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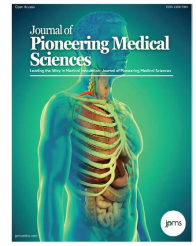

Serum levels of interleukin-15 (IL-15) and interleukin-40 (IL-40) in children with urinary tract infection

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Abstract The immune system is greatly impacted by urinary tract infections (UTIs), which cause both systemic and local immune responses meant to eradicate the infection and stop its spread. However, persistent or recurrent infections can result in immune dysregulation. The current study aimed to determine the serum levels of interleukin in the fifteenth and fortieth tests in a sample of children with UTI. This study was conducted at Al-Batoul Teaching Hospital for Women and Children in Diyala Governorate from August 2025 to January 2026. Ninety samples were collected from children, fifty of whom were diagnosed with urinary tract infection by the consultant doctor at the hospital, twenty of them female and thirty males. The sick children were divided into three age groups: the first group ranged in age from 2-3 year The second year is between 4-5 years and the third year is between 6-7 years, depending on their arrival at the hospital during the study. ELISA serological testing was used to determine the serum levels of interleukin 15 and interleukin 40 from blood serum. The study showed a decrease in the level of interleukin 15 when comparing patients and healthy individuals, By a huge margin, P = 0.001. The same samples also showed a big increase in interleukin 40 when comparing patients to healthy people (P = 0.001). In short: the rise of interleukin 40 in patients versus the drop in interleukin 15 matches inflammation that hasn't reached the kidneys yet, meaning the inflammation is acute or happens repeatedly.

Key Words: IL-15, IL-40, Urinary Tract Infection, Gross Hematuria, Malodorous Urine

INTRODUCTION

A urinary tract infection (UTI) is defined as an inflammatory condition resulting from excessive bacterial growth in the urinary tract, leading to dysfunction of the urinary tract and kidneys [1]. Humoral immunity plays a vital and effective role in combating urinary tract infections [2]. Immune interleukins play an important role in limiting the exacerbation of urinary tract infections. Interleukin-15 (IL-15) plays a critical role in regulating the survival, proliferation and activation of lymphocytes, as well as regulating the recruitment of neutrophils and phagocytes to infection sites. IL-15 is primarily expressed in monocytes, fibroblasts and some epithelial cells, while a wide range of immune cells respond to it. Including memory T cells, NK (Natural Killer) cells, NKT cells and CD+ memory T cells. It shares some functions with IL-2 while retaining structural characteristics, consisting of a large protein made of 114 amino acids and functionally, IL-15 prevents apoptosis in immune cells. It is distinguished in regulating innate lymphoid cells and long-lived cytotoxic T cells [3].

Interleukin-40 (IL-40), which is specifically associated with B cells, humoral immune response and antibody production; Interleukin-40 is a cytokine protein that does not belong to any of the known traditional cytokine families, such as the classical interleukin family or the TNF family. It was first characterised as a B-cell-associated cytokine and has a role in the formation and maturation of these cells in the bone marrow and periphery. [4]. Interleukin-40 (IL-40), the B cell-associated cytokine and local immune response, in the pathogenesis of UTI in children [5].

METHODS

Study Samples

This study was conducted from August 2025 to January 2026 and included 90 children aged between 2 and 7 years. The study samples were divided into two groups: 50 out of the 90 children had urinary tract infections, diagnosed by a specialist doctor at the pediatric unit of Al-Batoul Teaching Hospital for Women and Children. The lab cultures of their samples (30 boys and 20 girls) were grown on blood agar and MacConkey agar, which showed positive results for pathogenic bacteria, along with microscopic urine examination. The other forty children (20 boys and 20 girls) in the same age group as the children in the study hadn't had any urinary tract infections and represented the control group, as they were free from urinary tract infections after being examined by the consulting doctor and having their urine cultured on blood and MacConkey agar, with no signs of any diseases or infections and not taking any medication during the examination period.

Blood Sample Collection

We collected 5 ml of venous blood from all study participants using a sterile syringe and put it in a gel tube, then left it to clot for 15 minutes at room temperature (25-30°C). Next, we spun the tubes at 3000 rpm for 15 minutes to separate the serum, which we stored in Eppendorf tubes in three 1 ml portions, keeping one portion for each ELISA test to avoid thawing the frozen samples again.

Immunological Examination

- Interleukin 15 (IL15)
- SEA061Hu 96 Tests
- Enzyme-linked Immunosorbent Assay Kit
- Organism Species: Homo sapiens (Human)
- Instruction manual

Detection Range

15.6-1,000pg/mL. The standard curve concentrations used for the ELISA's were 1,000pg/mL, 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, 31.2pg/mL, 15.6pg/mL.

Sensitivity

- The minimum detectable dose of IL15 is typically less than 5.6pg/mL
- The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration
- that could be differentiated from zero. It was determined by adding two standard deviations to the mean optical
- density value of twenty zero standard replicates and calculating the corresponding concentration
- Use a microplate reader that has a 10 nm or smaller bandwidth and an optical density range of 0-3 O.D. at a wavelength of 450±10 nm to measure absorption

Interleukin-40

Catalog number ELK0969, Human IL-40 ELISA Kit, ELK Biotechnology, USA. Sensitivity of the current ELISA kit is 0.13 ng/mL, detection range: 0.32-20 ng/mL.

Human IL-40(Interleukin 40) ELISA Kit

Detectable Sample Type: Serum, plasma, tissue homogenates, cell lysates, cell culture

Set up 7 points of Diluted Standard such as 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.63 ng/mL, 0.32 ng/mL and the last EP tubes with Standard Diluent is the Blank as 0 ng/mL. Then, run the microplate reader and conduct measurement at 450 nm immediately.

Ethical Approval

This study was reviewed and approved by the Research Ethics Committee at the University of Diyala, Iraq (Approval No.: 29275 dated August 11, 2025). Approval was granted based on the Declaration of Helsinki, to ensure that all procedures followed comply with international ethical standards for research involving human participants and biological materials. Informed consent was obtained from all patients before sample collection.

Inclusion Criteria

The inclusion criteria in the current study included children aged between two and seven years and children who have clinical symptoms of urinary tract infection.

Exclusion Criteria

Children who are taking any kind of medication, children with chronic or associated diseases and children with congenital abnormalities in the urinary system were excluded.

Statistical Analysis

All statistical analyses were performed using SPSS version 26.0 to compare demographic factors (age (three categories), gender, residence) and their relationship with patients and healthy individuals. A one-way ANOVA test was used to determine the relationship between interleukin 15 and interleukin 40 among patients and healthy individuals. The ANOVA test was chosen because for each interleukin there are three groups based on age category and two groups based on gender and residence. A P-value of less than 0.001 and greater than 0.05 was considered.

RESULTS AND DISCUSSION

Distribution Demographic Characteristics of the Study Samples

Table 1 showed the demographic information of the study group, which includes 50 (55.6%) patients out of 90 patients who underwent immunological tests and 40 (44.4%) children as a control group. The patients constituted of the total sample compared to in the control group, reflecting the study structure of the dataset.

Table 1: Distribution of Demographic Characteristics of Patients and Healthy Individuals

Study group No.	Category	Patients	Controls	P value
90 individuals	2-3	5 (55.6%)	40 (44.4%)	
Age rang	4-5	20 (22.2%)	5 (5.6%)	>0.001
	6-7	20 (22.2%)	13 (14.4%)	
		10 (11.1%)	22 (24.4%)	
Sex	Male	30 (33.3%)	20 (22.2%)	>0.05
	Female	20 (22.2%)	20 (22.2%)	
Residence	Urban	44 (48.9%)	30 (33.3%)	>0.05
	Rural	6 (6.7%)	10(11.1%)	

Regarding to gender, the infected males had the highest proportion among the cases, numbering 30 (33.3%), compared to 20(22.2%)females, while equal numbers for male and females in the control group 20 children (22.2%) and did not show statistically significant differences (df = 1, p > 0.05 of two groups were similar).

In terms of age, the highest participation rate among patients was in the age groups 2-3 years and 4-5 years, with each group having 20 individuals out of 50, representing)22.2%(, followed by the older age group 6-7 years, which had 10 individuals out of 50 patients, making up)11.1%(. In the control group, the age group that had the highest participation was the oldest, 6-7 years, with 22 children accounting for)24.4%(, followed by the 4-5 years' group with 13 children, representing)14.1%(. As for the youngest ages (2-3years), they were the least, with only 5 children, representing)5.6%(. A statistically significant difference was found when comparing age groups, with a P value of 0.001, indicating the importance of age in the recurrence of infection. The study showed that the residence of the participants in urban areas was the most common in both the patient and control groups. In the patients, there were 44 children from urban areas, accounting for)84.9%(, compared to 6 children from rural areas, accounting for)6.7%(. As for the control group, there were 30 children from urban areas, accounting for)33.3%(and 10 children from rural areas, accounting for)11.1%(. No statistically significant differences were observed, as p>0.05.

The results in Table (1) showed that Age Groups: This study agreed with the study by [6] in Diyala, showing that younger age groups are more affected by bacterial urinary tract infections. There were 123 children aged 1-2 years infected, representing 51.77% and 66 children aged 3-4 years infected, representing 25.88%. For the older age group, 5-6 years, there were 57 children infected, making up 22.35%. This study also aligned with the study by [7] in China, where 47 children, representing 33.3% out of 141, were infected with urinary tract infections. The study by [8] in Poland showed that younger age groups had a higher infection rate of 63.4%, with a significant difference (P value = 0.0001), compared to older groups. Additionally, it agreed with the study by [9] in Finland that urinary tract infections decrease in circumcised boys at a young age, with a significant difference (p <0.05).

The genders: This study agreed with the study by [10] in Erbil, which included 130 patients, showing a higher incidence among uncircumcised males, numbering 91 (53.2%) compared to females, numbering 39 (30.2%). This study also agreed with the study by [11] in Saudi Arabia, where males had a higher incidence than females with a significant value of 0.0001 for the age group 1-6 years, reaching 153 for children under 5 years old and increasing as age decreased, reaching 483 for those under two years. Additionally, this study agreed with a Saudi study by [12], showing a higher incidence in males, numbering 85 (55.6%) versus females, numbering 68 (44.4%).

Table 2: Shows the Microscopic Study of the Urination in Sick

Patient	>10 less (n = 50) n = (%)	<10 Greater (n = 50) n = (%)	Non found (n = 50) n = (%)
Mucous cells	10(20.0%)	10(20.0%)	30(60.0%)
Epithelial cells	3(6.0%)	11(22.0%)	36(72.0%)
Red blood cells	1(2.0%)	49(98.0%)	0%
White blood cells	0%	50(100%)	0%

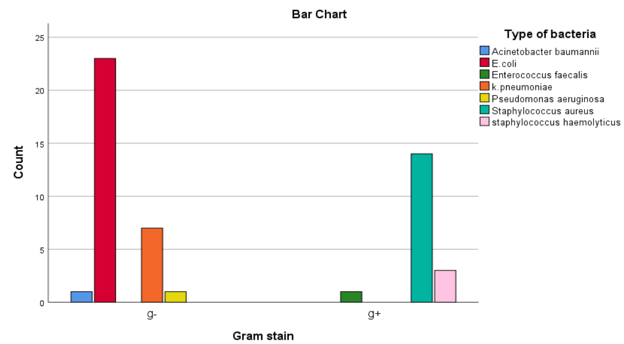

Figure 1: Shows The Distribution of Bacterial Infection Between Females and Males in Children with Urinary Tract Infections

General Analysis of Urine

The lab results in Table (2) showed that all patients had less than 10 white blood cells in their urine Red blood cells were found in only one sample <10 (2.0%), while the rest (98.0%) were >10. Mucus cells appeared in 10 samples with >10 and 10 samples with <10, with each group making up 20.0%. However, 30 patients had no mucus cells. Epithelial cells appeared in different amounts: only 3 samples were >10, making up 6.0%, 11 samples were <10, making up 22.0% and 36 samples had no epithelial cells at all, making up 72.0%, as shown in Table 2.

A study by [13] in Ethiopia, which included 224 children—118 boys (52.7%) and 79 girls (35.1%), with 27 children showing no cells in their microscopic field—found that the presence of epithelial and mucous cells in urine is important in confirming bacterial infection when present at more than or less than 10. This study agreed with a study by [14] in Sweden, which found that the presence of mucous and pus cells at levels more than or less than 10 indicates a bacterial infection. It also agreed with a study by [15] in the United States, where having pus and epithelial cells less than 5 is evidence of clear microbial infection.

Examination of Infection-Causing Bacteria

The study of urine samples, after being cultured on solid media to determine the type of infection, found that the bacteria types that appeared included 18 Gram-positive bacteria, making up 36.0% and 32 Gram-negative bacteria, making up 64.0%, Figure 1.

Based on the results of the laboratory cultures and the identification of Gram-positive and Gram-negative bacteria, it was found that the types of bacteria causing urinary tract infections in the children under study were seven types, but their frequency among the patients varied. Escherichia coli (E. coli) had the highest share with 23 cases, accounting for

Table 4: It Shows the Mean and Standard Deviation of Interleukin-15 In The Two Study Groups: Patients and Healthy Individuals

Type of interleukin	Children			P- value
	patients	Healthy individuals	total	Anova able
IL-15 Mean± Std	1.2±1 pg/ml	2.5±2 pg/ml	1.8±1 pg/ml	0.001

Table 5: It Shows the Mean and Standard Deviation of Interleukin-40 In My Study Groups: Patients and Healthy People

Type of interleukin	Sample type		total	P value Anova table
	patients	Healthy individuals		
IL-40 Mean± Std	8.1±5 ng/ml	1.9±1 ng/ml	5.3±5 ng/ml	0.001

46.0% of the infections, followed by Staphylococcus aureus and then Klebsiella pneumoniae with fewer cases, seven in total, representing 14.0%. Staphylococcus haemolyticus occurred in three children, accounting for 6.0%, while the other types appeared in just one case each out of fifty children with urinary tract infections, representing 2.0%, as shown in Table number 3.

This study agreed with [16]. That study included 11,749 children and found that the most common cause of urinary tract infections was E. coli, with 3,004 (31%) children infected, compared to other types like S. haemolyticus, which had 1,276 (12.8%) infections, K. pneumoniae with 746 (7.5%) cases and S. aureus with 479 (6%) infections. In [17], E. coli was also the dominant cause of UTIs. Similarly, [18], which included 155 children with urinary tract infections, showed that pathogenic E. coli (UPEC) was the most frequent pathogen with 58 cases (37.4%), more than other bacterial types. A study by [19] was consistent with this study, showing E. coli as the highest. Other Gram-negative bacteria, like Klebsiella pneumoniae and Pseudomonas aeruginosa, are also common in recurring infections.

Immunological Study

Evaluation of Interleukin-15 (IL-15) Levels

The results of this study showed a decrease in IL-15 levels in the blood serum of patients with urinary tract infections, with a value of 1.2±1 pg/ml, while its levels are higher in healthy individuals, with a value of 2.4±1 pg/ml, a significant difference of P ≤0.00.

Sensitivity = less than 5.6 pg/ml, detection range = 15.6–1000 pg/ml., as shown in Table 4.

This study aligns with the study by [20], which highlighted the inflammatory role of IL-15 and noted that its levels might decrease at the onset of inflammation or due to therapeutic failure. In the study by [21], it was found that IL-15 levels reduce damage and increase in infections, especially bacterial ones. However, it differed from the study by [22], where IL-15 levels in the serum of UTI patients caused by Klebsiella strains (K-ESBLs) were significantly elevated: IL-15 levels were 29.4±9.2 in patients compared to 22.1±5.1 in healthy individuals, with a significant difference of p <0.001. It also differed from the study by [23], where elevated IL-15 levels in patients indicated its inflammatory effect and its role in maintaining natural killer cells and $CD8^{+}$ T cells, as the difference compared to healthy individuals was significant (Pvalue ≥0.05). Furthermore, it differed from the study by [24], which noted elevated interleukin levels in UTI patients at the beginning of inflammation. Interleukin 15 (IL-15) belongs to the interleukin-2 family It activates immune cells like natural killer (NK) cells and T cells and contributes to the immune response against bacterial infections in urinary tract infections (UTIs) caused by bacteria. IL-15 has a protective role, helping to reduce inflammation in kidney cells. Changes in its levels, either up or down, are associated with the severity of the infection or the type of bacteria [25].

A decrease in IL-15 is due to weak activation of NK cells and T cells, leading to reduced efficiency in eliminating bacteria, which increases the likelihood of persistent and recurrent infections.

Evaluation of Interleukin-40 (IL-40) Levels

The results of this study showed elevated levels of the cytokine IL-40 in the blood serum of patients with urinary tract infections, with a value of 8.0±4 ng/ml, compared to healthy individuals whose cytokine levels were lower at 1±1.89 ng/ml, with a significant difference (P value ≤0.001), as shown in Table 4.

Sensitivity = 0.13 ng/ml and the sensitivity range = 0.32-20 ng/ml. as shown in Table 5.

This study agreed with the study by [26], which found that interleukin 40 levels were higher in rheumatoid arthritis patients than in healthy individuals with a highly significant difference P = 0.001. It also aligned with the study by [27], which reported a significant increase in interleukin 40 at the onset of inflammation and a decrease after recovery in rheumatoid arthritis patients, the study also agreed with a study in [28], showing elevated interleukin 40 in patients and lower levels in healthy individuals with a significant difference P = 0.001 in rheumatoid arthritis patients. Additionally, the study by [29] demonstrated the role of interleukin 40 (IL-40) in various body inflammations as an inflammatory cytokine, it also agreed with the study by [30], stating that interleukin 40 is inflammatory and rises at the onset of inflammation. In a study by [31], its levels were significantly higher in patients compared to healthy individuals, with a difference of P = 0.001 in rheumatoid arthritis Rheumatoid joints.

CONCLUSIONS

The immaturity of the immune system in the age group (2-3) years and (4-5) years may be the reason for the higher rate among them compared to the older age group (6-7) years. The participation of the age groups (2-3) and (4-5) years among patients was 22.2%, while the age group (6-7) years among healthy individuals had a higher participation of 42.4%. Male participation and their percentage was 33.3%, higher than females at 22.2%. Urban areas dominated over rural areas among patients and healthy individuals, with percentages of 48.9% and 33.3% respectively. The percentage of unhealthy eating was higher among patients at 46.7% compared to healthy eating among healthy individuals at 44.4%. Mucous cells in the urine of patients showed <10 and >10 equally at 20%, while samples free of mucous cells were 60%. As for epithelial cells, >10 were 22% and <10 were 6%, with 72% of patient urine samples free of epithelial cells. Red blood cells in the urine of patients were >10 in 98% of cases and white blood cells >10 were found in the urine of all patients at a rate of 100%.The fifteenth white blood cell decreased in patients, reaching 1.2±1 ng/ml, while the fortieth white blood cell increased to 8.0±4 ng/ml, with a significant difference compared to healthy individuals (P value <0.001).

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