Association of the IL-4 gene polymorphisms with developing of renal diseases and bacterial urinary tract infections

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Abstract

The current study was designed to determine the association between the genetic polymorphism of IL-4 to the frequency of urinary tract infection as one of the contributing factors in the development of kidney infection. Out of 135 cultured urine samples, 90 bacterial isolates were obtained, distributed among 45 bacterial isolates from patients with urinary tract infection only, but the number of isolated bacterial from group of patients with urinary tract infection and kidney failure, was 45 isolates bacteria. There were significant differences ($p \leq 0.001$) in the average level of interleukin 4 in the serum of the studied groups. Where the highest rates were recorded in the group of patients with renal failure only (295.81 ± 59.73) IU/ml compared to the control group (64.97 ± 36.06) IU/ml. There are significant difference in the distribution of genotypes ($p < 0.001$) and alleles ($p < 0.001$) for interleukin 4 among study groups compared to the control group. Where the C/T phenotype was dominant in the three studied groups compared to the control group from these results can be concluded that the association of different IL-4 genotypes with an increased risk of renal infection or UTI- related renal failure and there are no significant differences in the mean serum with the different genotypes of interleukin 4 at the probability level ($p > 0.05$).

Keywords: tract infections; renal diseases; IL-4 gene polymorphisms

Introduction

The kidneys are one of the important and complex organs in the human body, consisting of many specialized parts, which play an important role in regulating blood components and volume, as well as regulating blood pressure and purifying it from food waste (12). In addition to its ability to regulate the hydrogen function and maintaining the electrical balance of the body (3). The urinary tract is exposed to many microorganisms that begin to multiply and cause disease and infection in the lower urinary tract .When transform the initial infection into a complex injury that threatens the patient's life after reaches the upper parts of the urinary system represented by the kidneys (14). The continued development of upper and lower bacterial urinary tract infections may lead to the development of necrosis and renal abscess with increase in the inflammatory response and increase release of inflammatory mediators such as cytokines (11).

Interleukin-4 is one of the important multifunctional cytokines that may be involved in both autoimmunity and acquired immunity as a lymphocyte growth factor, which is able to stimulate the proliferation of B cells and activated T cells with ability to inhibit the synthesis of Pro-inflammatory cytokines (8). It is produced by a wide range of different cell types, is responsible for a wide range of antimicrobial functions, and physiologically mediates the host's defense against various bacteria, but it may exacerbate disease if its activities are unregulated (10). IL-4 plays an important role in the polarization of macrophages and dendritic cells which is important for recovery from acute kidney injury, thus IL-4 is required to effectively polarize macrophages and dendritic cells to their phenotype and to enhance Recovering from acute kidney injury (16).Therefor the important aim of this study was determined the effect of different IL-4 genotype as predisposing factors for UTI-related with renal failures.

Materials and Methods

Collection Specimen

the urine and blood specimens were collected from patients clinically diagnosed with urinary tract infections with or without renal failure and patients with renal failure only. aged from 16-85 years attending AL Hussein's Teaching hospital in Kerbala province and AL Diwaniyah Teaching hospital in AL Diwaniyah . This case-control study was conducted between March to July 2021 in Department of biology, College of science University of Kerbala.

Five milliliters of venous blood were taken from each person in the studied groups by disposable syringes. 1ml of blood specimen ware trans fend tip Eppendorf tubes and keep at -20 c units used in bench, while the remaining four milliliters of blood specimen was a centrifuge (1000rpm/5 minutes) to specimen the serum that was kept in Eppendorf tubes at -20 c units used in immunological Aspects loopful of midstream urine samples that were collected all person in studied groups were transported and cultured on gender, direction & selection culture medium then incubation at 37 for 24 h aerobically.

doi.org/10.31838/hiv22.02.604 Received: 01.04.22, Revised:14.07.22, Accepted: 28.08.22

Exclusion Criteria

All patients with one of the chronic diseases (such as blood pressure and diabetes), autoimmune diseases and patients with various bacterial and viral infections in other body sites were excluded .

Ethical Approval

this research was conducted according to the official instructions for the approval of the Research Ethics Committee in the Diwaniyah Health Department, according to Book No. 438 on 2/14/2021, and the Kerbala Health Department according to the numbered book 419 on 9/3/2021.

Diagnosis of isolated bacteria

The first diagnosis of isolated bacteria was dependent firstly on their cultural character in general and selective media[5]. then different biochemical tests were performed according to the methods in [9] and confirmed using the Vitek-2 diagnosis system.

Estimation of IL-4 level

The IL-4 level was determined in studied groups according to the manufacturer's instruction (BT-LAB-China).

Gene Polymorphisms Assay

a- DNA Extraction

The Genomic DNA Extraction of blood samples were performed according to the Manufacturer's instruction (Geneaid- Taiwan) and the concentration purity of the extraction DNA was it was measured by reading the absorbance using a nanodrop Spectro photometer and of the 260-280 nm.

b- (AS-PCR) Method

the (AS-PCR) Method was carried out to detect genotyping in the study groups according to [1].

Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 16 and Microsoft Office Excel 2007. Qualitative (categorical) variables were expressed as number and percentage, whereas, quantitative (numeric) variables were first evaluated for normality distribution using Kolmogorov-Smirnov test, and then accordingly normally distributed numeric variables were expressed as mean (an index of central tendency) and standard deviation (an index of dispersion) in addition to range.

Results & Discussion

Types of isolated bacteria

The culture results of urine samples culture in table(1) showed that 90 bacterial isolates were obtained, represented by 45 bacterial isolates from the group of patients with urinary tract infection only, which were 33 (73.3%) of Gram-negative bacteria and *E. coli* 24 (53.3%) the represented highest isolates that followed by *Acinetobacter baumannii* 4 (8.9%), *Proteus mirabilis* 3 (6.7%) and *Pseudomonas aeruginosa* 2 (4.4%). While Gram-positive bacteria, they were recorded as 12 (26.7%), represented by *Enterococcus faecalis* 4 (8.9%) , Streptococcus spp. 3 (6.7%), Staphylococcus spp. 3 (6.7%), *Staphylococcus aureus* 2 (4.4%).

As for the number of isolated bacteria from group of patients with urinary tract infection and kidney failure was 45 isolates distributed among (77.8%) 35 isolates of Gram-negative bacteria (*E. coli* 27 (60.0%), *Proteus mirabilis* 4 (8.9%) , *Acinetobacter baumannii* 2 (4.4 %) , *Pseudomonas aeruginosa* 1 (2.2%) , *Klebsiella pneumonia* 1 (2.2%) and 10 (22.2%) isolates of Gram-positive bacteria represented by *Streptococcus spp.* (8.9%) 4), *Staphylococcus aureus* 3 (6.7%), *Enterococcus faecalis* 2 (4.4%) and Staphylococcus spp. 1 (2.2%). This result was consistent with the findings of a previous study [7]. The reason for this may be due to the ability of *E.coli* bacteria to cause ascending urinary tract infections as a result of some it possesses special adhesion factors, and morphological structures known as filaments or flagella (fimbriae), which play an important role in their settlement on the mucous surfaces of the urinary tract and the formation of bacterial colonies that make them resistant to the force of the urine washing those surfaces, and the natural presence of these bacteria in human feces is one of the Pollutants to the urinary tract, especially in women, because of the female anatomy.

Table 1: Genus of isolated bacteria from studied groups

Bacteria	UTI		Renal UTI	
	n=45	%	*n*=45	%
Gram negative	**33**	**73.3**	**35**	**77.8**
E. coli	24	53.3	27	60
Acinetobacter baumannii	4	8.9	2	4.4
Proteus mirabilis	3	6.7	4	8.9
Pseudomonas aeruginosa	2	4.4	1	2.2
Klebsiella pneumonia	0	0	1	2.2
Gram positive	**12**	**26.7**	**10**	**22.2**
Enterococcus faecalis	4	8.9	2	4.4
Streptococcus spp.	3	6.7	4	8.9
Staphylococcus spp.	3	6.7	1	2.2
Staphylococcus aureus	2	4.4	3	6.7
Total	**45**	**100.0**	**45**	**100.0**

IL-4 Level in Serum of Studied groups

It was observed through the results shown in Table (2) a clear correlation of the level of interleukin-4 (IL-4) in the serum between studied groups ($p \leq 0.001$). Where the mean of IL-4 in the serum of patients with renal failure was (295.81 ±59.73), followed by patients with renal failure and UTI (215.42 ±66.07), and then patients with UTI only (132.51 ±56.23) compared to the control group (64.97 ±36.06).

Table 2 : Level of interleukin-4 in the serum of the studied groups

Characteristic	Control *n* = 45	Renal failure *n* = 45	UTI *n* = 45	RF and UTI *n* = 45	*p*
IL-4					
Mean ±SD	64.97 ±36.06 D	295.81 ±59.73 A	132.51 ±56.23 C	215.42 ±66.07 B	< 0.001 O **
Range	18.52 -169.34	193.49 -420.91	2.72 -215.84	68.89 -346.69	

n: number of cases; **O**: One way ANOVA; **: significant at $p \leq 0.01$

Despite the significant increase in the level of IL- 4 in the serum of the studied groups compared to control group, but it was clear that the association of high rates of interleukin 4 in patients with renal failure only compared to patients with renal failure and urinary tract infection, while patients with urinary tract infection recorded urinary tract only had the lowest level of elevation.

The rise in the rate of interleukin-4 in the current studied groups was higher than what was recorded by a previous study in which the average level of IL-4 in the serum was only 126.2 pg/mL and it is believed that the reason for this may be due to the high inflammatory level of patients Within the current study, and since IL-4 is an inflammatory cytokine, its levels were high, especially in patients with renal failure. Besides, the amount of enlarged tissue in the inflammatory process is much more than the tissue involved in urinary tract infection, and therefore the immune response will be higher and the amount of interleukin-4 will be Larger, patients with chronic kidney disease are also susceptible to ischemic heart disease, which is itself a chronic inflammatory response and can be a cause of elevated levels of the inflammatory cytokine IL-4 (6).

Genotype Aspect of IL-4 gene polymorphism

a-Genomic DNA Extraction

DNA was extracted and quality checked using electrophoresis as shown in Figure (1) before starting the PCR steps, and DNA concentration and purity were determined by Nano drop device.

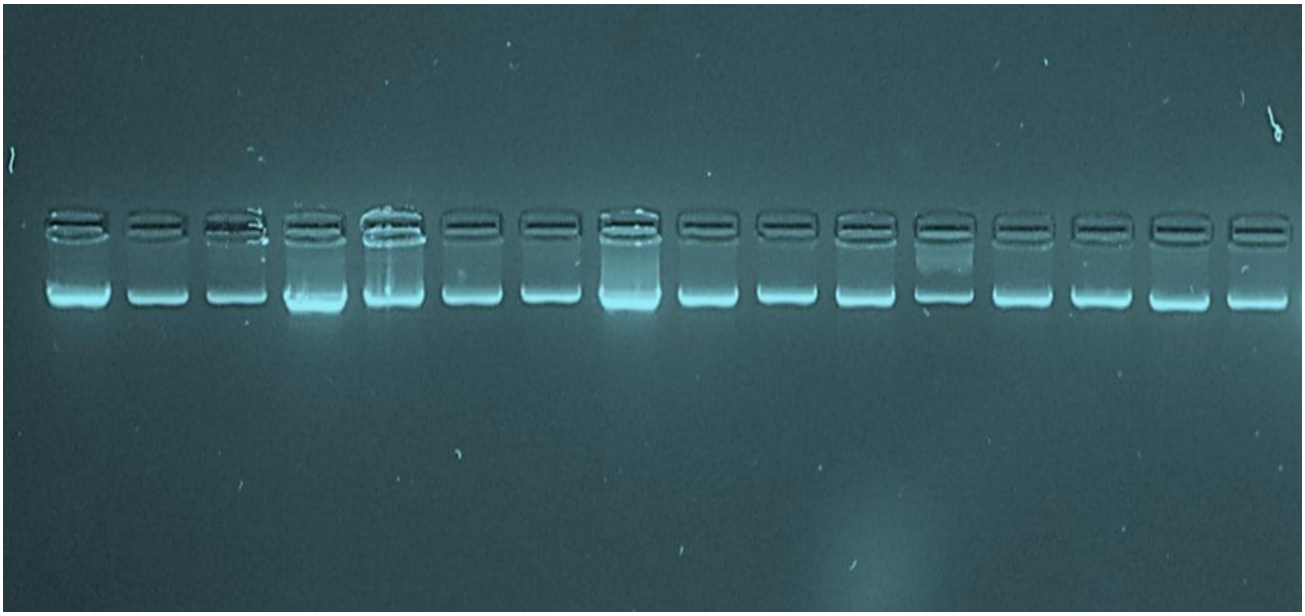

Figure (1) Electrophoresis of DNA samples using agarose gel electrophoresis at 0.7% concentration at 72 V for 45 minutes by ethidium bromide dye.

b- IL-4 gene polymorphism

The results of IL-4 single nucleotide polymorphism analysis at the IL-4-590 C>T gene polymorphism (rs2243250) site (rs2243250) by AS-PCR revealed the presence of three genotypes: CC, TT and T/C in patients and the group control, as show in Figure (2). The frequency distribution of the genotypes was according to the groups based on the (Hardy Weinberg) distribution, and there was no significant difference in the frequency distribution of the observed and expected number of IL-4 genotypes in the control group ($P = 0.796$) and renal failure ($p = 0.125$) .While there was a significant difference in the UTI group ($p = 0.028$) at the probability level ($p \leq 0.01$) genotypes. In the urinary tract and renal failure groups ($p = 0.001$). As show in Table (3).

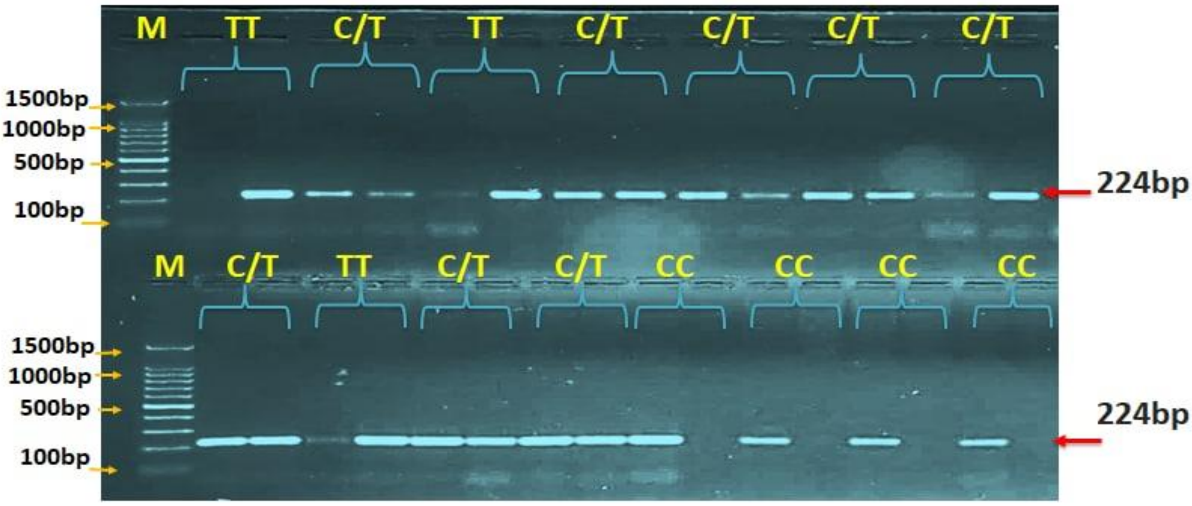

Figure (2) agarose migration images showing the results of the AS-PCR assay for the identification of the IL-4 -590C>T (rs2243250) gene variant. where M: DNA marker ladder 1500-100bp . The lines with the normal genotype (CC) represent the presence of the C allele only, the mutated genotype (TT) lines represent the presence of the T allele only, while the mixed genotype (C/T) lines represent the presence of the C allele and the T allele together. The size of the normal and mutant alleles was 224bp the size of the AS-PCR product.

Table (3): Frequency Distribution of Genotypes of studied Groups Based on the (Hardy Weinberg) Distribution

Genotype	Control *n* = 45		Renal failure *n* = 45		UTI *n* = 45		RF and UTI *n* = 45	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
CC	34	33.8	4	6.4	16	19.3	4	9.8
C/T	10	10.4	26	21.2	27	20.3	34	22.4
TT	1	0.8	15	17.4	2	5.3	7	12.8
χ^2	0.067		2.360		4.859		12.068	
p	0.796 NS		0.125 NS		0.028 *		0.001**	

n: number of cases; *: significant at *p* ≤ 0.05 ; **: significant at *p* ≤ 0.01; **NS**: not significant

The results of the current study showed the role of genetic variation in the interleukin-4 genes as one of the factors causing the development and exacerbation of urinary tract infection in both Kerbala and Al-Qadisiyah governorates.

Distribution of IL-4 gene Polymorphism in studied groups

The results shown in Table (4) showed that the C/T genotype was more common in the group of renal failure 26 (57.8 %) compared to the control group 10 (22.2%) (p < 0.001). The TT genotype was also more frequent in group 15 (33.3%) compared to control group 1 (2.2%) (*p* < 0.001), so it was considered a risk factor for kidney failure with an OR of(OR=127.50).

Allele T was more frequent in the renal failure group 56(62.2%) compared to the control group 12 (13.3%) (p < 0.001) .While the C allele was less frequent in Renal failure patients 34(37.8%) compared to the control group 78 (7.86%) (p < 0.001).Hence, the association of CT and TT genotypes and the T allele with the risk of kidney failure was clearly demonstrated. The C allele was associated with an increased risk of UTI, as the C/T genotype was more common in the UTI group 27 (0.60%) compared to the control group 10 (2.22%) (*p* < 0.001), so it was considered a risk factor for infection.

Whereas, Allele T was more common in UTI group 31 (4.34%) compared to control group 12 (3.13%) (*p* < 0.001) and therefore it was considered as a risk factor for UTI with an odds ratio of (OR=3.42) while the allele C was less frequent in UTI patients compared to the control group 78 (7.86%) and was considered a protective factor with an OR = 0.29) (71% protection).

The C/T genotype was more common in the renal failure and UTI group 34 (75.6%) compared to the control group 10 (22.2%) (p < 0.001).

Whereas, TT genotype was more frequent in renal failure and UTI group 7 (15.5%) compared to control group 1 (2.2%) (p < 0.001).

Allele T was more frequent in renal failure and UTI patients group 48 (53.3%) compared to the control group 12 (13.3%) (*p* < 0.001), while C allele was more frequent. Less frequent same group patients 78(86.7%) compared to the control group 42 (46.7%) and it was a protective factor with an odds of (OR=0.13) (87% protection).

From here it can be inferred that the CT and TT genotypes and the T allele were associated with the risk of developing combined renal failure and urinary tract infection .

groups

Table 4: Comparison of the frequency of IL-4 genotypes and alleles between the control group and studied

Genotype	Renal failure *n* = 45	Control *n* = 45	*p*	OR	95 % CI
CC	4 (8.9%)	34 (75.6%)	Reference	Reference	
C/T	26 (57.8%)	10 (22.2%)	< 0.001 **	22.10	6.22-78.46
TT	15 (33.3%)	1 (2.2%)	< 0.001 **	127.50	13.12-1239.08
Allele	Renal failure *n* = 90	Control *n* = 90	*p*	OR	95 % CI
C	34 (37.8%)	78 (86.7%)	< 0.001 **	0.09	0.04 -0.20
T	56 (62.2%)	12 (13.3%)		10.71	5.10 -22.49
Genotype	UTI *n* = 45	Control *n* = 45	*p*	OR	95 % CI
CC	16 (35.6%)	34 (75.6%)	Reference	Reference	
C/T	27 (60.0 %)	10 (22.2%)	< 0.001 **	5.74	2.25 -14.66
TT	2 (4.4%)	1 (2.2%)	0.218 NS	4.25	0.36 -50.39
Allele	UTI *n* = 90	Control *n* = 90	*p*	OR	95 % CI
C	59 (65.6%)	78 (86.7%)	< 0.001 **	0.29	0.14 -0.62
T	31(34.4%)	12 (13.3%)		3.42	1.62 -7.21
Genotype	RF and UTI *n* = 45	Control *n* = 45	*p*	OR	95 % CI
CC	4 (8.9%)	34 (75.6%)	Reference	Reference	
C/T	34 (75.6%)	10 (22.2%)	< 0.001 **	28.90	8.25-101.20
TT	7 (15.5%)	1 (2.2%)	< 0.001 **	59.50	5.75 -616.13
Allele	RF and UTI *n* = 90	Control *n* = 90	*p*	OR	95 % CI
C	42 (46.7%)	78 (86.7%)	< 0.001 **	0.13	0.06 -0.28
T	48 (53.3%)	12 (13.3%)		7.43	3.56 -15.50

n: number of cases; **OR**: odds ratio; **CI**: confidence interval; **: Significant at $p \leq 0.01$

The result of current study were in diffrent contrast to the findings of (15) as they did not find any significant association between IL-4 590 C/T gene polymorphism (rs 2243250) and CKD. On the other hand (2) showed a significant difference in genotype and frequencies of the -590C/T SNP allele between CKD patients and the control group in Rafsanjan population from southeast Iran. Similarly (13) found a relationship between IL4-590C/T polymorphisms and chronic kidney disease in North Indian patients, moreover (1) found IL4-590 (CT) heterozygous genotypes can be considered as risk factors. While it contradicts current results.Inaddition the (4) showed no association between the IL4-590C/T SNP and CKD.

Conclusions

The current result show that Gram-negative were the most common pathogen compared to Gram-positive bacteria isolated from the studied groups. An increase in the level of interleukin 4 in the serum of patients compared to the healthy ones, while no significant difference was found in the mean serum of interleukin 4 in the different genotypes within the study groups. Association of CT, TT, and T allele genotypes with risk of renal failure A and renal failure associated with urinary tract infection. Whereas, only the CT genotype and the T allele were associated with an increased risk of UTI.

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