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Exploring NLRP3, NLRC4, AIM 2 and their inflammatory cytokines in UTI of Basrah province, Iraq

Z.A. Eidan, W.S. Shani, M.A.-I. Almazini
College of Science, University of Basrah, Basrah, Iraq

Abstract

Background. Urinary tract infections (UTIs) are inflammation of the urinary tract epithelium resulting from invasion by microorganisms.

Purpose. This study aimed to determine the role of some innate immune receptors like NLRP3, NLRC4, AIM 2, and some inflammatory cytokines such as IL-1β, IL-18, and MCP-1 in inflammatory processes in UTI patients.

Materials and methods. A case-control study was conducted at (Al Basrah General Teaching Hospital, Al Mawani General Teaching Hospital, and Al Sadir Teaching Hospital) in Basrah province between October 2023 to March 2024. 35 patients with confirmed UTI by Vitek® 2 system and 16S rDNA and 30 healthy controls without UTI. Sera levels of receptors (NLRP3, NLRC4 and AIM2) and cytokines (IL-1β, IL-18, and MCP-1) were determined by using an enzyme-linked immunosorbent assay (ELISA) kit.

Results. *E.coli* is the most common species among the bacteria, NLRP3, NLRC4, AIM2, IL-1β, and IL-18 was highly significant in sera of UTIs but MCP-1 with no significant $p>0.303$ in sera of UTIs.

Conclusions. A positive correlation was recorded between *E.coli* infection and all studied receptors (NLRP3, NLRC4, and AIM2). The same is true in the studied inflammatory cytokines (IL-1β and IL-18), but *E.coli* infection with MCP-1 is not correlated. Data analysis indicated a positive correlation of NLRP3 and NLRC4 with IL-1β and IL-18 whereas no correlation with MCP-1. A positive correlation was also shown between AIM2 and IL-18 whereas no correlation between AIM2 with IL-1β, and MCP-1.

Keywords: urinary tract infections; *E.coli*; epithelium; receptors; immune; cytokines; Basrah province

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Introduction

One of the most prevalent bacterial illnesses worldwide, urinary tract infections (UTIs) significantly increase morbidity in otherwise healthy females [1]. This occurs as a result of the urethra acting as both a channel for urine to exit the body and a point of entry for germs to enter the urinary tract [2]. The most frequent cause of urinary tract infections is bacteria, specifically Gram-negative bacilli. UTIs are most often caused by Enterobacteriaceae, and 80–90% of infections are caused by uropathogenic *Escherichia coli* (UPEC) [2; 3]. Through type-1 fimbriae, UPEC binds to host receptors on the superficial bladder epithelial cells. Intracellular bacterial communities are created by UPEC after it invades the cells after attachment [4]. Additionally, UPEC can penetrate the urothelium's deeper layers, develop quiescent intracellular reservoirs, and remain there for months at a time before reoccurring infections [4]. The submucosa of the urinary tract contains a large population of macrophages, and additional cells are attracted to these areas after infection [5]. When activated, macrophages create cytokines and chemokines that affect the activity of nearby immune cells, impacting the timing and strength of inflammatory responses during UTIs [6; 7]. Excessive inflammation may lead to chronic or secondary conditions, which are further involved in tissue damage and illness severity to the host. The host's innate reaction serves as the initial line of defense against UPEC infection and the ensuing tissue damage [8]. The first line of defense against pathogenic microbes is provided by innate immune receptors. These receptors can be classified as either intracellular, including absent in Melanoma 2 (AIM2), Nod-like receptor family pyrin domain containing 3 (NLRP3), Nod-like receptor family CARD domain-containing protein 4 (NLRC4) or extracellular receptors include toll-like receptors (TLRs) [9]. Receptors activate when pathogen-associated molecular patterns (PAMPs) are sensed by pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), or danger-associated molecular patterns (DAMPs) are sensed by nucleotide oligomerization domain (NOD)-like receptors (NLRs) [4]. This study aimed to determine the role of some innate immune receptors like NLRP3, NLRC4, AIM 2 and some inflammatory cytokines such as IL-1β, IL-18 and MCP-1 in inflammatory processes in UTI patients.

Materials and methods

Samples collection

Urine and blood samples were collected from Al Basrah General Teaching Hospital, Al Mawani General Teaching Hospital, and Al Sadir Teaching Hospital in Basrah province from October 2023 to March 2024. A total of 200 individuals suspected of having a urinary tract infection (UTI) were provided clinical samples. The participants' ages ranged from 20 to 60 years. Each patient's urine and blood samples were classified as +ve *E. coli*. After a general urine examination (GUE) and bacterial culture, the isolates were examined under a microscope. Biochemical tests were used to identify Gram-positive bacteria, while Gram-negative isolates were identified using the Vitek® 2 system. The results of 16S rDNA sequencing also confirmed the identification of *E. coli* in the isolates. The positive patients were included in this study, and additional urine and blood samples were collected from a control group, which consisted of individuals who tested negative in the GUE and culture. For each patient, 10 ml of urine and 5 ml of blood were collected, and the serum was separated from the blood by centrifugation for 20 minutes at 1000 × g.

Patients

A case-control study was conducted involving 35 patients with confirmed urinary tract infections (UTIs) and 30 healthy controls who did not have UTIs. Serum levels of receptors (NLRP3, NLRC4, and AIM2) and inflammatory cytokines (IL-1, IL-18, and MCP-1) were measured using enzyme-linked immunosorbent assay (ELISA) technology. The specific kits used in this study included the Human NLRP3 ELISA Kit RDEEH4202, Human NLRC4 ELISA Kit RDEEH1796, Human AIM2 ELISA Kit RDEEH2125, Human IL-1β ELISA Kit RDEEH0185, Human IL-18 ELISA Kit RDEEH0011, and Human MCP-1 ELISA Kit RDEEH0222, all of which are commercially available from MyBioSource/American. These kits utilize a sandwich enzyme-linked immunosorbent assay format. The experiments were conducted as factorial experiments with two replications, and differences between the averages were analyzed using the least significant difference (LSD) method.

Statistical analysis

Data from the current study were analyzed by using the Chi-square (X2) test to compare between percentages. Numeric data were described by (Mean ± SD). An independent sample t-test is used to compare two numeric variables, while an F test (ANOVA) is used to compare three numeric variables or more. Pearson correlation (R) accounted to explain the type and strength of the relationship between variables. ROC curve used to measure sensitivity and specificity of diagnostic tests (detection of the best test for diagnosis). A level of significance of α=0.05 was applied to the test. (SPSS v.26 and Excel 2013) programs used to analyze current data.

Results

Distribution of Patients with UTI According to gender

The present study included 100 patients with UTIs. The distribution of UTI patients according to gender is shown in (Figure 1). In the present study, the proportion of female patients with UTI was higher than that of male subjects, 78 versus 22, respectively.

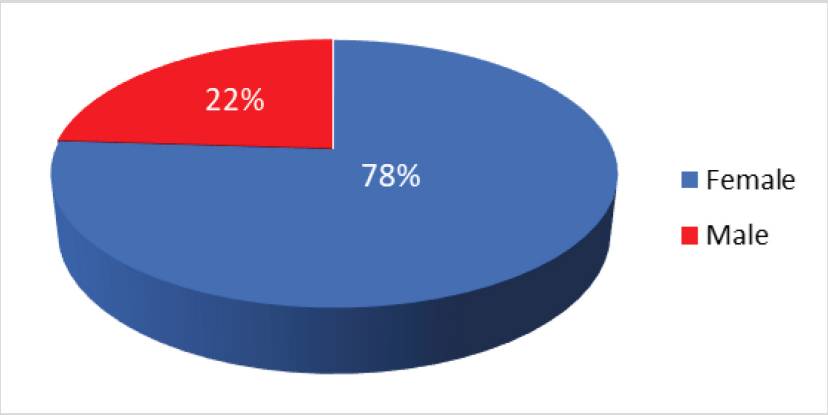

Fig. 1. Distribution of the patients with UTI according to gender

Distribution of patients and control according to age groups:

According to recent data, UTI patients of (20–30) age group had the highest proportion of infection, while those between the ages of (51–60) had the lowest percentage, with significant differences ($p \leq 0.001$). (Figure 2) and (Table 1).

Table 1.

Distribution of patients and control according to age

Age range groups (Years)	Number of patients	Percentage of patients	Number of control	Percentage of control	Total number	Percentage of total	The *p*-value for each period	*p*-value for total
20-30	14	40%	14	36.67%	28	43.08 %	0.595	
31-40	8	22.86%	7	26.67%	15	23.08 %	0.964	
41-50	7	20%	5	23.33%	12	18.46 %	0.735	0.001
51-60	6	17.14%	4	13.33%	10	15.38 %	0.677	
Total	35	100%	30	100%	65	100%	0.018	

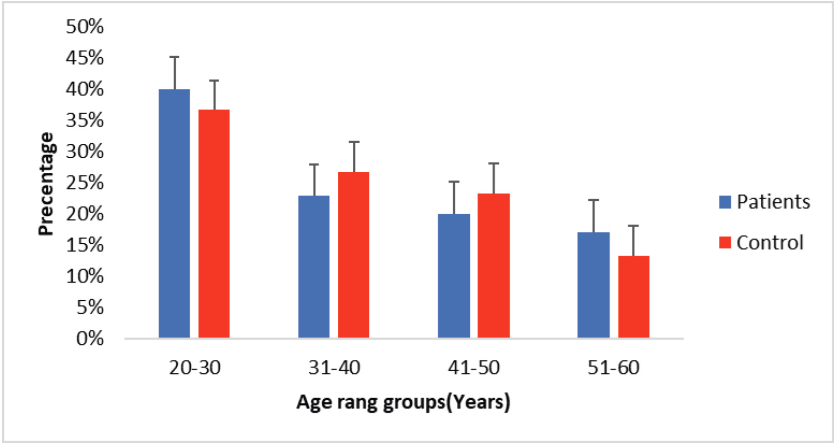

Fig. 2. Distribution of patients and control according

Distribution of patients according to type of bacteria

Present work indicated that the percentages of uropathogen. *E.coli* 35(50%) isolates, *S. aureus* 18(25.71%) isolates, *K. pneumoniae* 5(7.14%) isolates follows by *Staphylococcus spp.* 4(5.71%), *Streptococcus spp.*3(4.29%) isolates, *Psedomonas* 3(4.29%) isolates and 2(2.86%) *Proteus mirbalies* isolates as shown in (Figure 3), with significant differences $p \leq 0.005$.

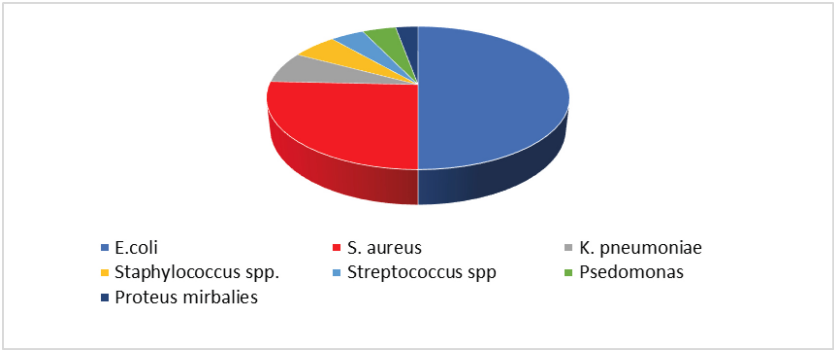

Fig. 3. Frequency of bacterial isolates in the urine of patients

Determination of NLRP3, NLRC4 and AIM2 concentration

Present data recorded highly significant elevation $p \leq 0.001$ of NLRP3 and NLRC4 in sera of UTI patients in comparison with control. While current study

showed that there were significant differences $p \leq 0.003$ of AIM2 in sera of UTI patients in comparison with control groups (Table 2) and (Figures 4,5 and 6).

Table 2.

NLRP3, NLRC4, and AIM2 concentration in sera of patients and control groups.

Groups		N	Mean	SD±	*p*-value
NLRP3 (ng/ml)	Patients	35	6.447	±2.328	0.001
	Control	30	2.597	±0.645	
NLRC4 (pg/ml)	Patients	35	127.61	±9.74	0.001
	Control	30	107.02	±14.71	
AIM2 (ng/ml)	Patients	35	0.35	±0.181	0.003
	Control	30	0.24	±0.039	

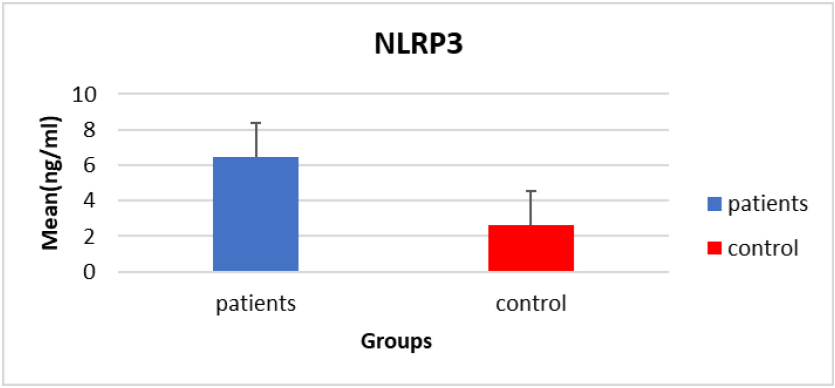

Fig. 4. Mean serum levels of NLRP3 in patients and control groups.

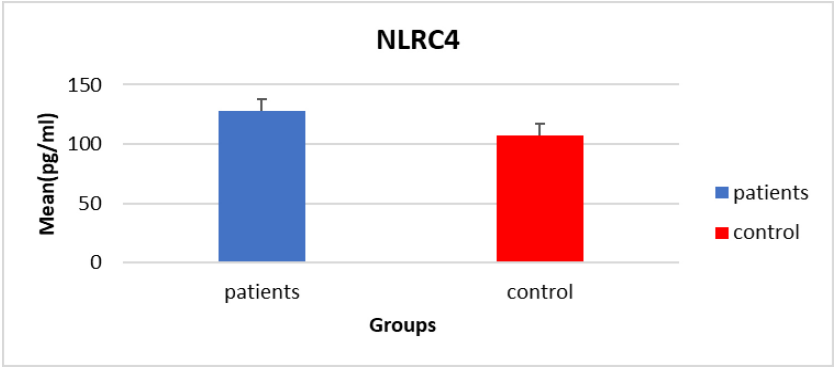

Fig.5 . Mean serum levels of NLRC4 in patients and control groups

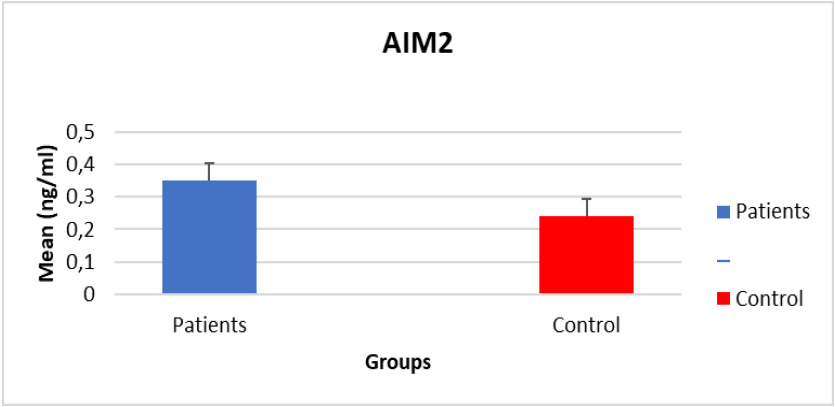

Fig. 6. Mean serum levels of AIM2 in patients and control groups

Concentration of NLRP3, NLRC4, and AIM2 in patients according to age groups

Recent data documented that the mean value of NLRP3 was higher in (the 31-40) age group than in the (20-30) age group with high significant differences ($p \leq 0.001$) among all studied age groups.

The concentration of NLRC4 was higher in (the 41-50) age group than in the (20-30) age group with a significant difference ($p \leq 0.01$) among all studied age groups. In the case of AIM2 current data do not record any statistical difference among studied age groups where the highest concentration was recorded in the (31-40) age group and the lowest in the (20-30) age group (Figure 7) and (Table 3).

Table 3.

Concentration of NLRP3, NLRC4 and AIM2 in patients according to patients age groups

Age range groups	Concentration of receptors								
	NLRP3 (ng/ml)			NLRC4 (pg/ml)			AIM2 (ng/ml)		
	Mean	SD±	*p*-value	Mean	SD±	*p*-value	Mean	SD±	*p*-value
20-30(G1)	5.005	±1.244	0.001	121.85	3.51	0.001	0.2771	±0.0542	0.236
31-40(G2)	9.267	±2.270		124.114	2.407		0.4496	±0.2519	
41-50(G3)	5.832	±1.426		139.65	12.50		0.3591	±0.2198	
51-60(G4)	5.587	±1.455		124.468	2.309		0.3192	±0.0866	

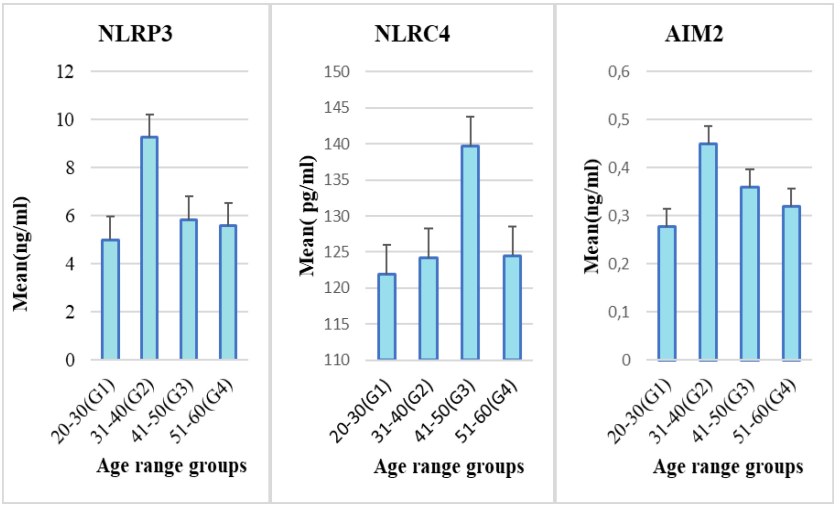

Fig. 7. Concentration of NLRP3, NLRC4, and AIM2 in patients according to age groups

Determination of sera IL-1β, IL-18 and MCP-1 concentration by ELISA

The results demonstrated that there was an increase in IL-1β levels in sera of UTI patients than the control group with highly significant differences of $p \leq 0.001$ in (Table 4) and (Figure 8).

In the case of IL-18 recent data documented that there was a highly significant elevation in UTI patients than control, (Table 4) and (Figure 9). MCP-1 concentration not showed any statistical differences between the two studied groups (Table 4) and (Figure 10)

Table 4.

IL-1β, IL-18 and MCP-1 concentration in sera of patients and control groups

Groups		N	Mean	SD±	*p*-value
IL-1β (pg/ml)	Patients	35	151.48	±39.702	0.001
	Control	30	62.41	±12.161	
IL-18 (pg/ml)	Patients	35	299.41	±11.926	0.001
	Control	30	144.05	±1.969	
MCP-1 (Pg/ml)	Patients	35	27.10	±5.636	0.303
	Control	30	25.96	±2.147	

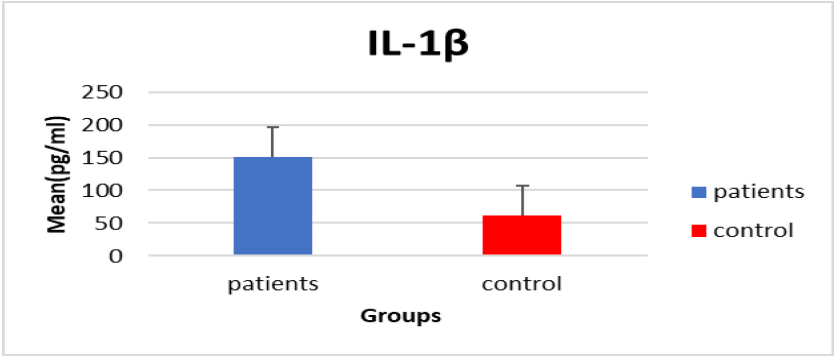

Fig. 8. Mean serum levels of IL- 1β in patients and control groups

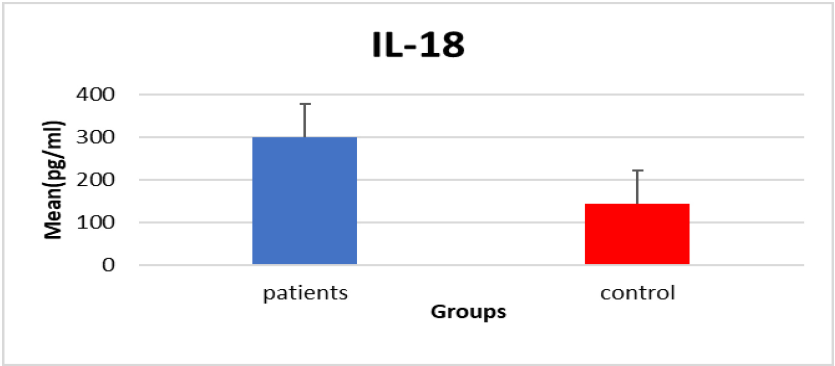

Fig. 9. Mean serum levels of IL-18 in patients and control groups

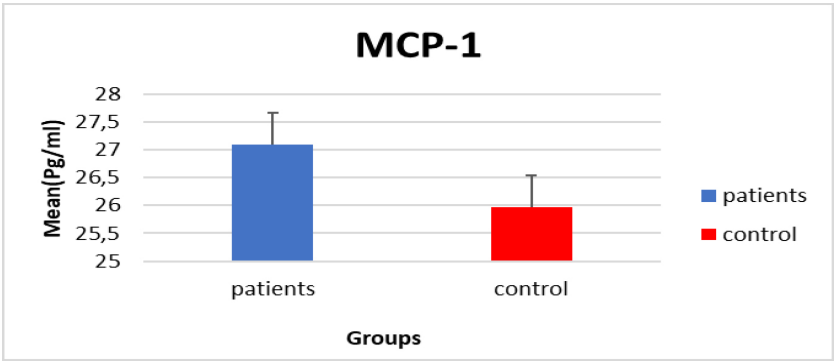

Fig. 10. Mean serum levels of MCP-1 in patients and control groups

Concentration of IL-1β, IL-18, and MCP-1 in patients according to age groups.

Recent data documented that the mean value of IL-1β was higher in the (41-50) age group than in (the 20-30) age group with high significant differences ($p \leq 0.01$) among all studied age groups. Current results showed that the concentration of IL-18 was higher in the (51-60) age group than in (the 20-30) age group with significant differences ($p \leq 0.010$) among all studied age groups. In the case of MCP-1 current data did not record any statistical differences among studied age groups where the highest concentration was showed in the (51-60) age group and the lowest in (the 20-30) age group (Figure 11) and (Table 5).

Table 5.

Concentration of IL-1β, IL-18, and MCP-1 in patients according to age groups

Age range groups	Concentration of cytokines								
	IL-1β			IL-18			MCP-1		
	Mean	S.D ±	*p*-value	Mean	S.D ±	*p*-value	Mean	S.D ±	*p*-value
20-30(G1)	94.8	± 42.6	0.001	285.55	± 16.34	0.001	25.64	± 5.62	0.611
31-40(G2)	159.91	± 7.80		303.23	± 3.86		25.88	± 3.80	
41-50(G3)	183.04	± 21.91		305.15	± 3.94		27.99	± 6.79	
51-60(G4)	161.86	± 5.74		304.25	± 3.98		28.70	± 6.27	

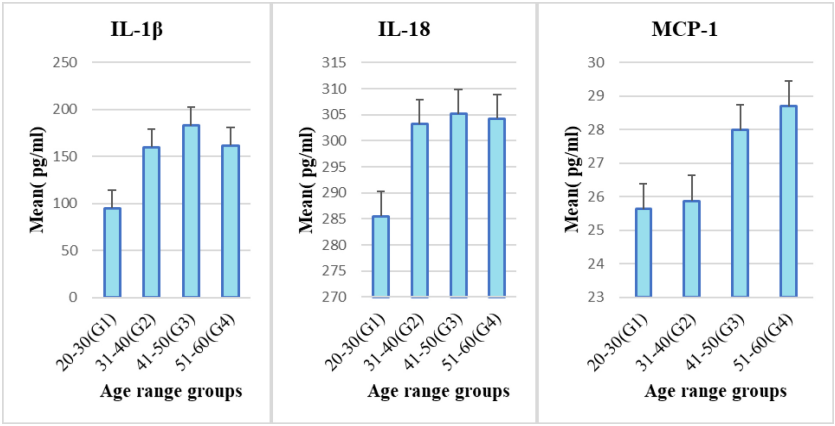

Fig. 11. Concentration of IL-1β, IL-18 and MCP-1 in patients according to age groups

Correlation of *E.coli* infection with receptors and cytokines

A positive correlation was recorded between *E.coli* infection and all studied receptors (NLRP3, NLRC4, and AIM2), (Table 4) The same is true in the case

of studied inflammatory cytokines (IL-1β and IL-18), whereas no correlation *E.coli* infection with MCP-1, (Table 6).

Table 6.

Correlation of E.coli infection with receptors and cytokines

Receptors	*E.coli*	
	R-value	*p*-value
NLRP3	0.741	0.001
NLRC4	0.647	0.001
AIM2	0.363	0.003
cytokines	R-value	P-value
IL-1β	0.830	0.001
IL-18	0.994	0.001
MCP-1	0.130	0.302

Correlation between receptors and cytokines

Data analysis indicated that there was a positive correlation between NLRP3 and NLRC4 with IL-1β and IL-18, whereas no correlation with MCP-1 (Table 7). A positive correlation was also showed between AIM2 with IL-18, whereas no correlation between AIM2 with IL-1β, and MCP-1 (Table 7).

Table 7.

Correlation between receptors and cytokines

Receptors	Cytokines					
	IL-1β		IL-18		MCP-1	
	R-value	*p*-value	R-value	*p*-value	R-value	*p*-value
NLRP3	0.609	0.001	0.773	0.001	0.142	0.258
NLRC4	0.558	0.001	0.646	0.001	0.082	0.516
AIM2	0.153	0.225	0.359	0.003	-0.175	0.170

Sensitivity, Specificity, and off for comparison parameters of current study ROC curve in serum

As shown in (Table 8) and (Figure 12) of receptors, serum NLRC4 showed a high sensitivity when compared with serum NLRP3 and AIM2 (89.5%% vs.85.7% and 54.2%), respectively, while serum NLRP3, was high Specificity when compared with serum NLRC4 and AIM2 (90%% vs.80% and 89%) respectively. As shown in (Table 8) and (Figure 12) of cytokines, serum IL-18 showed a high sensitivity when compared with serum IL-1β and MCP-1 (91%% vs. 85.7%% and 34.3%%) respectively, also IL-18, serum was high Specificity

when compared with IL-1β serum and MCP-1 serum (92% vs.90% and 90%), respectively.

Table 8.

Sensitivity, Specificity, and Cut-off for comparison studied receptors and Cytokines by ROC curve

serum								
parameters	Sensitivity %	Specificity %	Cut off	AUC	C.195		p-value	S.erroe
					Lower	Upper		
NLRP3	85.7%	90%	4.2085	0.979	0.822	0.899	0.000	0.13
NLRC4	89.5%	80%	109.381	0.877	0.775	0.842	0.000	0.52
AIM2	54.2%	89%	0.589	0.542	0.401	0.683	0.563	0.72
serum								
parameters	Sensitivity %	Specificity %	Cut off	AUC	C.195		P value	S.erroe
					Lower	Upper		
IL-1β	85.7%	90%	119.889	0.953	0.829	0.894	0.001	0.027
IL-18	91%	92%	201.054	0.989	0.893	0.922	0.001	0.024
MCP-1	34.3%	90%	28.648	0.484	0.332	0.636	0.823	0.077

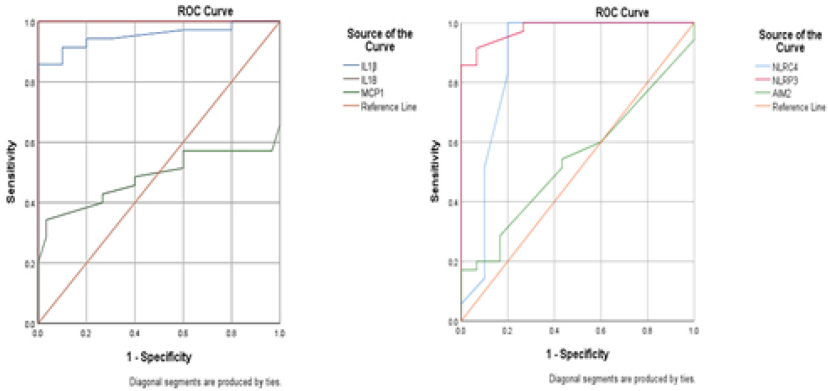

Fig. 12. ROC curve for comparison of studied receptors and cytokines

The ROC curve of studied receptors showed that the Area under the curve (AUC) for serum NLRP3, NLRC4, and AIM2 (AUC= 0.979, 0.877, 0.542) respectively whereas the standard error for serum NLRP3, NLRC4 and AIM2 (0.13, 0.52 and 0.72) respectively. (Figure 12) while the confidence Interval of 95% for serum NLRP3, NLRC4, and AIM2 were 0.822 to 0.899, 0.775 to 0.842, and 0.401 to 0.683 respectively as shown in (Table 8). ROC curve of

studied Cytokines showed that the area under the curve (AUC) for serum IL-1β, IL-18, and MCP-1 (AUC= 0.953, 0.989, 0.484) respectively whereas standard error for serum IL-1β, IL-18 and MCP-1 (0.027, 0.024 and 0.077) respectively. (Figure 12) while confidence Interval of 95% for serum IL-1β, IL-18, and MCP-1 were 0.829 to 0.894, 0.893 to 0.922, and 0.332 to 0.636 respectively as shown in (Table 8).

Discussion

Urinary tract infections (UTIs) are characterized by inflammation of the urinary tract epithelium caused by the invasion of microorganisms. This is a common and significant health issue that affects individuals of all ages [10,11], both men and women. According to the current study, females accounted for 72% of UTI cases, while males comprised 28%. Previous studies support these findings. For instance, a study conducted by Kamel and Ali (2024) found that females represented 77% of UTI cases, with males at 23% [12]. Another study in Iraq reported that females made up 58.3% of UTI cases and males 41.7%. Alfetlawi and Jasim (2022) indicated that in Al-Diwaniyah, females were the most susceptible to UTIs, with a rate of 79.7% compared to 20.3% for males [13]. These results align with the anatomical differences between males and females, as the urethra in women is shorter, reducing the distance bacteria must travel to reach the bladder. Additionally, hormonal fluctuations in women throughout the menstrual cycle play a significant role in increasing their susceptibility to UTIs [14]. Present data revealed that the distribution of UTI patients was highest percentage with ages between (20-30), which was in agreement with Alfetlawi and Jasim, (2022) who referred the highest percentage with ages between (18-30) [13]. Study done by Assafi *et al.,* (2022) revealed that the highest percentage with ages between of (20-39) [15]. Another study showed that the highest proportion of UTIs was detected among aged (27-35) years with high parity Kamel and Ali (2024) [12]. A study conducted in Iraq found that the predominant age group was (20-29) years, stated that the risk factors for UTI in women include: sexual intercourse could lead to minor urethral damage and also transfer bacteria from the perineum to the urethra and bladder which made them more susceptible to UTI [16].

UTI in the current study is frequently seen with increasing gestational age, which coincides with the findings of (Ahmed,2021) showed that pregnancy can temporarily weaken the immune system and make it more susceptible to infections [17]. In our research, despite changes in the etiology of UTIs over recent years, *E. coli* emerged as the most common urinary pathogen encoun-

tered. These findings correlate strongly with numerous studies conducted both regionally and globally. Specifically, our study found that *E. coli* was the most common bacteria isolated from UTIs, at a rate of 50%, which aligns with Abbood (2024) who reported the same rate in Baghdad, Iraq reported the rate of *E. coli* (50%) [18]. According to (Jalil and Atbee 2022) findings from 82 (68.3%) urine samples revealed *E. Coli* as the most common pathogen, followed by 38 (31.7%) urine samples containing *K. pneumonia* [19]. A study conducted in Lebanon found that the most common organisms causing UTI were *E.coli* (67.1%), *klebsiella pneumonia* (10%), and *Proteus mirabilis* (3.7%) [20]. A study on patients with community-acquired UTI in Libya found that *E.coli* was the most frequently isolated organism and occurred in (66.7%) of patients [21]. Another study was done in Iraq showed that *E.coli* were found to be the most common cause of UTIs [22].

One reason for *E.coli* dominance over other bacterial species that cause UTIs is that it is part of the normal flora in the intestines, where it can enter the urinary tract and cause infection due to its possession of virulence factors such as fimbriae were utilized to bind to mannose-rich receptors on bladder epithelial cells. Which increases their persistence in the urinary tract, P pili facilitated attachment to glycolipid receptors in the kidneys, contributing to the development of pyelonephritis. This strong adhesion capability distinguished *E. coli* from many other bacteria associated with UTIs [17]. Not only did *E. coli* adhere to the cells, but they were able to induce cell lysis and inflammation by secreting toxins such as hemolysin (HlyA) which stretch tissue invasion and immune evasion [23]. The cytotoxicity of hemolysin enabled this bacterium to break through epithelial barriers and infect the underlying tissues. They also contributed to synergistic inhibition of the immune response during bacterial infection by altering host signaling pathways. In addition, *E. coli* also depended on immune evasion strategies to maintain infections such as producing capsules made of K antigens. This capsule protected the bacterium from phagocytosis and complement-mediated killing thereby aiding it in survival within the urinary tract. Biofilms provide physical and chemical barriers that protect the bacteria from the immune system [24].

In order to investigate the hypothesis that NLRP3 is essential for host defense against *E. coli* UTI, the present study investigates the relation among the measuring the soluble form of NLRP3 in sera of UTI patients with positive culture. Present data indicated an increase in the concentration of NLRP3 compared to the control; this result is in agreement with Verma *et al,* (2020) [25], who showed a high significant concentration of NLRP3 in the sera of

UTI patients, which is higher than the level of the healthy group. Demirel *et al,* (2020) referred that, the NLRP3 inflammasome has been linked to the severity of uropathogenic Escherichia *coli* (UPEC)-mediated urinary tract infection [26]. UPEC hemolysin can induce the formation of the NLRP3 inflammasome by initiating deubiquitylation of NLRP3, whereas proHlyA failed to do so.

NLRP3 levels are elevated in UPEC-infected patients, which could be a contributing factor to the development of this disease. Hughes *et al*, (2016) found that the NLRP3 inflammasome plays a key role in the initiation and advancement of inflammation, bladder outlet obstruction, and bladder dysfunction in female mice and found that inhibiting inflammasomal activation (NLRP3) can lower bladder inflammation by directly influencing IL-1β and IL-18 secretion levels [27]. It could be due to the inhibition of functional changes associated with bladder inflammation (voiding frequency and volume) mediated by the NLRP3 inflammasome.

Present data indicated an increase in the concentration of NLRC4 comparable to the control; this result is in agreement with Verma *et al*, (2020) who showed a high significant concentration of NLRC4 in the serum of UTI patients, which is higher than the level of the healthy group [25]. UPEC carries flagellin and a type 3 secretion system for survival and host invasion, which can effectively activate the NLRC4 inflammasome. NAIP5 and NAIP6 detect the presence of flagellin in the cytosol, whereas NAIP1 and NAIP2 detect bacterial needle and inner rod T3SS proteins, respectively [28]. Rod proteins from the T3SS apparatus have a secondary structure similar to the flagellin D0 domain, which is responsible for ligand binding. This function is important for the co-oligomerization of NAIPs with the NLR family, including NLRC4, and explains the molecular basis for ligand detection and inflammasome complex formation [29].

Upregulated NLRC4 expression could be linked to the presence of flagellin as a virulence factor in UPEC strains, which allows them to progress through the human urinary system [25]. The exact mechanism of the NLRC4 inflammasome expression remains unknown. The involvement of NLRC4 inflammasomes in blood samples of UPEC-infected UTI patients. Increased gene expression, protein expression, and higher levels of pro-inflammatory cytokines in UTI patients may be regarded as risk factors for UTI progression.

Current results revealed that there was a significant increase of AIM2 in sera of UTI patients compared with the control. According to Inouye *et al., (*2018) showed that the AIM2 inflammasome may be activated by uropathogenic bacterial infections that enter the cytoplasm by type 3 secretion system (

T3SS) or bacteriolysis and cause cystitis [30]. A study done by Russell *et al.,* (2023) [1] showed that the AIM2 has a critical role in UTI patients by *E. coli.* Whereas Verma *et al.,* (2019) revealed no significant concentration of AIM2 in UTI patients [25].

AIM2 is a cytoplasmic sensor that can recognize double-stranded DNA (dsDNA), typically from pathogens or damaged host cells [1]. *E. coli* bacteria incorporate into the urinary tract and expel their DNA out to the environment [31]. AIM2 senses this bacterial DNA and activates the immune system. The inflammatory response might cause increased levels of AIM2 in the bloodstream among people suffering from urinary tract infections. AIM2 levels in the serum of patients suffering from UTI can be affected by this inflammatory response. Several mechanisms might also explain the increase in AIM2 levels in the serum of UTI patients: Released directly from the activated cell(s): When AIM2 is activated when it binds to bacterial DNA it may get released from the cells into the bloodstream, hence increasing the serum levels [32]. Induced production occurs when an inflammatory response is triggered by AIM2. During this time, AIM2 can also be produced or synthesized in increased amounts by other cells in the body [32].

IL-1β is one of the first cytokines that are released during the inflammatory process directly after some infection occurs. If *E. coli* asserts an infection in the urinary tract, IL-1β would usually be produced and injected at once to attract phagocytic cells such as neutrophils and monocytes to the focus of infection. This helps in the clearance of the bacteria but also results in a systemic increase in IL-1β levels, which can be detected in the sera [26].

Present data indicated an increase in the concentration of IL- 1β compared to the control this result is in agreement with Asker and Ali (2022); Kasid *et al*, (2023); Al-Saowdy and Abbas, (2024) showed a significantly high concentration of IL- 1β in the sera of UTI patients [34, 35,36]. Kannian *et al., (*2020) referred that IL-1β has recently been linked to the severity of uropathogenic *Escherichia coli* (UPEC)-mediated urinary tract infection [37]. Human neutrophils' antimicrobial activity against Uropathogenic *Escherichia coli* (UPEC) is regulated by the release of IL-1β from them when exposed to UPEC (Demirel *et al*, 2020) [26]. According to Engelsöy *et al*, (2019) UPEC carrying virulence factors such as pyelonephritis-associated pili (pap), type-1 fmbriae (fmH), α-hemolysin (hlyA) and Toll/interleukin-1 receptor domain containing protein C (TcpC), increase the production of IL-1β from various cell types *E. coli,* especially uropathogenic strains (UPEC), can ignite the NLRP3 inflammasome activation in immune cells like macrophages for example [38]. These inflammasomes are capable of getting

hold of bacterial invaders such as lipopolysaccharide (LPS) components, bacterial toxins, or simply invasive bacteria. Once these are activated, the inflammasomes can provoke the cleavage of pro-IL-1 beta to its active form which is later released into the bloodstream during an inflammatory response [4].

Urinary tract infections (UTIs) have been shown to damage the epithelial tissues of the urinary tract, as well as the bladder's epithelium. This damage can trigger immune cells to produce more interleukin-1 beta (IL-1β) to assist in tissue repair. Once the tissue is ruptured, it releases danger-associated molecular patterns (DAMPs), which further elevate the levels of inflammasomes and enhance IL-1β production [34]. Additionally, there is a reduced likelihood of immune cell infiltration at the sites of infection. Recent data demonstrates a significant increase in serum levels of interleukin-18 (IL-18) in patients with UTIs.

Comparably with control and this was also coincident with the result of Asker and Ali (2022) whom showed a high significant concentration of IL-18 in the serum of UTI patients [34]. This result does not contradict the result of the study of (Shaker *et al*.,2023) which stated that the serum IL-18 level in patients with glomerulonephritis (which is a type of urinary tract infection) is higher compared to healthy controls [39].

UPEC, possess various virulence factors such as fimbriae and toxins that help them adhere to the urothelium and stimulate immune responses [40], these virulence factors trigger the activation of immune cells and pro-inflammatory pathways, leading to elevated IL-18 production.

In UTI, urothelial cells (lining of the urinary tract) are directly exposed to *E. coli,* which can stimulate them to release IL-18 as part of their innate immune response to infection [41]. IL-18 also amplifies the recruitment and activation of neutrophils, key immune cells in UTIs. Neutrophil-mediated responses are important for clearing bacteria but can also contribute to excessive inflammation, raising IL-18 levels further [42].

The current study showed that there were no significant differences ($p>0.05$) in MCP-1 levels between patients and control. This result agreement the result of the study of (Verma *et al,*.2019) which stated no significant difference was observed in the expression of MCP-1 in UT patients [25]. The body may deliberately reduce MCP-1 levels as a protective mechanism to prevent overwhelming inflammation. MCP-1 is involved in recruiting immune cells to infection sites, and if too many immune cells accumulate, it could result in significant inflammation and damage to the urinary tract tissues [43].

Decrease level of MCP-1 (Monocyte Chemoattractant Protein-1) in the sera of UTI patients caused by Uropathogenic *E. coli* (UPEC) can utilize several

mechanisms to avoid the immune system [44], involving suppressing the production of key cytokines like MCP-1. *E. coli* inhibits the recruitment of immune cells, involving monocytes and macrophages, allowing the bacterium to keep in the urinary tract.

During chronic or recurrent UTIs the immune response may become improperly regulated within the immunological process and down-regulate inflammatory pathways by default. This may result in the downregulation of MCP-1 as part of the mechanism to curb inflammation to avoid extensive tissue destruction due to an excessive immune response [45]. Virulence factors such as α-hemolysin (HlyA) and toxins released by *E. coli* suppress the signaling pathways that mediate MCP-1 production, leading to ineffective inhibition of medullary congestion. In this way, the pathogen escapes the immune response by effectively lowering chemotactic signals that would call for immune cells to migrate to the biological focus of an infection [46]. In active, prolonged, or recurrent infections, the immune system could be less active, resulting in a state called immune exhaustion. This results in lower levels of immune mediators like MCP-1, as the body is less capable of sustaining a robust immune response.

The low level of MCP-1 also may impair the recruitment of important immune cells including macrophages and monocytes [47], which result in an inability of the immune system to effectively clear the *E. coli* infection and may lead to persistent or recurrent UTIs.

Conclusion

E. coli is the most common species among the bacteria UTI patients of Basrah province. Elevated level of innate immune receptors (NLRP3, NLRC4, AIM2) and inflammatory cytokines (IL-1β and IL-18) in sera of UTI patients as a possible candidate marker for UTI.MCP in sera can't be used as a potential marker in UTI patient's marker. There is a positive correlation between NLRP3, NLRC4, AIM2, IL-1β, and IL-18 in sera and *E. coli* of UTI patients. Further study on exploring the role of MCP-1 in modulating immune responses in UTIs, especially its interactions with other cytokines and receptors.

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DATA ABOUT THE AUTHORS

Zainab Ali Eidan, PhD, Prof., Department of Biology
College of Science, University of Basrah
Basrah, Iraq
zainabmoha2025@gmail.com
ORCID: https://orcid.org/0009-0009-7009-0164

Wafaa S. Shani, PhD, Prof., Department of Biology
College of Science, University of Basrah
Basrah, Iraq
wafaa.shain@uobasrah.edu.iq
ORCID: https://orcid.org/0000-0003-4688-780X

Muna Abdul-Imam Almazini, PhD, Prof., Department of Biology
College of Science, University of Basrah
Basrah, Iraq
muna.abdul-ima@uobasrah.edu.iq
ORCID: https://orcid.org/0000-0001-9544-1535

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