

# Assessment Of IL-22 Receptor As A Diagnostic Biomarker For Recurrent Utis

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## Abstract :

**Background:** The surface of epithelial cells expresses IL-22R in mucosal tissues such as the urinary tract, and its elevated levels in urine indicate an inflammatory response. This receptor is part of the cytokine family and interacts with IL-22.

**Objective :** Assessment of IL-22R levels in urine from individuals who have recurrent UTIs.

**Methods:** This study included only 68 urine samples from individuals aged 6 to 60 years and older, males and females suffering from recurrent urinary tract infections from Imam Al-Sadiq Hospital (AS) in Babylon Governorate, Iraq, during the period enzyme-linked immunosorbent assay (ELISA), which is an indicator of urinary tract infection. We also compared it with 30 control samples.

**Results :** The mean level of IL-22R in urine samples of patients was 1562 ng/l, while that of the control group was 1076 ng/l, with statistically significant differences ( $P=0.003$ ), It indicates a statistically significant variation in IL-22R levels between patients and the healthy control group. Since  $P$  is less than 0.05, the difference is significant and statistically significant at the 0.05 threshold of significance. Also, the level of this interleukin in urine samples of males was 1401 ng/l and in urine samples of females was 1548 ng/l, with a value of ( $P=0.484$ ), which means that there is no statistically significant difference between males and females in IL-22R levels in urine. This indicates that gender does not clearly affect the concentration of this biomarker in urine.

**Conclusion:** IL-22R levels are elevated in cases of urinary tract infections, and this study found that testing for IL-22R protein in urine can be used as a biomarker for diagnosing urinary tract infections.

**Keywords:** IL-22R, recurrent, UTI, E.coli, Klebsiella pneumonia, urine.

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## INTRODUCTION:

Any infection of the kidneys, bladder, or urethra that results in billions of dollars in medical expenses each year is referred to as a urinary tract infection (UTI). *Escherichia coli* is the most common cause of these infections, but other pathogens, such as *Klebsiella*, *Enterococcus*, *Pseudomonas aeruginosa*, and *Staphylococcus*, also play a major role. Both men and women, healthy people and immunocompromised patients can contract these illnesses. (1) Urinary tract infections are one of the most prevalent infection forms in healthcare settings. In certain situations, if untreated, may lead to pyelonephritis and urine poisoning. In some cases, they resolve without treatment. Clinical diagnosis usually based on urine analysis and the patient's symptoms (2) Over half of women in their adult years will have at least one urinary tract infection (UTI) during their lifetime, and nearly a quarter of them will have recurrent urinary tract infections (UTIs), which are defined as two infections within a 6-month period or three infections within a 12-month period (3) In the Netherlands, urinary tract infections are the most common reason for primary care consultations, accounting for approximately 149,000 patient visits, equivalent to 2.6 million visits in 2022 (4) Prior research has demonstrated that the immune response is meticulously controlled to maintain the epithelial barrier's structural integrity. Macrophages and mast cells play a major role in the immunological control of the urinary system, which includes organizing the recruitment and start of neutrophil responses that result in the elimination of bladder microorganisms (5) Cytokines are powerful mediators of inflammation and their effectiveness is regulated in numerous ways to protect the body. Many cytokines, including IL-22, contain co-binding proteins or soluble receptors that bind to the cytokine and prevent interaction with the cellular receptor, thus inhibiting cellular signaling (6) The IL-10R $\beta$  and IL-22R $\alpha$  subunits make up the IL-22 receptor (7) Studies have shown that the IL-22R receptor is expressed on urinary epithelial cells, including those in the bladder and renal tubules. This expression enables IL-22 to directly act on these cells to enhance antimicrobial defenses (8) IL-22 receptors are made up of two subunits: IL-22RA1 (alpha1): The primary subunit that binds IL-22 directly and is designated IL-22RA1 and is also located on chromosome 1p36.11.

IL-10B2 (beta 2): This subunit shares receptors with other cytokines such as IL-26 and IL-10. Another subunit, called IL-22RA2 (alpha2), acts as a soluble receptor that regulates IL-22 activity. It is designated IL-22A2 and On chromosome 6q24.1-24.2, it stands (9).

**This study aims:**

to evaluate the expression of Interleukin-22 Receptor (IL-22R) as a potential biomarker in the diagnosis Recurrent recurring infections of the bladder (rUTIs). By analyzing IL-22R expression levels in patient samples and comparing them to healthy controls, the research seeks to determine its diagnostic value and its possible role in the pathogenesis of rUTIs.

**MATERIALS AND METHODS:**

**Sample collection:**

One hundred urine samples in all were taken from male and female patients aged between 5 and 75 years who were suspected of having urinary tract infections, during the period from November 2024 to February 2025 at Imam Al-Sadiq Hospital, located in the center of Babylon City. Sterile conditions were used to collect the samples. Containers and transported to the laboratory, where they were cultured within two hours of collection. Urinalysis was performed using dipstick test strips to assess several parameters, includes red blood cells, pH, and specific gravity, white blood cells, glucose, protein, bilirubin, ketone bodies, and nitrates. Additionally, inspection of urine sediment under a microscope was conducted under a 10x objective lens. The samples were inoculated onto various culture media, including Eosin methylene blue agar, mannitol salt agar, blood agar, MacConkey agar, and chromogenic UTI agar. The inoculated plates were incubated at 37°C for 24 hours, after which The proliferation of microorganisms was noted and documented.

**Urine culture:**

Blood agar was inoculated using urine samples. Which was quenched by adding 5 ml of sheep blood, Mannitol agar, methylene eosin blue, and MacConkey agar and UTI chromogenic agar. These plates were then incubated in an incubator for 24 hours at 37°C. The plates were then analyzed for bacterial growth or any colonies present on the plate and the type of pathogens was identified by chemical methods. Biochemical tests such as oxidase test, catalase test and IMVIC tests, such as voges, methyl red, and indole -Proskauer were also performed(10).

**Identification of Bacteria:**

Isolated bacteria are identified by characterizing them on plates and by observing their shape and biochemical properties. The properties that can be observed include shape, size, texture, and color. Also, the bacteria and their type are identified, whether they are positive or negative, based on the composition of their cell wall through Gram staining and microscopic examination of the Gram-stained slide. Also, bacteria are identified through IMVIC chemical tests some samples were identified using the Vitek 2 automated microbial identification system (11).

**Estimation of IL-22R in UTI:**

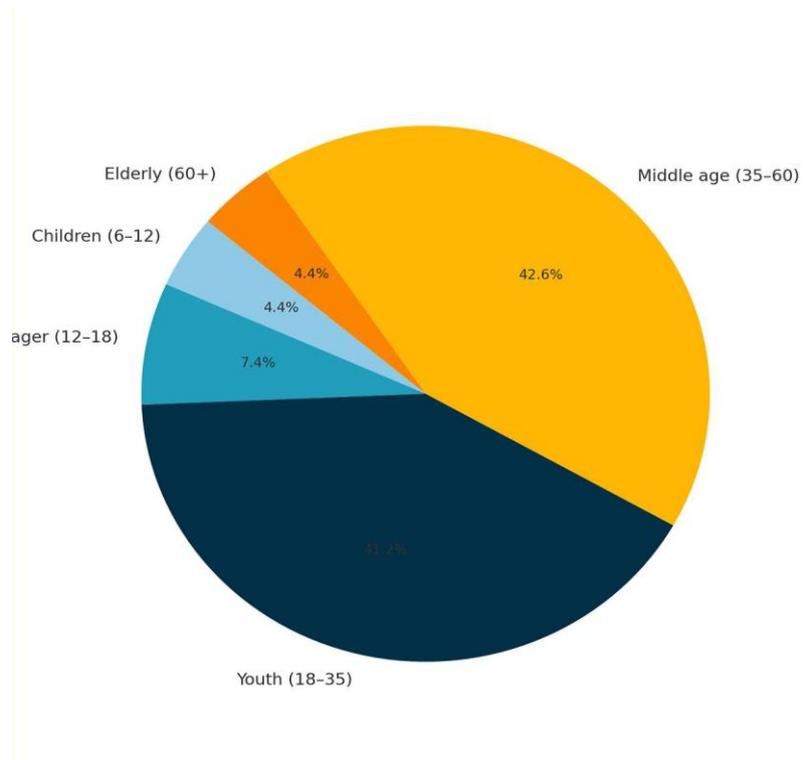
The urinary tract's immune response is significantly influenced by the interleukin 22 receptor (IL-22R). It is expressed on urothelial cells and also helps to trigger the signaling cascade pathways that lead to the production of antimicrobial peptides and promote tissue regeneration after infection, making it an important component of the immune defense against urinary tract infections (12) The concentration of IL-22R in urine samples was measured using an ELSA kit from the Chinese manufacturer BT LAB to assess the changes between patients and the control group, as well as between males and females. The data were then analyzed using a statistical T-test to compare the means and determine whether the differences between the two groups were statistically significant. This analysis will contribute to clarifying the role of IL-22R as a diagnostic tool in the early detection of urinary tract infection.

**Ethical approval:**

The protocol for the study was authorized by the Babil Health Directorate's Ethical Committee on November 1, 2024. Precautions were taken to guarantee the participants' safety during the sampling process, and the patients' verbal consent was acquired prior to sample collection. The Ethics Committee of the Iraqi Ministry of Health also conducted this work, adhering to all national regulations.

## RESULTS:

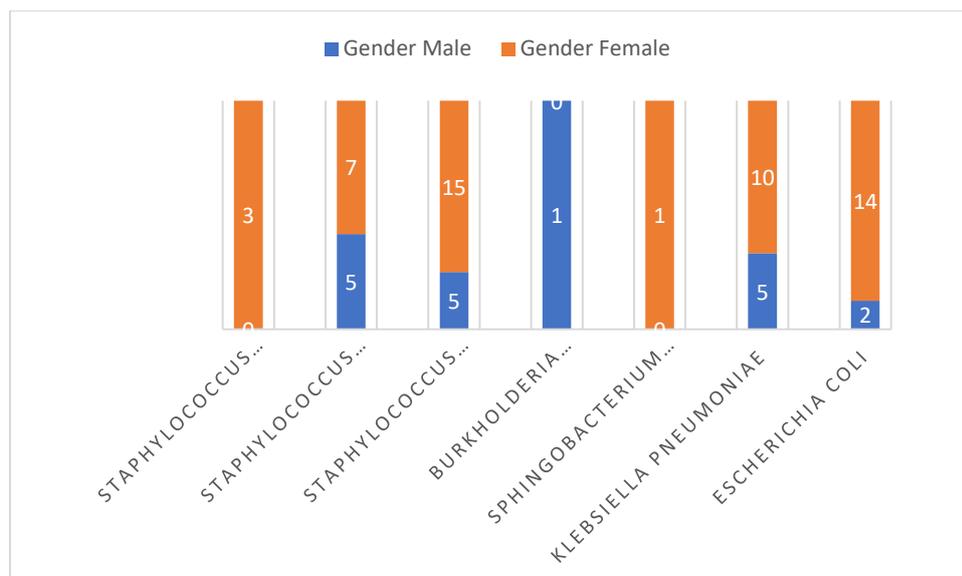
Recurrent urinary tract infection sufferers in the age range (35-60) are the most affected, followed by the age group (18-35) and then the age group (12-18), as shown in Figure 1. This study showed that the older a person is, the more likely he is to develop urinary tract infections. This is consistent with a study conducted in North Africa and the Middle East (MENA) that the infection increases with age in males due to prostate problems and is concentrated in females of reproductive age (13). It is also consistent with the American study that indicated that aging is linked to a higher rate of infection, especially after the age of 65 (14). This study confirmed that the infection rate among females is much higher than that among males and is consistent with the Arab trilateral study (Egypt - Sudan - Kingdom of Saudi Arabia), which showed that 68.45% of cases of recurrent urinary tract infections are among women(15) .



**Figure( 1) Rate of urinary tract infection by age group.**

In our study, our urine culture showed growth in 68% of the samples, while 32% did not show growth. The samples were divided into two sections, one containing negative bacteria and the other containing positive bacteria. The predominant It was discovered that the bacteria were Gram-negative, including *Escherichia coli* and *Klebsiella*. Among the positive group was *Staphylococcus aureus*. This study is consistent with a study conducted in Turkey (16), where *Escherichia coli* was more prominent among the negative group, and *Staphylococcus* was more prominent among the positive type of bacteria, especially among young women of reproductive age. This study is consistent with another study conducted in Nigeria (17), whose results showed that *Escherichia coli* was more prevalent among the negative group, and *Staphylococcus* was among the positive group, especially among women with urinary tract infections.

Figure (2) shows the gender distribution of bacterial isolates found in this study as shown. *Escherichia coli* was the most common bacteria, with 14 isolates appearing from infected females compared to two isolates from males. *Klebsiella pneumoniae* was found in females (10) compared to males (5). *Sphingobacterium thalpopilum* and *Burkholderia cepacia* were the only two Gram-negative non-fermenting bacteria that were rarely found, as only one sample was found in females and the other in males. As for Gram-positive cocci, *Staphylococcus aureus* was more evenly distributed (7) in females and (5) in males, but saprophytic *Staphylococcus aureus* was found mostly in females, with (15) samples and (5) samples in males. Females contained only three samples of hemolytic *Staphylococcus*, in line with established epidemiological trends in urinary tract infections. These results show a clear predominance of females for the majority of Urinary tract pathogens.



**Figure 2 : Distribution of Bacterial Isolates According to Patient Gender.**

The table displays the results of five commonly used biochemical tests performed on various bacterial species frequently found in clinical and environmental settings. These assays use urease, citrate, methyl red, catalase, and indole to differentiate between different bacterial species based on their metabolic capabilities. Results are shown as either positive (+) or negative (-). The phrase "does not match" indicates that the test is either not frequently used for specific species, such as Gram-positive cocci like *Staphylococcus* spp., or does not yield accurate results for certain organisms. For example, Methyl Red and Citrate are primarily used for Gram-negative rods, particularly those in the Enterobacteriaceae family, and are not commonly used for *Staphylococcus* species. as shown in table 1.

**Table 1 :Biochemical Test of Selected Bacterial Species .**

| Bacteria species              | Catalase | Indole | Methyl Red     | Citrate        | Urease |
|-------------------------------|----------|--------|----------------|----------------|--------|
| Escherichia coli              | +        | +      | +              | -              | -      |
| Klebsiella pneumoniae         | +        | -      | -              | +              | +      |
| Sphingobacterium thalpophilum | +        | -      | -              | +              | -      |
| Burkholderia cepacian         | +        | -      | +              | +              | -      |
| Staphylococcus saprophyticus  | +        | -      | Does not match | Does not match | +      |
| Staphylococcus aureus         | +        | -      | Does not match | Does not match | +      |
| Staphylococcus haemolyticus   | +        | -      | Does not match | Does not match | +      |

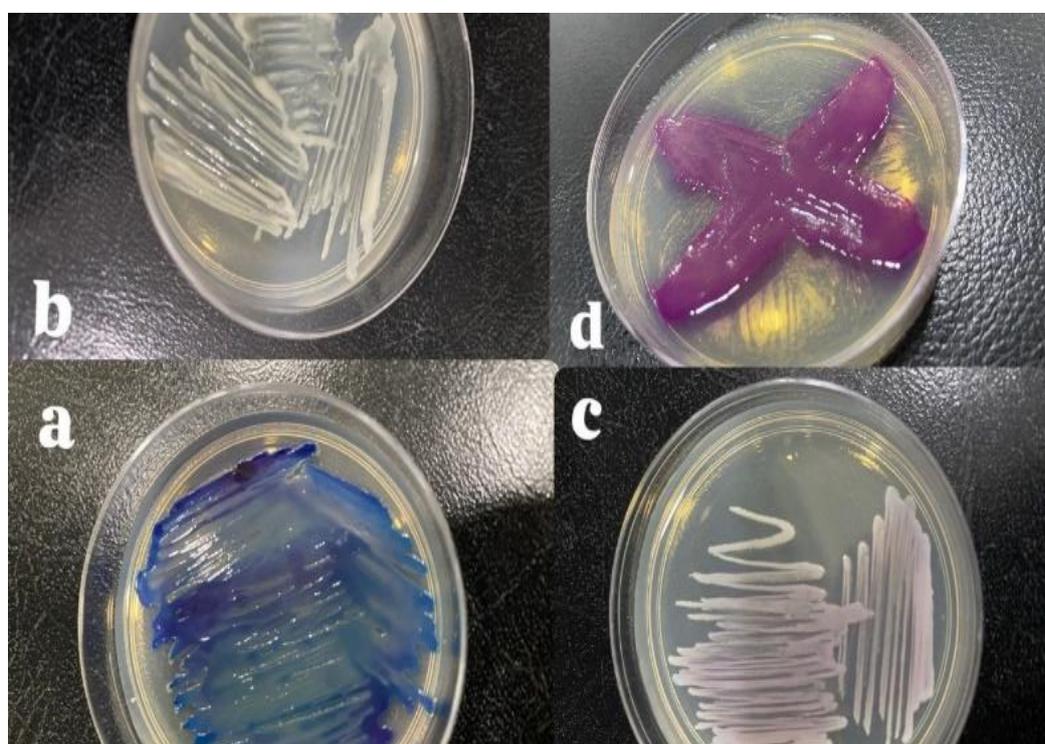
Chromogenic agar (UTI) is used to isolate and diagnose bacteria found in urinary tract infections. This medium is considered an effective medium for diagnosing primary infections because it contains chromogenic substrates that are hydrolyzed by specific enzymes produced by different types of bacteria. This leads to the appearance of distinctive colors on the plate, which makes it easier for us to distinguish between bacteria. For example, *Escherichia coli* appears as a distinctive pink balloon, *Klebsiella pneumoniae* appears as colonies with a dark blue to purple color as a result of the activity of the enzyme  $\beta$ -galactosidase, *Sphingobacterium thalpophilum* appears as pale colonies, which indicates weak or no interaction with chromogenic substrates, and *Burkholderia cepacia* appears as gray to transparent colonies, and it does not interact with dyes, which means it does not produce colors. *Staphylococcus saprophyticus* appears as white with a pink halo, which is considered an important feature to distinguish

it from other types of staphylococci, and staphylococci produce *Staphylococcus aureus* is a white, non-pigmented colony that does not produce enzymes that react with the dyes. Hemolytic staphylococci appear white-pink in color, indicating limited or partial reaction with the dyes (18,19). As in table 2 and Figure

**Table 2: Colony Color of Bacterial Isolates on Chromogenic UTI Agar .**

| Bacterial Species                    | Colony Color Description     | Color Symbol |
|--------------------------------------|------------------------------|--------------|
| <i>Escherichia coli</i>              | Pink                         | 🌸            |
| <i>Klebsiella pneumoniae</i>         | Dark Blue                    | ◆            |
| <i>Sphingobacterium thalpophilum</i> | Pale / Faint                 | ○            |
| <i>Burkholderia cepacia</i>          | Gray to white or transparent | ● / ○        |
| <i>Staphylococcus saprophyticus</i>  | White with a pink halo       | ○ + 🌸        |
| <i>Staphylococcus aureus</i>         | Non-pigmented, white         | ○            |
| <i>Staphylococcus haemolyticus</i>   | Light pink to pale gray      | 🌸 / ●        |

**Note:** The symbols represent approximate visual interpretations of colony colors and may vary slightly depending on the growth medium and incubation conditions



**Figure 3 : UTI bacteria colors on chromogenic medium .**

**a : *Klebsiella pneumoniae*      b: *Staphylococcus aureus***

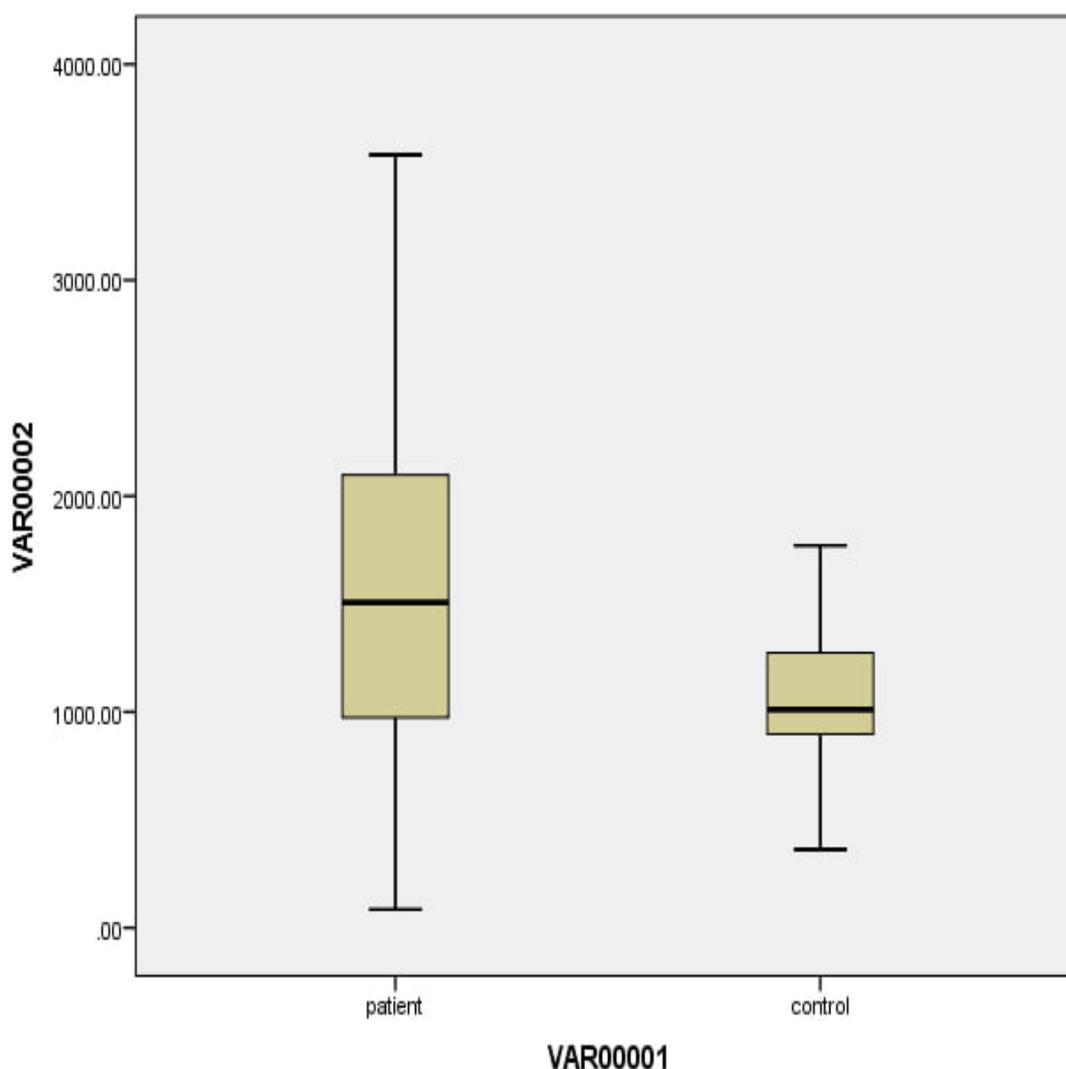
**c : *Staphylococcus saprophyticus*      d: *Escherichia coli***

In our study, we used the IL-22 receptor ELISA kit, which is part of the immune axis that regulates the inflammatory response in the urothelium, as it works to enhance immune defenses against bacterial infection through tissue repair. We used it to detect the level of this protein in urinary tract infection patients' urine samples as opposed to those of healthy individuals, which indicates the existence of a relationship between the elevated IL-22 level receptor in urine and the occurrence of urinary tract infections. We used the standard curve fitting equation to calculate the test results, as the level of IL-22R concentration in urine samples of patients reached 1562.2854 ng/ml, while it reached 1076.3749 ng/ml in control samples, with some statistically significant differences, as the value reached ( $P = 0.003$ ). as shown in Table 3.

**Table (3): IL-22R in urine of patient and control**

| IL-22R  | M±SD ng\L           | P value |
|---------|---------------------|---------|
| Patient | 1562.2854±771.8845  | 0.003*  |
| Control | 1076.3749±316.68365 |         |

\*Significant at  $P \leq 0.05$



Statistical analysis using T-test. There was no discernible difference between males and females in the samples' in the levels of IL-22R in urine, as the average concentration of IL-22R in females was 1548.3012 ng/ml and in males 1401.6325 ng/ml, with a value of ( $P=0.484$ ), which is higher than the approved significance level (0.05), indicating that there is no effect of gender on the concentration of this protein in the studied samples. As shown in Table 4.

\*Significant at  $P \leq 0.0$

| IL-22R | M±SD ng\L            | P value |
|--------|----------------------|---------|
| Male   | 1401.6325 ±418.62761 | 0.484*  |
| Female |                      |         |

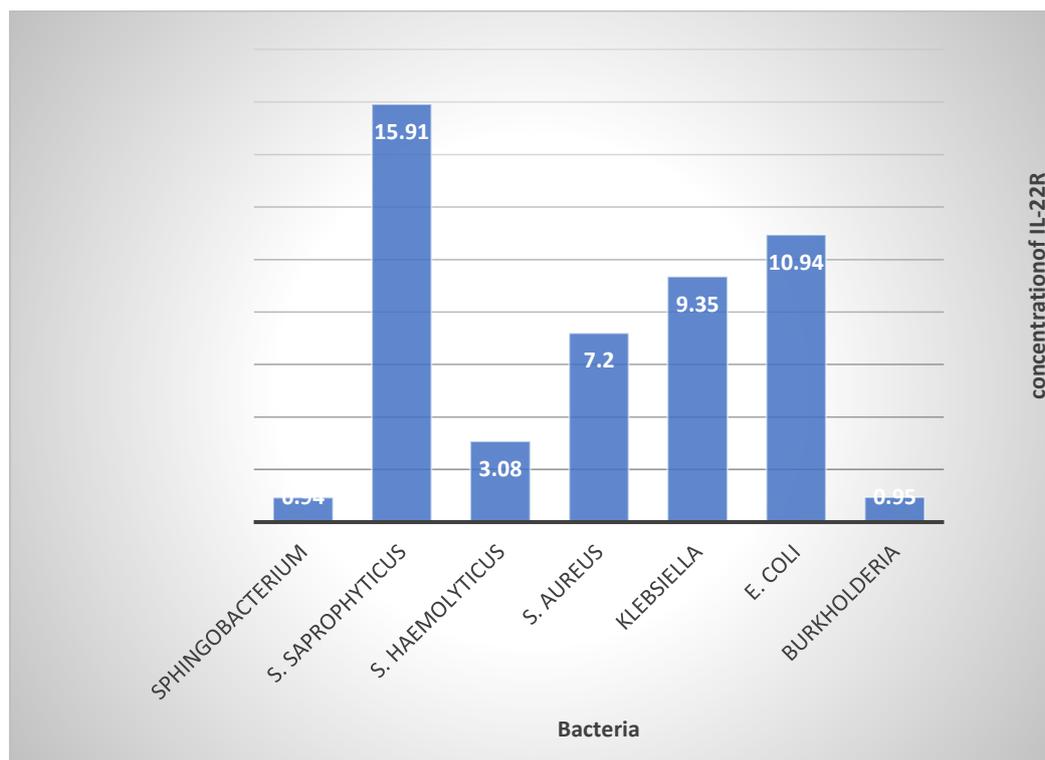
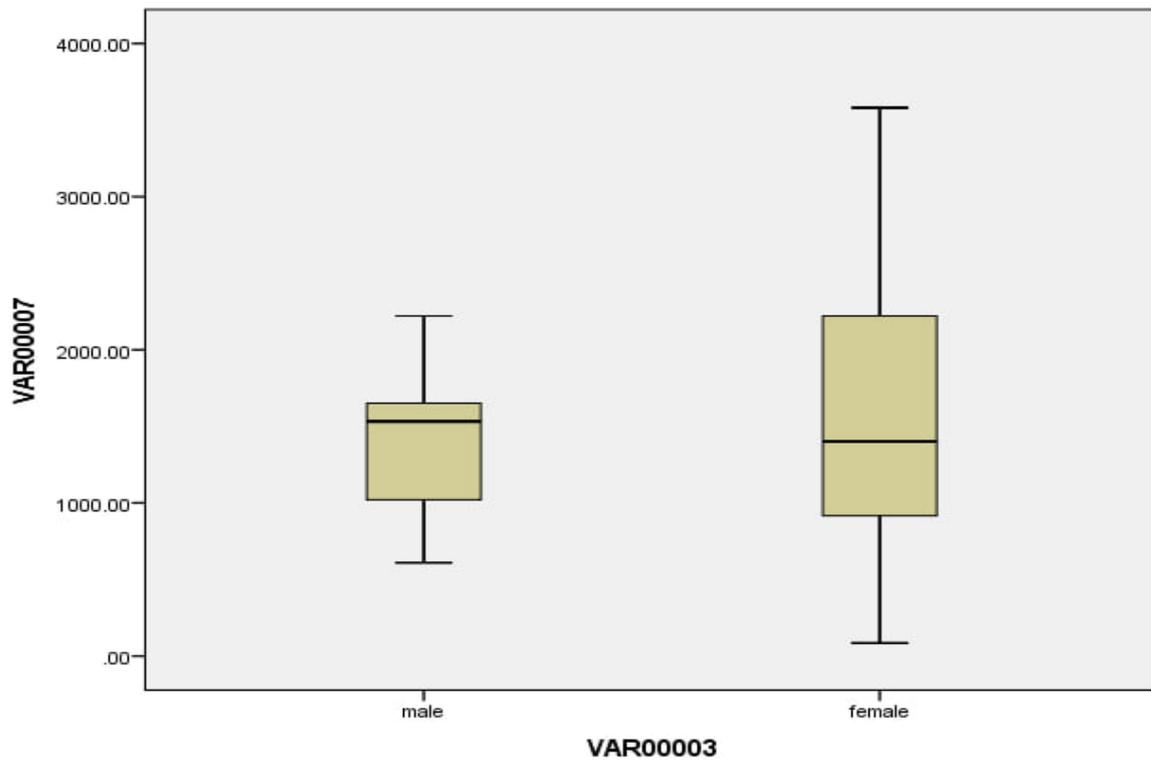


Figure 4 : IL-22R concentration in patients with urinary tract infections.

This graph shows the distribution of total IL-22R expression among different types of inflammatory bacteria. It appears that the highest levels of receptor expression were with *Staphylococcus saprophyticus*, followed by *Escherichia coli* and *Klebsiella pneumoniae*, while expression levels were relatively low with *Sphingobacterium thalpophilum* and *Burkholderia cepacia*. These results indicate that there is a clear difference in the interaction of the IL-22R receptor with different types of bacteria, which reflects a difference in the immune system response. We have a study in (2004) by Wolk et al. indicating that IL-22 plays an important role in immunity against certain bacteria, especially *E.coli*, as it contributes to safeguarding tissues and bolstering the epithelial barrier from infection and inflammation(20) Another study in (2020) by Kawasaki et al. showed that IL-22R was regulated differently depending on the type of bacteria, which affects immune signaling pathways and the degree of inflammation, which is consistent with the variation in IL-22R expression in our previously presented scheme(21) However, there are studies that show opposite or different results, such as the study (2017) by Smith et al., which found that some bacteria, such as *Staphylococcus haemolyticus*, may not significantly activate IL-22R expression, which may indicate different mechanisms to avoid the immune response (22).

### DISCUSSION:

The results showed an increase in the concentration of IL-22R in patients with urinary tract infections compared to healthy controls. IL-22R is a protein classified as a cytokine and plays a dual role in regulating the immune response and balancing defense against infection and tissue regeneration. When IL-22 binds to this receptor, cellular signaling pathways are activated, leading to the stimulation of the production of antimicrobial peptides and the promotion and repair of tissue. The study, published in the journal *Clinical & Translational Immunology*, indicates that The two subunits that make up the IL-22 receptor are IL-22R1 and IL-22R2, is expressed in renal tubule and urothelial epithelial cells. When IL-22 is activated, it leads to antimicrobial responses. When IL-22 attaches itself to its receptor., signals such as JAK/STAT3 and ERK1/2 are activated, leading to enhanced production of antimicrobial peptides and tissue regeneration (23) IL-22R is expressed exclusively on non-hematopoietic cell populations in different tissues, such as the intestinal mucosa (24). This also due to bacterial virulence and role of its pathogenicity in induce immunity (25-26-27 -28).

### Acknowledgments

The authors gratefully acknowledge University of Babylon, Babil, Iraq.

### Declarations

**Ethical Approval:** Rights of Humans and Animals The current study accepts human participants, according to the authors. The authors further declare that this paper is a genuine work that has never been published in any language or format before.

**Competing interests:** No conflicts of interest are disclosed by the authors.

### Author contributions

Russell Muhammad Jassim Conceptualization and methodology,.; formal analysis, writing—original draft preparation, Shaimaa Jassim ALSultany., and Yazi Abdullah Jassim.; All authors have read and approved the published version of the text after it was written, reviewed, and edited.

**Funding:** Not applicable

**Data availability statement:** Not applicable.

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