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# Association between Single Nucleotide Polymorphism in the Promoter of CD14 *Gene* and Development of Urinary Tract Infection

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## Abstract

The tetra-arm method was employed in this investigation to identify the SNP (rs2569190) in the promoter region of the CD14 gene as a risk factor for acquiring chronic urinary tract infections in a sample of Iraqis with a history of these illnesses in various forms. Using sandwich ELISA to estimate the serum level of CD14 in conjunction with TLR4 and other associated cytokines (TNF-α, TGF-β, and IFN-γ). A Gram-negative co-receptor for LPS, CD14 is expressed by innate immune cells. Through their effects on neutrophil expression levels, which have been associated with pyelonephritis, recurrent cystitis, and asymptomatic bacteriuria, its genetic variations have been connected to susceptibility to various types of UTIs The frequency of genotypes distribution and alleles of rs2569190 for two investigated groups presented The GG, GA, and AA genotype frequencies of rs2569190 were (26.1 %, 60.5 % and 13.4 %) respectively among the patients while 77.5 % for GG, 17.5% for GA and 5% for AA genotypes of healthy control.

**Keywords:** Nucleotide Polymorphism, Promoter**,** CD14 *Gene,* Urinary Tract Infection, SNP (rs2569190)

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## Introduction

Humans frequently experience upper and lower urinary tract infections, which significantly reduce many people's quality of life. It is the most common infectious disease among the aged population and a serious health concern in various nations (Al–Ubaidy and Al-Ibadi 2010)(Nicolle et al. 2019) A lipopolysaccharide-binding protein called CD14 functions as a co-receptor of TLR4, which can be found on the surface of monocytes and the majority of tissue macrophages as a membrane-bound receptor or as a soluble protein. According to (Xu et al. 2019)CD14 plays a crucial and vital role in inducing inflammatory responses that fight infections. The development of infection is triggered by the presence of bacterial infection and the increased production of pro-inflammatory cytokines and chemokines(AlChalabi et al. 2020). Environmental and genetic factors control the inter-individual variations in the inflammatory response to bacterial infections of the urinary

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system(Wujcicka et al. 2018)(Allami et al. 2023). Inflammatory reactions to bacterial colonization, immune cells implicated in inflammation, the production of pro-inflammatory mediators, and the activation of innate and adaptive immunity can all be affected by genetic variables (Xu et al. 2019)(AlChalabi et al. 2016)

Gene polymorphism refers to variations in DNA sequence among people, groups, or populations. The most prevalent sort of genetic variation that manifests a difference in a single nucleotide is known as SNP(Kozak et al. 2020). A gene's polymorphic variant might result in the creation or expression of an aberrant protein, which can either cause or contribute to disease (Varela and Amos 2010)(AlChalabi et al. 2020)(El-Zayat, Sibaii, and Mannaa 2019). The highly polymorphic CD14 gene is characterized by a huge number of SNPs, some of which are present at high rates in the different regions of the gene, which affects susceptibility to bacterial infections and speeds up the development of UTIs. According to research by (Guo et al. 2020)and (Frick-Cheng et al. 2020) the genetic variant rs2569190 of the CD14 gene (a G-to-A transition, 159 G/A) has been linked to disease severity(Jia et al. 2019)(Samson et al. 2022).

## Methodology

This study included 189 individuals of both sexes who visited the Urology Unit at Al-Yarmouk Teaching Hospital in Baghdad between the first of October (2020) and the first of July (2021); 113 females and 76 males. The participants were divided into two groups mostly by their clinical diagnosis, which was established by their signs, symptoms, and laboratory examinations. Blood was drawn from each person using a plastic disposable syringe, which was then used to extract the DNA from the blood after adding EDTA and mixing it inverted to prevent blood clotting. The blood gDNA Miniprep System is an efficient, straightforward method for the extraction of genomic DNA from blood samples. To detect the presence of (rs2569190) in the human *CD14* gene by tetra arms technique, specific primers were designed according to their sequence in NCBI https://www.ncbi.nlm.nih.gov and by free online primer designing tool http://primer1.soton.ac.uk/primer1.html. The primer sequence and product listed in Table (1) were purchased from Macrogen Company/Korea.

**Table (1): Primers for Detection of (rs2569190) in Human *CD14* Gene**

| SNPs | Size (bp) | Annealing Temp.(℃ ) | Primer | Sequence |
|---|---|---|---|---|
| rs2569190 | A allele: 176 | 60 | Inner F | /5- GGATGTTTCAGGGAGGGTGA -/3 |
| | G allele : 245 | | Inner R | /5- TGCAGAATCCTTCCTGTTACTGC -/3 |
| | Size of two outer primer:378 | | Outer F | /5-GCTCTGGAAGTGCTTTAGCTTCTTT -/3 |
| | | | Outer R | /5-CTAAGGCACTGAGGATCATCCTTT -/3 |

According to the manual, the Nanodrop was used to quantify the DNA concentration. The ratio of sample absorbance at 260 and 280 nanometers was used to determine purity. Agarose gel (1.2%) was used to separate the DNA fragments, while a concentration of (2%) was utilized to confirm the size of the PCR products. The 1 X TBE buffer was used to run the gel horizontally. DNA samples and loading buffer (2:5 v/v); the master mix contains a component that increases the sample's density with blue dyes, which serve as a loading dye when reaction products are evaluated by gel electrophoresis; loading buffer was not used for PCR product verification. TBE buffer (1X) was applied to the gel, and it was electrophoresed at 5 volts/cm for 1-2 hours. For 20–30 minutes, an agarose gel was stained with ethidium bromide to reveal bands. Nuclease-free water was used to dissolve the lyophilized primer to create a solution of 100 pmol/**µ**l in the master tube. Following this, 10 pmol/**µ**l of the solution was generated as the working solution by transferring 10**µ**l from the master tube to another tube, and the volume was then brought to 100 **µ**l by adding additional nuclease-free water. PCR reactions were carried out using sterilized 25 **µ**l PCR tubes. The mixture for the reaction was used It contained $MgCl_2$ (1.5 Mm), Taq polymerase 1 U, and each dNTP 200 mM, which are the reaction's optimum concentrations for each component. A negative control blank, which contained all of the PCR reaction mixture's ingredients minus template DNA, was used in each amplification experiment. The reaction mixture, which is shown in Table (2), was centrifuged for three seconds to collect wall droplets and make sure the reaction's final volume was 25 **µ**l. After that, the extracted DNA was subjected to amplification, as shown in Table (3).

The Sandwich ELISA technique is used for estimating serum levels of determined immunological parameters because of its high specificity and sensitivity

**Table (2): Master Mix to Identify rs2569190 in The Promoter of *CD14* Gene**

| Material | Volume ((**µl)** |
|---|---|
| Master Mix | 5 |
| Inner forward primer | 1.5 |
| Inner reverse primer | 1.5 |
| Outer forward primer | 1 |
| Outer revers primer | 1 |
| Nuclease free water | 12 |
| Template DNA | 3 |
| Total | 25 |

**Table (3) PCR Program for Detection of rs2569190 in *CD14* Gene**

| **Steps** | **Temp.(°C)** | **Time(min.)** | **No. of Cycle** |
|---|---|---|---|
| Initial Denaturation | 95 | 3 | 1 |
| Denaturation | 95 | 0:30 | 35 |
| Annealing | 60 | 0:30 | |
| Extension | 72 | 1 | |
| Final Extension. | 72 | 5 | 1 |

## Results and Discussion

It was shown that CD14 gene polymorphisms are more prevalent in UTI patients than in healthy controls, and they are crucial to the genetic susceptibility to UTIs. Changes in CD14 protein levels, which affect the likelihood of developing UTIs, are to blame. Tables (4) and (5) show the frequency of genotype distribution and rs2569190 alleles for the two groups under investigation. Results showed that severe UTI cases had significantly greater frequencies of the GA genotype and G allele. These results revealed that the occurrence of severe urinary system infections was related to the polymorphism (rs2569190) in the CD14 gene promoter. The GG, GA, and AA genotype frequencies of rs2569190 were (26.1 %, 60.5 %, and 13.4 %) respectively among the patients while 77.5 % for GG, 17.5% for GA, and 5% for AA genotypes of healthy control. This polymorphism, which is present in the gene's promoter region, increases transcription, changing the serum level of the CD14 protein. Acute and chronic UTI susceptibility is influenced by genetic factors based on modifications in gene expression as well as the presence of certain alleles connected to the disease phenotype.

Table (4): Distribution of rs2569190 between groups.

| Genotype | Patients (N = 149) | | | | Control (N = 40) | | | |
|---|---|---|---|---|---|---|---|---|
| | Observed | | Expected | | Observed | | Expected | |
| | N | % | N | % | N | % | N | % |
| GG | 39 | 26.18 | 48.8 | 32.8 | 31 | 77.5 | 28.8 | 72.2 |
| GA | 90 | 60.40 | 73.01 | 49.0 | 7 | 17.5 | 10.24 | 25.6 |
| AA | 20 | 13.42 | 27.12 | 18.2 | 2 | 5 | 1 | 2.2 |
| HWE *p*-value | **0.002** | | | | 0.130 | | | |

**Table (5): Statistical Evolution of rs2569190 Genotypes or Alleles.**

| Allele/ Genotype | Patients (N = 149) | | Control (N = 40) | | OR | 95% C.I. | *p*-value |
|---|---|---|---|---|---|---|---|
| | N | % | N | % | | | |
| *G* | 168 | 56.38 | 69 | 86.25 | Reference | | |
| *A* | 130 | 43.62 | 11 | 13.75 | 4.20 | 2.49 - 7.06 | < **0.001** |
| GG | 39 | 26.18 | 31 | 77.5 | Reference | | |
| GA | 90 | 60.40 | 7 | 17.5 | 3.94 | 2.11 - 7.33 | < **0.001** |
| AA | 20 | 13.42 | 2 | 5 | 19.17 | 1.14 -321.52 | **0.002** |

The Hardy-Weinberg equilibrium predicts that genetic variation in a population will stay stable from generation to generation in the absence of perturbing events. The rule states that both genotype and allele frequencies will remain constant since they are in equilibrium in vast populations with random mating and no disruptive events. The allele frequency depicts the prevalence of a gene variant in a population. Alleles are distinct forms of a gene that have the same genomic location on a chromosome in common(AlChalabi et al. 2020). CD14 is a multifunctional receptor expressed on many cell types and has been shown to mediate immune response resulting in the activation of an inflammatory cascade, with polymorphism of its promoter (rs2569190) found to be associated with susceptibility to several diseases via its association with transcriptional activity of the promoter it could impact the production of CD14 then affect pro-inflammatory

response in human leucocytes(Ojurongbe et al. 2017). The balance of Th1/Th2 cells is very important for regulating the immune system. when urinary tracts are infected, pro-inflammatory cytokines such as IL-2,IFN-γ , TGF-β, IL-6, and IL-8 secreted by Th1 cells to reduce infection by inhibiting by promoting microbial clearance(Al-Kaabi and Al-Khalidi 2020). Toll-like receptors are pattern recognition receptors (PRRs) that form the cornerstone of the innate immune response as an important first line of defense against microbes which can recognize the invading pathogens or endogenous molecules and act as an immunomodulatory factor of the immune response (Rawaa et al. n.d.). After estimation of the serum level of CD14, TLR4, and various associated cytokines by sandwich ELISA for subjects of the investigated groups. The results were illustrated in table (6) The mean serum level of all patients was significantly higher than healthy control.

TLR4 is expressed in a number of immune system cells, including macrophages, neutrophils, dendritic cells, eosinophils, and monocytes. These immune system cells play a crucial role in the regulation of inflammatory responses, signaling cascades, and the production of effector molecules, such as cytokines and chemokines. TLR4 was found to be more abundant in patient serum as a result of exposure to uropathogens and acute inflammation. In this study, the mean of serum levels of TGF-β, TNF-α, and IFN-γof chronic UTIs patients elevated when compared to serum levels of healthy individuals thus, suggested that it plays a significant role in UTIs progression and severity. (Jiménez-Sousa et al. 2018)reported that CD14 SNPs overexpressed, that effect on CD14 protein expression and activity thus the variation plays a role in the etiology of many diseases. The results in Table (3-7)showed that the sera level of CD14 in patients who had GA genotype was (72.69) pg/ml. A significant decrease was recorded in healthy (16. 61 pg/ml). with GG and AA genotypes, CD14 level was (56.51) pg/ml (53.54 pg/ml) respectively in comparison to healthy (16. 57) pg/ml with significant differences.

**Conflict of interest:**

There are no conflicts of interest.

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**Table (6).** Serum level of cytokines (CD14, TLR4 TGF-β, TNF-α, and IFN-γ).

| Groups | Serum level (Mean ± S.E pg/ml) | | | | |
|---|---|---|---|---|---|
| | **CD14** | **TGF-β** | **TNF-α** | **IFN-γ** | **TLR4** |
| **Patients** | 67.60±5.26 | 47.15±2.25 | 70.36±5.12 | 58.84±2.97 | 84.10±20.07 |
| **Healthy** | 13.4±1.45 | 10.13±0.55 | 8.61±0. 45 | 17.53±2.26 | 19.85±4.76 |
| **P Value** | 0.001 | 0.00041 | 0.0001 | 0.001 | 0.0001 |

**Table (7): Association between Different Genotypes and CD14 Level**

| Genotype | Mean Serum level of Soluble CD14 Mean± SE) pg/ml | | |
|---|---|---|---|
| | Patients | Healthy | P-value |
| GA | 72.69 ± 5.52 | 16.61 ± 0.79 | **0.0027** |
| GG | 56.51 ± 3.57 | 22.38 ± 3.21 | 0.0001 |
| AA | 53.54 ± 6.54 | 16.57 ± 1.78 | 0.0001 |
| LSD value | 15.355 NS** | 11.163 NS** | 0.0001 |

***(Significant)** Not Significant**

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