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# Investigation of Interleukine-1 Beta in Urinary Tract Infection Patients

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**Abstract** **Objective:** The study aims to investigate interleukin -1 beta (IL-1$\beta$) in patients with urinary tract infections in the holy governorate of Karbala. **Methods:** This study was conducted during the period from October 2023 to February 2024 at Imam Hussein Hospital in the holy city of Karbala and the laboratories of the College of Applied Medical Sciences/University of Kerbala. The current study included collecting urine samples (for use in general urine examination) and blood (using blood directly to measure the complete blood count CBC and serum to measure interleukin -1 beta (IL-1$\beta$) from 70 patients with urinary tract infection (35 patients with positive bacterial growth and 35 patients with negative bacterial growth) in addition to 70 healthy people. The following general criteria were also investigated: age, sex, height, and weight. **Results:** Eight bacterial species were obtained. From the findings of the study is the increase in concentration of IL-1$\beta$ in patients with urinary tract infections compared to the control group. The presence of a negative correlation between LBP and IL-1$\beta$. **Conclusions:** Increase the concentration of IL-1$\beta$ in patients with urinary tract infections. There is a negative correlation in patients with bacterial growth between LBP and IL-1$\beta$.

**Key Words** urinary tract infection , IL-$\beta$ , bacterial infection , E. coli

## 1. Introduction

Urinary tract infection (UTI), in humans, inflammation of the renal system characterized by frequent and painful urination and caused by the invasion of microorganisms, usually bacteria, into the urethra and bladder. Infection of the urinary tract can result in either minor or major illness [1].

UTIs is a common clinical problem that comprises 1–6% of medical referrals and includes urinary tract, bladder, and kidney infections [2]. Urinary tract infections (UTIs) represent the most common bacterial illnesses that occur in various settings, including community and clinical environments. Bacteria are the primary etiological agents of these infections, however less frequently, other species, such as fungi and some viruses, have been documented as the causal agents of UTIs [3].

UTIs are accompanied by inflammation, which involves several cytokines such as TNF-$\alpha$, interleukin (IL)-1$\beta$, IL-6, and IL-8 [4]. The origins of interleukin-1 (IL-1) can be traced back to the 1940s when researchers first identified the fever-inducing properties of "soluble factors" produced by leukocytes stimulated by endotoxins. Since then, the field of IL-1 has expanded to include the discovery of inflammasomes, the clinical advantages of anti-IL-1$\beta$ therapy, and the study of inflammatory cytokines, Toll-like receptors (TLRs), and innate immune responses [5]. IL-1 consists of two agonists, IL-1$\alpha$ and IL-1$\beta$, that initiate signals by binding to IL-1 receptor 1 (IL-1R1) and enlisting an additional peptide chain for assistance [6].

Despite the relatively low homology (27%) in terms of amino acid sequences, IL-1$\alpha$ and IL-1$\beta$ exhibit structural similarities and perform similar functions. They both interact with the IL-1 type 1 receptor (IL-1R1) and possess a core $\beta$-barrel structure with adjacent loops [7].The reason for having two IL-1 agonists may lie in the difference in robustness or specific functions between them [8].

Interleukin-1 beta (IL-1$\beta$) is a pro-inflammatory cytokine, meaning it plays a role in promoting inflammation as part of the immune response. It is produced by various cells, including immune cells (such as macrophages and monocytes) and non-immune cells (such as epithelial cells). IL-1$\beta$ is one of the most potent pro-inflammatory cytokines and it has been linked to dysregulated inflammation and to the severity of the UTI [9].

IL-1$\beta$ is induced by inflammatory signals in a broad num-

ber of immune cell types [10]. Monocytes and macrophages are the main sources of IL-1$\beta$ secretion. It causes harm to the tissue and the entry of neutrophils into it. In order to prevent unregulated inflammation, the release of the active form of IL-1$\beta$ is carefully controlled and influenced by a molecular complex known as the inflammasome [4]. IL-1$\beta$ is synthesized as a 269-amino acid precursor protein and undergoes processing by caspase-1, also known as IL-1$\beta$-converting enzyme (ICE), which is activated in inflammasomes. This processing results in the production of mature IL-1$\beta$, consisting of the C-terminal 153 amino acids [11]. During the interaction between the host cell and the pathogen, there is a sudden release of cytokines. This release is aimed at attracting the cells of the innate immune system and strengthening the body's defense against pathogens. Cytokines in urinary tract infections (UTIs) are mostly generated within the uroepithelial cell lining of the bladder and released into the urine [12].

IL-1$\beta$ shows potential as a useful indicator for distinguishing between upper and lower urinary tract infections (UTIs) [13]. Interleukin-1 beta (IL-1$\beta$), a substance commonly seen in the blood of children with urinary tract infections (UTIs), has been utilized as an indicator for acute pyelonephritis [14].

## 2. Materials and Methods

**Collection of the samples:** A positive urinary tract infection (UTI) was diagnosed in patients following a general urine examination (G.U.E.) and control.

**Collecting the Blood Sample:** Each participant provided a 5 ml sample of venous blood using a disposable syringe. The serum samples were concentrated using a centrifuge at an approximate speed of 3000 revolutions per minute (rpm) for a duration of 10 minutes. The serum was carefully transferred into two eppendorf tubes and stored at -20°C to prevent any potential damage from repeated freezing-thawing cycles. This preserved the serum for further measurement of Lipopolysaccharide Binding Protein.

### *A. Preparation of Culture Media*

**Blood Agar:** A suspension of 40 g of blood agar was made in 1Lof distilled water (DW). The mixture was heated until it completely dissolved. Next, the sterilization process involves subjecting the material to a temperature of 121°C for a duration of 15 minutes. The agar was cooled to a temperature of 45 - 50°C and then 7% of sterilized defibrinated blood was added. The media was utilized for culturing and activating bacteria that had been collected from various samples. The bacteria that was collected from samples was activated [15].

**MacConkey Agar:** MacConkey Agar is a type of agar used in laboratory settings. It is commonly used to differentiate between different types of bacteria based on their ability to ferment lactose. The agar contains specific indicators that change color depending on whether lactose fermentation has occurred. This allows researchers to analyze and identify different bacterial species. To prepare this medium, dissolve 40gm of agar in 1000 ml of D.W and sterilize it in an autoclave at 121C° for 20 minutes. Once cooled, the mixture was carefully poured onto the plates. These plates were specifically designed to selectively culture gram-negative bacteria [16].

**Muller Hinton Agar:** The steps of weighing 38 g of media, dissolving it in 1L of D.W, and autoclaving it for 15 minutes was carried out in accordance with the instructions provided by the company [17].

**Identification through the use of automated methods The VITEK2 system:** Automated methods are highly efficient and accurate when it comes to identifying bacteria. The VITEK2 system is composed of plastic reagent cards that contain small amounts of various biochemical test media in 30 wells. These wells provide a biochemical profile that is used to diagnose organisms. The inoculum is transferred from cultured samples into the card, and a photometer periodically measures the color changes in the card resulting from the microbe's metabolic activity. The data was thoroughly analyzed and efficiently stored in a computerized database. A variety of cards, such as those for Gram-negative identification (GN) and Gram-positive identification (GP), are available. The work of [18].

### *B. Estimation the level of IL-1$\beta$*

**Principle:** The test principle utilized in this kit is the Sandwich enzyme immunoassay. The microtiter plate included in this kit comes pre-coated with an antibody that targets Interleukin 1 Beta (IL-1$\beta$). Standards or samples are added to the appropriate microtiter plate wells, followed by the addition of a biotin-conjugated antibody that specifically targets IL-1$\beta$.Then, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated, just like a scientist carefully conducting an experiment. When the TMB substrate solution is added, the wells that have IL-1$\beta$, biotin-conjugated antibody, and enzyme-conjugated Avidin will be the ones that show a color change. Termination of the enzyme-substrate reaction involves the addition of a sulphuric acid solution, followed by the measurement of the resulting color change using spectrophotometry at an approximately 450 nm ± 10 nm in wavelength. The concentration of IL-1$\beta$ in the samples is determined by comparing the OD of the samples to the standard curve.

**KIT Components and Storage :** The components and storage information for the IL-1$\beta$ ELISA Kit were provided in Table 1.

### *C. Preparing the reagents*

1) The kit components and samples were brought to room temperature (18-25°C) before being used.
2) Mix the 25×Wash Buffer with double-distilled Water to create a 1×Wash Buffer.
3) Follow the standard working solution protocol by centrifuging the standard at 1000 × g for 1 minute. Prepare the Standard by adding 1.0 mL of Standard Diluent Buffer and allowing it to sit at room temperature for 10 minutes. Gently shake the mixture to avoid foaming.

| Reagents | Quantity | | Storage Condition |
|---|---|---|---|
| | 48 T | 96 T | |
| Pre-Coated Microplate | 6 strips x 8 wells | 12 strips x 8 wells | -20ºC (6 months) |
| Standard (Lyophilized) | 1 vial | 2 vials | -20°C (6 months) |
| Biotinylated Antibody (100X) | 60 $\mu$L | 120 $\mu$L | -20°C (6 months) |
| Streptavidin-HRP (100X) | 60 $\mu$L | 120 $\mu$L | -20°C (6 months) |
| Standard/Sample Diluent Buffer | 10 mL | 20 mL | 4°C |
| Biotinylated Antibody Diluent | 6 mL | 12 mL | 4°C |
| HRP Diluent | 6 mL | 12 mL | 4°C |
| Wash Buffer (25X) | 10 mL | 20 mL | 4°C |
| TMB Substrate Solution | 6 mL | 10 mL | 4°C(store in dark) |
| Stop Reagent | 3 mL | 6 mL | 4°C |
| Plate Covers | 1 Piece | 2 Pieces | 4°C |

Table 1: Components and Storage of the IL-1$\beta$ ELISA Kit

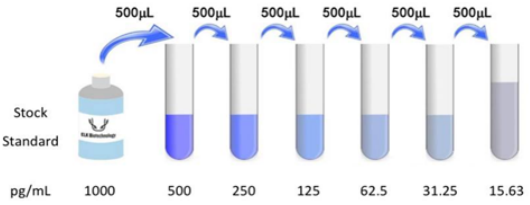


Figure 1: TMB Substrate Solution aspirated with sterile tips; excess not returned to the vial

The concentration of the standard in the stock solution is 1000 pg/mL. There are seven tubes containing 0.5 mL of Standard Diluent Buffer. These tubes are utilized for the purpose of generating a double dilution series, as depicted in the picture provided below. Make sure to properly mix each tube before moving on to the next transfer by repeatedly pipetting the solution up and down.

4) Arrange 7 points of Diluted Standard with varying concentrations, including 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, and 15.63 pg/mL. The last EP tubes containing Standard Diluent serve as the Blank with a concentration of 0 pg/mL. To ensure the validity of the experimental results, it is essential to use the new Standard Solution for each experiment. When diluting the Standard from high concentration to low concentration, it is important to replace the pipette tip for each dilution. **Important:** The final tube should be treated as a blank and no solution should be transferred into it from the previous tube.
5) Before used, it is recommended to briefly spin or centrifuge the stock Biotinylated Antibody and Streptavidin-HRP. Prepare a 100-fold dilution of the antibodies using Biotinylated Antibody Diluent and HRP Diluent.
6) The required amount of TMB Substrate Solution was carefully aspirated using sterilized tips, and the remaining solution was not discarded into the vial Figure 1.

### *D. Preparing Samples*

1) Ensure that all materials and prepared reagents are at room temperature before use. Thoroughly mix all reagents, ensuring that no foam is created within the vials.
2) It is important to determine the total number of samples used in the entire test.
3) Anticipate the concentration prior to conducting the assay. Whether the values fall within or outside the range of the Standard curve is being considered.

### *E. Procedure for conducting the assay*

1) Identify the wells for the Diluted Standard, Blank, and Sample. Set up 7 wells for the Standard and 1 well for the Blank. Place 100 $\mu$L of either the Standard Working Solution or the samples into the appropriate wells. Place the Plate Cover on top. Set the incubation time to 80 minutes at a temperature of 37°C. Please ensure that solutions are carefully added to the bottom of the micro ELISA plate well, taking care to avoid touching the inside wall and causing any unnecessary foaming.
2) Remove the solution and rinse each well with 200 $\mu$L of 1×Wash Solution. Allow it to sit for 1-2 minutes. Ensure that all remaining liquid is thoroughly removed from each well by firmly attaching the plate to absorbent paper. Wash three times in total. Following the final wash, ensure that all remaining Wash Buffer is removed by either aspirating or decanting. Turn the plate upside down and press it onto absorbent paper. Reminder: To prevent contamination, be careful not to let the pipette tip touch the well walls when adding the Washing Solution. (b) Take care to pour the washing liquid directly to prevent any contamination of other wells.
3) 100 $\mu$L of Biotinylated Antibody Working Solution was carefully added to each well, ensuring that the wells were properly covered.
4) The aspiration and wash process were repeated a total of three times, as conducted in the previous step. 2.
5) 100 $\mu$L of Streptavidin-HRP Working Solution were added to each well. Cover the wells with the plate

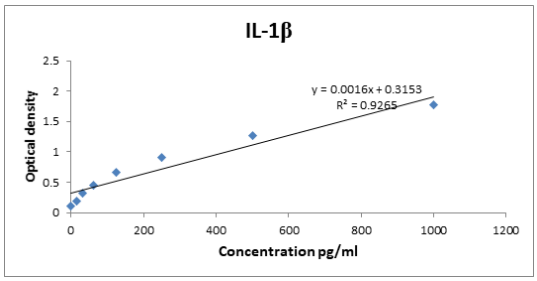


Figure 2: The standard curve of IL-1$\beta$ concentration (pg/ml) and trend linear equation that display on chart Y

sealer and incubate for 50 minutes at 37°C.

6) The aspiration and wash process were repeated a total of 5 times, following the procedure conducted in step. 2.
7) Each well received 90 $\mu$L of TMB Substrate Solution. Replace the existing plate cover with a new one. Leave to incubate for 20 minutes at 37°C, ensuring not to exceed 30 minutes, and keep in a dark environment. By adding the TMB Substrate Solution, the liquid will undergo a color change and turn blue. It is recommended to preheat the Microplate Reader for approximately 15 minutes prior to taking OD measurements.
8) Stop Reagent was added to each well, in a precise amount of 50 $\mu$L. By adding Stop Reagent, the liquid will undergo a color change and turn yellow. Ensure thorough mixing by gently tapping the side of the plate. To ensure thorough mixing, gently tap the plate if the color change does not appear uniform. It is important to maintain the same insertion order for the Stop Reagent as the TMB Substrate Solution.
9) Every single drop of water was meticulously wiped off, along with any fingerprints on the bottom of the plate. It was ensured that there were no bubbles present on the surface of the liquid. Next, operate the microplate reader and perform a measurement at 450 nm.

## 3. Results

The results in Table 2 showed that there was a highly significant increase ($P < 0.05$) in the concentration of IL-1$\beta$ (pg/ml) in two groups of patients compared with control group.

The results showed that there was a highly significant increase ($P < 0.05$) in the concentration of IL-1$\beta$ (pg/ml) in tow patient groups (female and male) compared to control group. We find that the concentration of IL-1$\beta$ increases significantly ($P<0.05$) in the tow patient groups compared to the control group in all groups. Based on the BMI criterion, a significant increase ($P<0.05$) was observed in the concentration of IL-1$\beta$ for all BMI categories, whether Normal, Overweight, or Obese, in tow patient groups compared to Control, as show in the Table 3.

The results was approved that there was non-significant difference in IL-1$\beta$ concentration according to the types of bacterial isolates as show in Table 4.

## 4. Discussion

The results of current study not similar with results of Alfadul et al. founded there is a relationship between diabetes and elevation of levels of IL-1$\beta$ [19]. IL-1$\beta$ could be a promising marker for differentiation between upper and lower UTIs [20]. Butler et al. who suggested IL-1$\beta$ release to be important for the progression of urinary tract infection (UTI) [21]. The current study agreed with a study of [22]. which showed highly significant relationship between the level of IL-1$\beta$ (pg/ml) in patient group and would not agree highly significant relationship between the level of IL-1$\beta$ (pg/ml) and bacterial infections ($P < 0.01$). Inversely , found that IL-1b level were higher in patient with growth bacterial compared with no growth bacterial [23].

Maculewicz et al. [24] were observed relationship between BMI and IL-1$\beta$, in patients with infection and BMI $\geq$ 30 they have high significant , obesity is a major factor that leads to increase IL-1$\beta$. The similar study reported by [25]. founding the level of IL-1$\beta$ increase with age , when age $\geq$ 18 observed highly significant ($P<0.05$) in IL-1$\beta$.

The results of current study disagreement with [26]they founded that the release of IL- 1$\beta$ was linked with E. coli infections.

## Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

## Authors Contribution

All authors contributed equally in this paper.

## References

[1] Rogers, S. A. & Kara, M. (2024). "Urinary tract infection". The Current Scenario and Future Prospects. *Pathogens. Jan 44*(16), 326-335.
[2] Tegegne, K. D., Wagaw, G. B., Gebeyehu, N. A., Yirdaw, L. T., Shewangashaw, N. E., & Kassaw, M. W. (2023). Prevalence of urinary tract infections and risk factors among diabetic patients in Ethiopia, a systematic review and meta-analysis. *PloS one, 18*(1), e0278028.
[3] Mancuso, G., Midiri, A., Gerace, E., Marra, M., Zummo, S., & Biondo, C. (2023). Urinary tract infections: the current scenario and future prospects. *Pathogens, 12*(4), 623.
[4] Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology, 13*(5), 269-284.
[5] Dinarello, C. A. (2018). The IL-1 Cytokine and Receptor Family. Immunol Rev 281. Oxford, UK: John Wiley & Sons Ltd. 247.
[6] Fields, J. K., Günther, S., & Sundberg, E. J. (2019). Structural basis of IL-1 family cytokine signaling. *Frontiers in Immunology, 10,* 1412.
[7] Kaneko, N., Kurata, M., Yamamoto, T., Morikawa, S., & Masumoto, J. (2019). The role of interleukin-1 in general pathology. *Inflammation and Regeneration, 39,* 1-16.
[8] Mantovani, A., Dinarello, C. A., Molgora, M., & Garlanda, C. (2019). Interleukin-1 and related cytokines in the regulation of inflammation and immunity. *Immunity, 50*(4), 778-795.
[9] Masajtis-Zagajewska, A., & Nowicki, M. (2017). New markers of urinary tract infection. *Clinica Chimica Acta, 471*, 286-291.
[10] Bent, R., Moll, L., Grabbe, S., & Bros, M. (2018). Interleukin-1 beta—a friend or foe in malignancies?. *International Journal of Molecular Sciences, 19*(8), 2155.
[11] Lachman, L. B., Hacker, M. P., & Handschumacher, R. E. (1977). Partial purification of human lymphocyte-activating factor (LAF) by ultrafiltration and electrophoretic techniques. *The Journal of Immunology, 119*(6), 2019-2023.

| Groups | | Mean ± SE | P value | LSD |
|---|---|---|---|---|
| Case | Bacterial Growth | 165.66 ± 10.31 | 0.0000 ** | 31.145 |
| | No Bacterial Growth | 229.79 ± 10.94 | | |
| Control | | 127.24 ± 5.54 | | |
| NS: Non significant P value * : Significant P value ** : Highly Significant P value | | | | |

Table 2: The mean of IL-1$\beta$ (pg/ml) in study groups

| Criteria | Class | N | Mean ± SE of IL-1$\beta$ in studied Groups | | | P value | LSD |
|---|---|---|---|---|---|---|---|
| | | | Control (70) | Patients Growth(35) | Patients No Growth(35) | | |
| Sex | Female (N) | 112 | 132.7±6.12 (56) | 164.2±12.07 (28) | 231.2±13.20 (28) | 0.0000* | 27.53 |
| | Male (N) | 28 | 136.1±9.96 (14) | 171.9±18.63 (7) | 212.8±24.83 (7) | 0.0076* | 52.78 |
| P value | | 0.00001* | 0.7981 | 0.7689 | 0.5333 | | |
| Age (years) | 18 – 37 (N) | 62 | 130.53±8.15 (31) | 188.2±14.87 (16) | 234.9±12.23 (15) | 0.0000* | 32.736 |
| | 38 – 57 (N) | 52 | 141.18±8.80 (26) | 144.9±15.02 (14) | 205.8±22.02 (12) | 0.0053* | 15.769 |
| | 58 – 77 (N) | 26 | 92.32±9.73 (13) | 164.1±29.82 (5) | 263.3±20.04 (8) | 0.0000* | 46.792 |
| P value<br>LSD | | 0.00091* | 0.0056*<br>14.628 | 0.1506<br>NS | 0.1278<br>NS | | |
| BMI (kg/m2) | Normal <25 | 28 | 135.8±11.59 (14) | 160.7±23.90 (3) | 207.9±16.22 (11) | 0.0037* | 50.11 |
| | Overweight 25 – 29.9 | 88 | 147.7±5.96 (44) | 165.8±11.40 (29) | 239.7±11.87 (15) | 0.0000* | 21.89 |
| | Obese $\geq$ 30 | 24 | 94.7±10.21 (12) | 170.7±46.70 (3) | 235.9±32.23 (9) | 0.0006* | 83.07 |
| P value<br>LSD | | 0.00001* | 0.0006*<br>15.63 | 0.9805<br>NS | 0.4391<br>NS | | |

Table 3: Mean of IL-1$\beta$ (pg/ml) among studied groups according to sex, age and BMI

| Type of bacteria | N | Mean | SE | P value | LSD |
|---|---|---|---|---|---|
| E. coli | 13 | 173.2 | 15.03 | 0.5193 | NS |
| Enterococcus faecalis | 1 | 268.0 | 0.0 | | |
| Enterococcus faecium | 1 | 203.6 | 0.0 | | |
| Klebsiella aerogenes | 3 | 151.9 | 27.42 | | |
| Klebsiella pneumoniae ssp | 2 | 99.2 | 12.44 | | |
| Staphylococcus aureus | 4 | 168.8 | 42.2 | | |
| Staphylococcus haemolyticus | 3 | 145.7 | 25.46 | | |
| Staphylococcus saprophyticus | 8 | 167.2 | 25.80 | | |

Table 4: The mean of IL-1$\beta$ in patient group with bacterial growth according to type of bacteria


[12] Sundvall, P. D., Elm, M., Ulleryd, P., Mölstad, S., Rodhe, N., Jonsson, L., ... & Gunnarsson, R. (2014). Interleukin-6 concentrations in the urine and dipstick analyses were related to bacteriuria but not symptoms in the elderly: a cross sectional study of 421 nursing home residents. *Bmc Geriatrics, 14,* 1-9.

[13] Nanda, N., & Juthani-Mehta, M. (2009). Novel biomarkers for the diagnosis of urinary tract infection—A systematic review. *Biomarker Insights, 4,* BMI-S3155.

[14] Sheu, J. N., Chen, M. C., Cheng, S. L., Lee, I. C., Chen, S. M., & Tsay, G. J. (2007). Urine interleukin-1$\beta$ in children with acute pyelonephritis and renal scarring. *Nephrology, 12*(5), 487-493.

[15] Yeh, E., Pinsky, B. A., Banaei, N., & Baron, E. J. (2009). Hair sheep blood, citrated or defibrinated, fulfills all requirements of blood agar for diagnostic microbiology laboratory tests. *PloS one, 4*(7), e6141.

[16] Mac Faddin, J. F. (1980). Biochemical tests for identification of medical bacteria. (No Title).

[17] Murray, P. R., & Zeitinger, J. R. (1983). Evaluation of Mueller-Hinton agar for disk diffusion susceptibility tests. *Journal of Clinical Microbiology, 18*(5), 1269-1271.

[18] Maina, D., & Kagotho, E. (2014). Suitability of Vitek 2 System in Identification and Susceptibility Testing of Gram Negative Bacteremias by Direct Inoculation. *East African Medical Journal, 91*(4), 115-118.

[19] Alfadul, H., Sabico, S., & Al-Daghri, N. M. (2022). The role of interleukin-1$\beta$ in type 2 diabetes mellitus: A systematic review and meta-analysis. *Frontiers in Endocrinology, 13*, 901616.

[20] Horváth, J., Wullt, B., Naber, K. G., & Köves, B. (2020). Biomarkers in urinary tract infections–which ones are suitable for diagnostics and follow-up?. *GMS Infectious Diseases, 8*, Doc24.

[21] Butler, D., Ambite, I., Wan, M. L. Y., Tran, T. H., Wullt, B., & Svanborg, C. (2022). Immunomodulation therapy offers new molecular strategies to treat UTI. *Nature Reviews Urology, 19*(7), 419-437.

[22] Wasnaa, J., & Ibrahim, N. A. K. Levels of IL-1$\beta$ and IL-8 in Iraqi women with Bacterial vaginosis and Trichomoniasis, *Cihan University-Erbil Scientific Journal,* 253-264.

[23] Kim, H. J., Lee, J. P., Kim, H. K., Kim, S. G., Oh, J. E., & Kim, Y. S. (2017). Association between cytokine levels and arteriovenous fistula dysfunction in hemodialysis patients. *Kidney Research and Clinical Practice, 36*(2), 177-184.

[24] Maculewicz, E., Dzitkowska-Zabielska, M., Antkowiak, B., Antkowiak, O., Mastalerz, A., Garbacz, A., ... & Cięszczyk, P. (2022). Association of Interleukin Genes IL10 and IL10RB with Parameters of Overweight in Military Students. *Genes, 13*(2), 291.

[25] Kato, A., Gabay, C., & Okaya, T. (2023). Mechanisms of dysregulated production of interleukin-1 beta in bacterial infection. *Arthritis Research & Therapy, 15*(3), 1-11.

[26] Demirel, I., Persson, A., Brauner, A., Särndahl, E., Kruse, R., & Persson, K. (2020). Activation of NLRP3 by uropathogenic Escherichia coli is associated with IL-1$\beta$ release and regulation of antimicrobial properties in human neutrophils.*Scientific Reports, 10*(1), 21837.