, the largest proportion was 31–40 years 34.55% . The consistent with prior studies highlighting that UTI are particularly common in adults between (30–50) years [5].

Furthermore present study indicated a robust systemic and local inflammatory response to E. coli infection. The elevated WBC count and neutrophil percentage reflect acute bacterial infection, which stimulates bone marrow production of neutrophils and mobilizes them to the site of infection. CRP, acute-phase reactant synthesized by the liver in response to IL-6, was markedly increased [15]. The presence of RBCs and pus cells in urine corroborates direct damage to the urinary tract epithelium by bacterial colonization and inflammatory infiltration. Hemoglobin levels was lower in UTI patients than to controls (11.2 ± 1.5 g/dL vs. 12.6 ± 1.3 g/dL), but the different was not statistically different significant ($p = 0.08$), possibly indicating mild anemia of inflammation.

Serum IL-6 level serum were significantly elevated in UTI patients (47.9 ±17.6 pg/mL) than to healthy controls (10.4± 4.8 pg/mL) $p < 0.001$,with a large effect size Cohen's $d = 2.65$ and a strong rank- biserial correlation ($r = 0.71$). IL-6 is a key proinflammatory cytokine that stimulated acute phase protein production including CRP and mediated immune cell activation . level of IL-6 elevated confirm its central role paly important in the immune response to E. coli infection [16].

TLR4 Polymorphism the study investigated two SNP TLR4 (rs4986790 – Asp299Gly, rs4986791 – Thr399Ile) . the most prevalent genotypes were CC (rs 4986791) and AA (rs 4986790) in both UTI patients healthy controls .heterozygous and mutant genotypes did not show statistically significantly associated with UTI susceptibility [17] .Population specific effect may exist as TLR4 polymorphism can alter receptor recognition of bacteria LPS , affect innate immunity , Elevated hematology markers , CRP , urine abnormalities and IL-6 provide strong evidence for active infection and systemic inflammation . IL-6 may be particularly useful as a biomarker for UTI

severity . Although TLR4 polymorphism did not reach significant , understanding genetic susceptibility can inform future personalized therapies .

Another study also pointed to the weak selective pressure of the variant (TLR4 Thr399Ile) in fighting infection. Given the diversity of alleles among Iranian ethnic groups, including Iranian Kurds) [18], it has been proven that the polymorphism (TLR) is associated with a reduced response to bacterial infection and increased susceptibility to Gram-negative bacterial infection as a result of weak signaling (LPS)[19] .In addition, many studies have focused on laboratory mice or on other ethnic groups, including Caucasians, Koreans [19], Iranians, [20, 21] and sub-Saharan Africans [22], where the percentage of single nucleotide polymorphisms SNPs only in Africans, the TLR4 Thr399Ile SNPs (rs4986791) is only 0.79% in Africans, in Europeans (8.51%), and 0% in East Asians. Therefore, this variant is considered likely to be detrimental to protein function and structure [22] . Interestingly, the genotype (TLR4 Thr399Ile) shows a percentage (17.7%) of heterogeneous variation in Caucasian populations without any homogeneous mutations

No articles were found in Pubmed- NCBI data base when searching for key words as 'TLR4 SNP Iraq'. Although, when searched for 'TLR4 polymorphism Iraq' only one article was published that was about TLR4 Asp299Gly SNP (rs4986790) and the samples were taken from Jordanian populations but one of its authors is from Iraq [23] . Similarly, no data were found for TLR4 SNP in Syria as a country of having Kurdish populations .

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TLR4 Polymorphism the study investigated two SNP TLR4 (rs4986790 – Asp299Gly, rs4986791 – Thr399Ile) . the most prevalent genotypes were CC (rs 4986791) and AA (rs 4986790) in both UTI patients healthy controls .heterozygous and mutant genotypes did not show statistically significantly associated with UTI susceptibility [17] .Population specific effect may exist as TLR4 polymorphism can alter receptor recognition of bacteria LPS , affect innate immunity , Elevated hematology markers , CRP , urine abnormalities and IL-6 provide strong evidence for active infection and systemic inflammation . IL-6 may be particularly useful as a biomarker for UTI severity . Although TLR4 polymorphism did not reach significant , understanding genetic susceptibility can inform future personalized therapies .

Another study also pointed to the weak selective pressure of the variant (TLR4 Thr399Ile) in fighting infection. Given the diversity of alleles among Iranian ethnic groups, including Iranian Kurds) [18], it has been proven that the polymorphism (TLR) is associated with a reduced response to bacterial infection and increased susceptibility to Gram-negative bacterial infection as a result of weak signaling (LPS)[19] .In addition, many studies have focused on laboratory mice or on other ethnic groups, including Caucasians, Koreans [19], Iranians, [20, 21] and sub-Saharan Africans [22], where the percentage of single nucleotide polymorphisms SNPs only in Africans, the TLR4 Thr399Ile SNPs (rs4986791) is only 0.79% in Africans, in Europeans (8.51%), and 0% in East Asians. Therefore, this variant is considered likely to be detrimental to protein function and structure [22] . Interestingly, the genotype (TLR4 Thr399Ile) shows a percentage (17.7%) of heterogeneous variation in Caucasian populations without any homogeneous mutations

No articles were found in Pubmed- NCBI data base when searching for key words as 'TLR4 SNP Iraq'. Although, when searched for 'TLR4 polymorphism Iraq' only one article was published that was about TLR4 Asp299Gly SNP (rs4986790) and the samples were taken from Jordanian populations but one of its authors is from Iraq [23] . Similarly, no data were found for TLR4 SNP in Syria as a country of having Kurdish populations.

6.Conclusion

UTI patients showed significant systemic and local inflammatory responses compared to healthy controls. TLR4 polymorphisms (rs4986790 and rs4986791) were not significantly associated with UTI susceptibility. These results highlight the diagnostic value of inflammatory markers, particularly IL-6, and suggest further studies on host genetic factors

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Conflict of interest: The authors discloses no conflicts of interest.

Ethical Considerations:

This study was done in agreement with clinical ethical concern of hospitals in within Al-Zahraa General Hospital, Wasit province and verbal informed consent were obtained from all the participants

Authors' contribution:

The first author originated and structured the study, gathered and analysed data, interpreted results, composed and amended the text, and sanctioned the final version.

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