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# PATTERNS OF URINARY BIOMARKERS AND BLOOD IMMUNOLOGICAL MARKERS OF PATIENTS WITH URINARY TRACT INFECTIONS

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## ABSTRACT

**Urinary tract infection (UTI) is a serious public health problem caused by a variety of microorganisms, the most prevalent of which are bacteria. The diversity of the immune system in the urinary tract is a critical factor in the defense against infections. This research included 127 clinically suspected UTI patients that all are treated with urine analysis and complete blood count test in order to observe the increased effects of neutrophils, total white blood cells, mainly granulocytes, and lymphocytes, in response to bacteria in urinary tract infection patients. All were classified into three groups: adult males, females, and children's girls, boys were excluded as they rarely encounter with this infection. In males, significant differences appear between bacteria and pus cells, bacteria and white blood cells as well as bacteria and granulocytes. Regarding females, a significant difference was seen between bacteria and lymphocytes in asymptomatic UTI cases, in contrast, the symptomatic cases showed a significant difference between bacteria and pus cells. Girls with asymptomatic UTI showed a significant difference between bacteria and pus cells, and bacteria and white blood cells. The significant difference between men and women appeared in bacterial presence in urine, white blood cells, and granulocytes. Conclusion; this study supports the great role of neutrophil cells that fight bacteria in blood circulation and later become pus cells in urine.**

***KEYWORDS:*** Adaptive immunity, Bacteria, Innate immunity, Pus cells, Urine analysis test.

## 1. INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections caused by both gram-negative and gram-positive bacteria, as well as by certain fungi (Bitew et al., 2022). In 2021, Gad and AbdelAziz observed that seasonal variations in weather, lifestyle, immune-compromised patients, and catheterization all increase the risk of urinary tract infections (Gad and AbdelAziz., 2021). It was also discovered that there is a strong correlation between the type of bacteria present and factors such as age, the number of pus cells present, and education level (Alsamarai et al., 2017).

microscopic urinalysis reveals that ≥10 white blood cells per cubic millimeter refer to pyuria (Shaikh, N., et al 2016). The best cutoff for detecting bacteriuria was discovered to be a urine white blood cell count of more than 25 cell/hpf, this refers to symptomatic UTI. While less than 25 cell/hpf, is considered asymptomatic UTI. (Cheng et al,. 2022)

Clinically, UTIs are categorized as uncomplicated or complicated (Sundquist and Jansker, 2022). Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities. These infections are differentiated into lower UTIs: cystitis and

upper UTIs: pyelonephritis (Alrumyyan et al., 2021).

When bacteria infect the bladder or kidney, they colonize the mucous membrane and face a strong immune response from the host. The first immune cells secreted are neutrophils, which engulf bacterial cells, then die and become pus cells, so more pus cells indicate a good immune response and also indicate a high dose of bacteria in infected patients (Almaiman et al., 2021). It was established that bacteriuria was associated with the presence of pus cells in the urine, and it was shown that a high urine white

blood cell count was the most accurate criterion for identifying bacteriuria (Cheng et al., 2022). Moreover, the immune response to a urinary tract infection is divided into two categories: non-specific innate immunity and specific adaptive immunity (Joseph and Enting, 2019). Host defense mechanisms are critically important for the protection of the urinary tract against pathogenic microorganisms. In recent years, considerable advances have been made in our understanding of the mechanisms underlying the immune homeostasis of the kidney and urinary tract, dysfunctions in these immune mechanisms may result in acute disease, tissue destruction, and overwhelming infection (Spencer et al., 2014).

This study's goal is to understand how white blood cells contribute to second- and third-line defenses against bacteria in the urinary tract. Additionally, a complete blood count and a general urine examination were conducted in order to look at and assess potential changes in immunological indicators in patients with urinary tract infections in Duhok city, Iraq

## 2. MATERIALS AND METHOD

This retrospective cross-sectional study included the participation of 127 patients clinically were suspected with UTI. Urine and blood samples were collected from participants at various hospitals, including Azadi Teaching, Golan, Vin, and Emergency hospitals, during the period of five months (October 2021 to March 2022). The ages of the enrolled patients ranged from three to fifty years old. The girl child group was 3-12 years old, while the adult female and male groups were 13-50 and 14-50 years old, respectively.

A general urine examination analysis to determine the relationship between pyuria and bacteriuria) and a complete blood count was performed by a full-automated Coulter analyzer (Medonic and Swelab) to verify for white blood cells, granulocytes, and lymphocyte cells in blood samples of enrolled participants in Duhok city, Iraq. .

### 2.1 Microscopic examination of sediment urinalysis

This test was performed according to (Ince et al., 2016).

Firstly, centrifuge tubes were filled with 10 ml of mid-stream morning urine and then centrifuged for 5 min at 1500 rpm for manual microscopic examination. The supernatant was discarded until 0.5 mL of urine remained at the bottom of the tube, which contained a concentrated pellet of formed elements and was visible at the bottom of the tube. Then one drop of sediment was placed on a microscope slide, covered with a cover slip, and examined by a light compound microscope at 40X.

### 2.2 Complete blood count (CBC):

Several steps were followed during the blood draw for the CBC test: Firstly, the area where the needle will be put in the arm of a patient was cleaned with an antiseptic alcohol swab. Secondly, a tourniquet is tied around the upper arm to make the vein in the arm more visible and easier to access with a needle. Then, the needle was placed in the vein, and a test tube was attached to the needle and filled with blood. After the test tube is filled, it is directly analyzed by basic laboratory equipment, an automated hematology analyzer, which counts blood cells and collects information on their size and structure.

### 2.3 Statistical analysis:

Statistical calculations were performed using R-statistic version 4.2.0, categorical variables were presented as numbers and percentages, the Chi-square test was used to show the association between the categorical variables, the t-test was used to determine to mean differences between the groups, p-value $\leq$ 0.05 was considered relevant Statistical significance.

## 3. RESULTS

**Table (1):** The Activity of UTI among patients:

| Participants | symptomatic | asymptomatic | Age range(Years) | P-value |
|---|---|---|---|---|
| **Adult males (28)** | (1)3.57% | (27)96.42% | (14-50) | 0.063* (between UTI and Adult Gender) |
| **Adult females(55)** | (10)18.18% | (45)81.81% | (13-50) | |
| **Children(female 44)** | (7)20.45% | (37)79.54% | (3-12) | |

Table 1, shows that females are more susceptible to these infections than males and children.
*P-value ≤ 0.05 (statistically significant) (between UTI and Adult Gender).

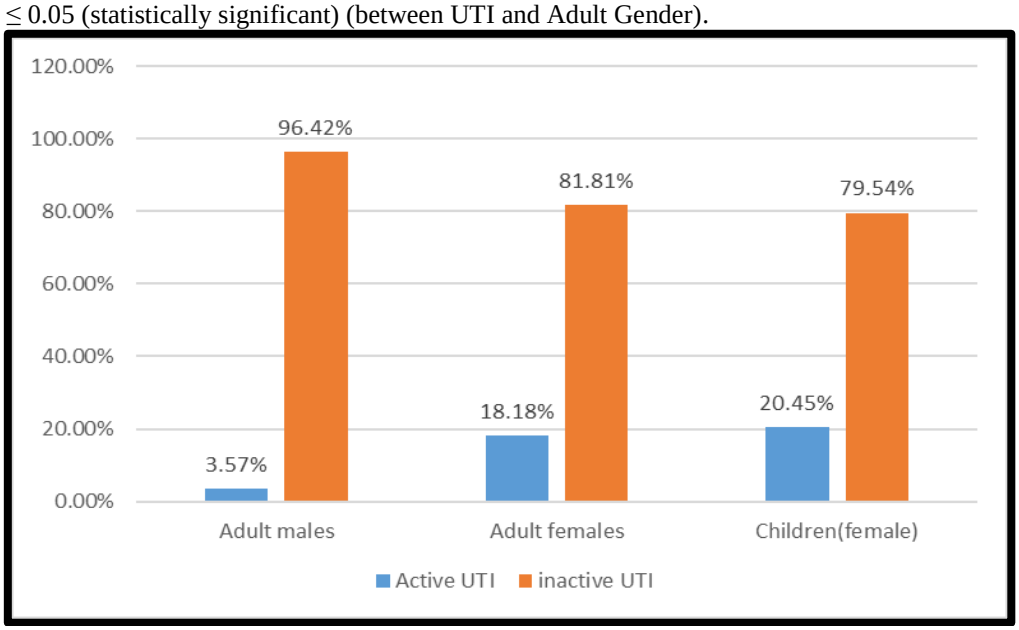


**Fig. (1):** symptomatic or asymptomatic UTI among patients.

**Table (2):** urinary tract infection biomarkers and blood count markers in males

| Gender | Mean of pus cells / HPF | Mean Of WBC(*10^9) | Mean Of lymphocytes (*10^9) | Mean of granulocytes (*10^9) |
|---|---|---|---|---|
| **Males** | 5.888 | 6.944 | 2.326 | 4.273 |
| **Mean of bacteria/ml** | 0.607 | 0.607 | 0.607 | 0.607 |
| **P –value** | 0.002* | 0** | 0.33 | 0.004*** |

Table 2, shows that neutrophils and granulocytes are secreated against UTI bacteria in males.
*t = 3.31, p-value ≤ 0.05 (statistically significant)
**t = 5.92, p-value ≤ 0.05 (statistically significant)
***t = 3.0935, p-value ≤ 0.05 (statistically significant).

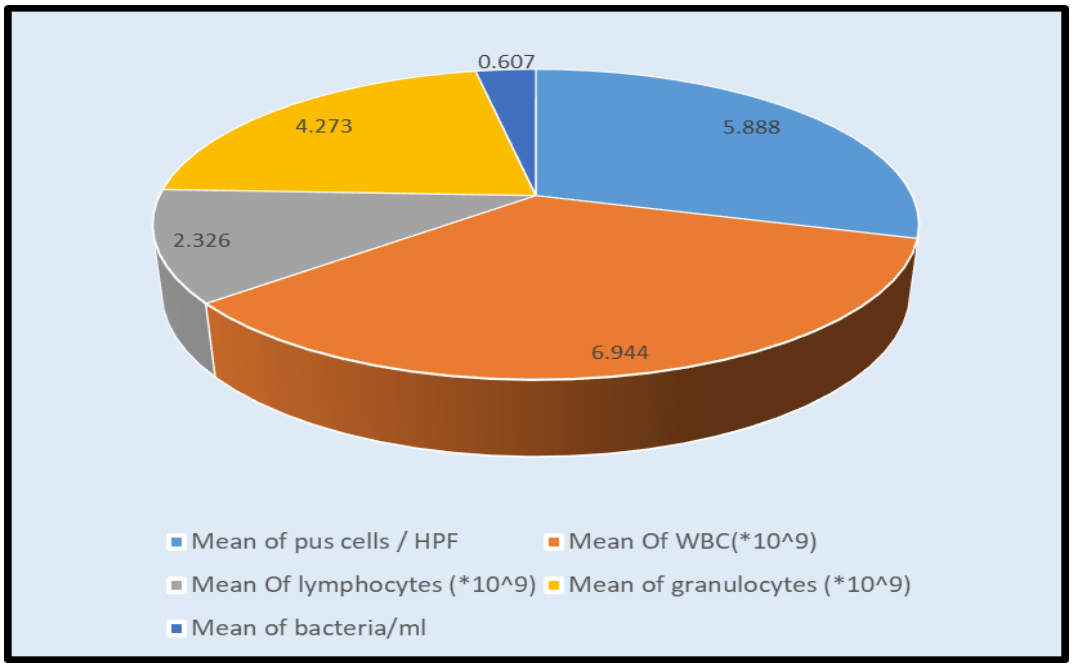


**Fig. (2):** urinary tract infection biomarkers in males.

**Table (3):** Symptomatic-urinary tract infection biomarkers and blood count markers in females

| Gender | Mean Of pus cells / HPF | Mean Of WBC (*10^9) | Mean Of lymphocytes (*10^9) | MeanOf granulocytes (*10^9) |
|---|---|---|---|---|
| **Females** | 5.333 | 8.62 | 2.353 | 4.567 |
| **Mean of bacteria/ml** | 7.022 | 7.022 | 7.022 | 7.022 |
| **P –value** | 0.245 | 0.33 | 0.0007* | 0.33 |

Table 3, shows that there is a relationship between the second line of the immune response (lymphocytes) and UTI bacteria.
*t=3.63, p-value ≤ 0.05 (statistically significant).

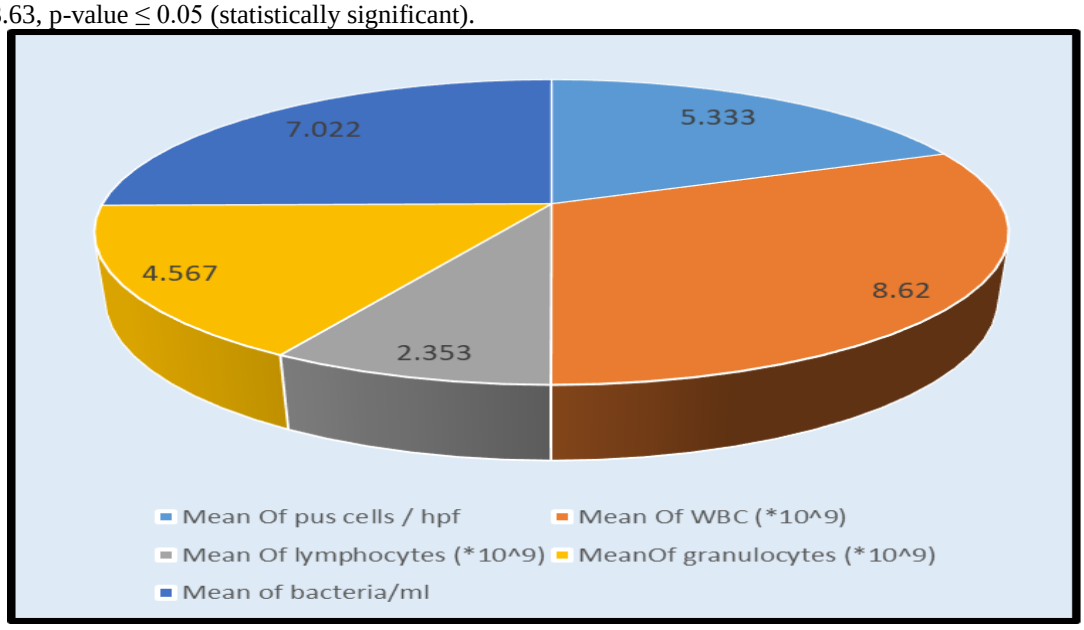


**Fig. (3):** Symptomatic urinary tract infection in females biomarkers.

**Table (4):** urinary tract infection biomarkers and blood count markers in both males and females

| Gender | Mean of bacteria/ml | Mean of pus cells /HPF | Mean of WBC (*10^9) | Mean Of lymphocytes (*10^9) | Mean Of granulocytes (*10^9) |
|---|---|---|---|---|---|
| **Males** | 0.607 | 5.888 | 6.944 | 2.326 | 4.273 |
| **Females** | 7.022 | 5.333 | 8.62 | 2.353 | 4.567 |
| **P -value** | 0.0007* | 0.61 | 0.043** | 0.8 | 0.008*** |

Table 4, shows that there are differences between male and female biomarkers (bacteria) and hematological markers (granulocytes) in UTIs.
*test = -3.56, p-value ≤ 0.05 (statistically significant)
**test = -2.07, p-value ≤ 0.05 (statistically significant)
***test = -2.71, p-value ≤ 0.05 (statistically significant)

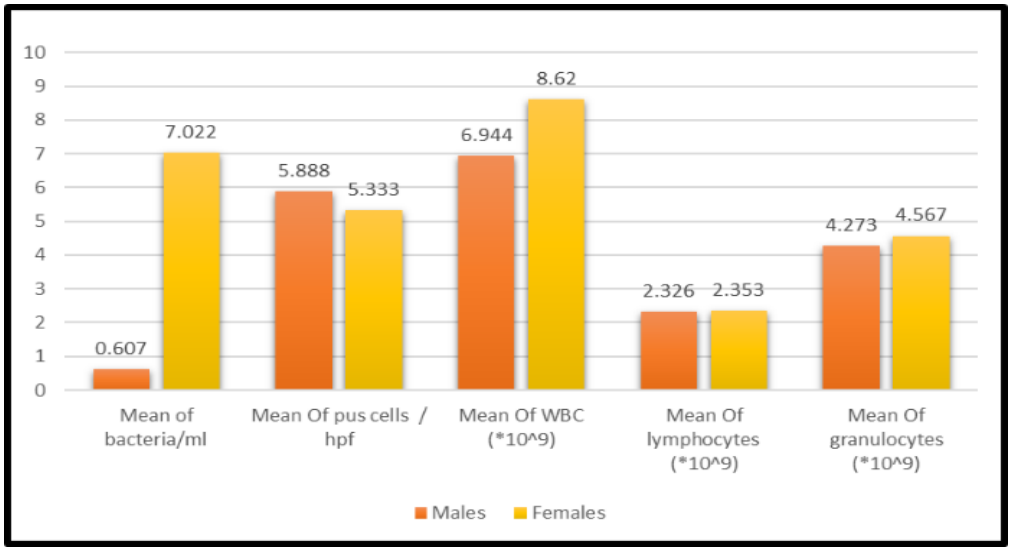


**Fig. (4):** urinary tract infection biomarkers and blood count markers in both males and females

**Table (5):** Asymptomatic urinary tract infection biomarkers and blood count markers in girls.

| Gender | Mean of pus cells / HPF | Mean of WBC (*10^9) | Mean of lymphocytes (*10^9) | Mean of granulocytes (*10^9) |
|---|---|---|---|---|
| **Girls** | 3.027 | 8.883 | 3.437 | 4.668 |
| **Mean of bacteria/ml** | 0.919 | 0.919 | 0.919 | 0.919 |
| **P -value** | 0* | 0** | 0.55 | 0.1 |

Table 5, shows that asymptomatic girls have neutrophils secreated against UTI bacteria.
*t = 6.9632, p-value ≤ 0.05 (statistically significant)
**t = 5.433, p-value ≤ 0.05 (statistically significant)

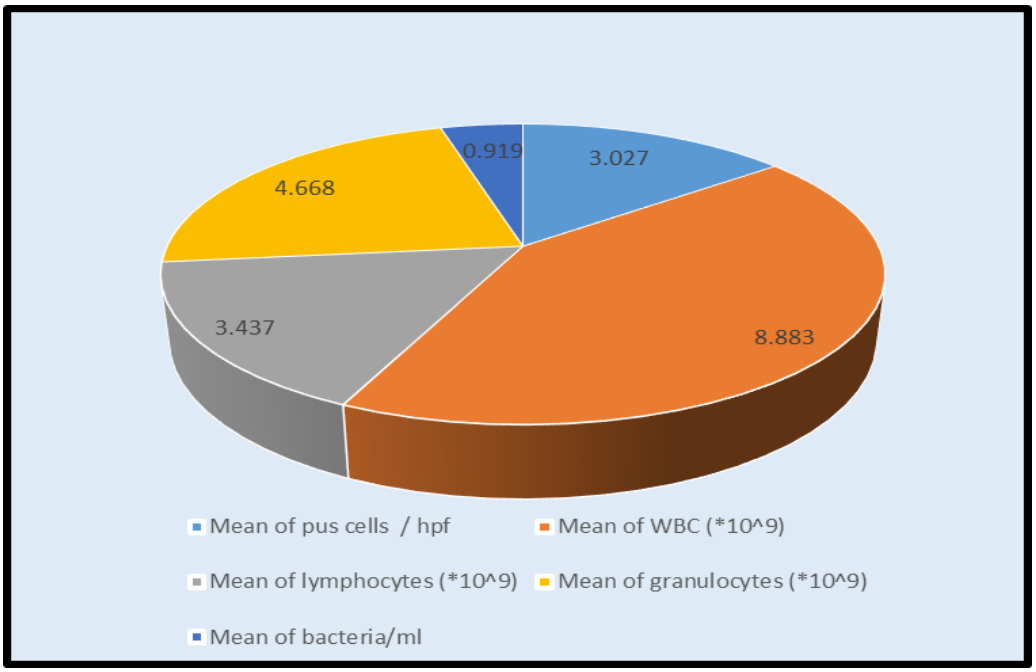


**Fig. (5):** urinary tract infection biomarkers and blood count markers in girls.

**Table (6):** Symptomatic urinary tract infection biomarkers and blood count markers in girls

| Gender | Mean of pus cells/ HPF | Mean of WBC (*10^9) | Mean of lymphocytes (*10^9) | Mean of granulocytes (*10^9) |
|---|---|---|---|---|
| **Girls** | 56.428 | 7.671 | 3.728 | 3.085 |
| **Mean of bacteria/ml** | 12.857 | 12.857 | 12.857 | 12.857 |
| **P –value** | 0.157 | 0.33 | 0.142 | 0.13 |

Table 6, shows that symptomatic girls have no relationship between biomarkers and haematologic markers.

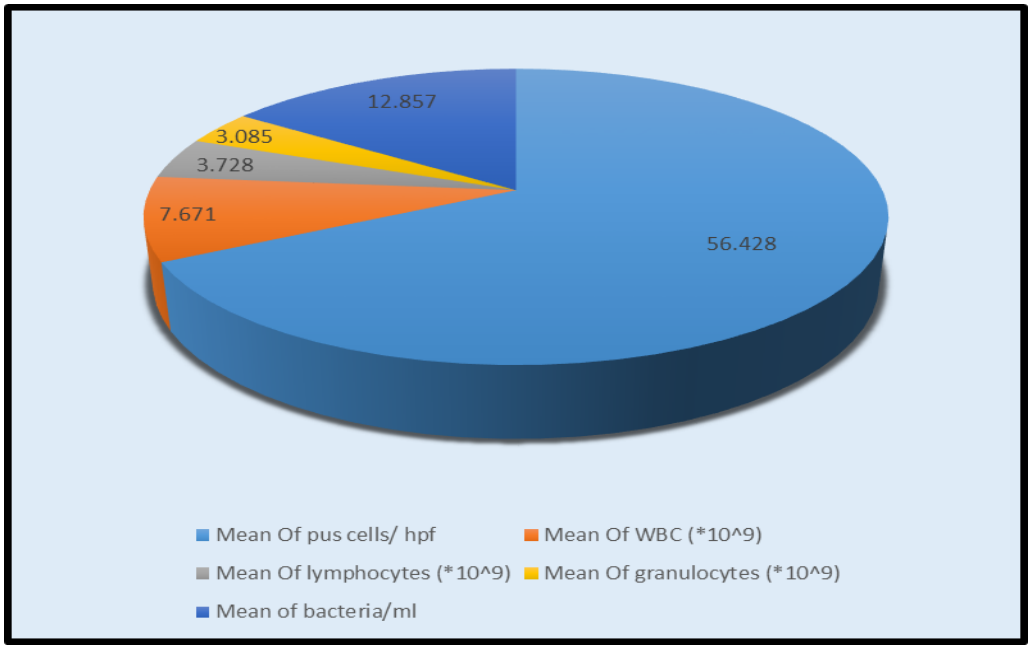


**Fig. (6):** urinary tract infection biomarkers and blood count markers in girls

## 4. DISCUSSION

Current Study subjects were grouped into symptomatic and asymptomatic urinary tract infection patients on the basis of the presence of more than 25 pus cells per mm3 in the urine sample (symptomatic) (Cheng et al., 2022). This refers to severe pyuria, which is characterized by an increased quantity of white blood cells and neutrophils (pus cells) in the urine (Kwon et al., 2020).////As shown in table (1), 28 (16.66%) of the 127 UTI patients are men and 55 (32.73%) are women. Adult males are between the ages of 14 and 50, while adult females are between the ages of 13 and 50. In the case of children, there were 44 children girls between the ages of 3 to 12. Boys were excluded because they are less susceptible to urinary tract infections.

According to table 1, there is a statistically significant association between adults and age in urinary tract infection as demonstrated by Hantoosh et al. (2016).

One (3.57%) of the 28 male patients had a significant UTI because this patient had diabetes mellitus (Utku, A. Ç. 2022). while the other 27 (96.42%) were non-significant UTIs. Of 55 females, ten patients (18.18 %) have symptomatic UTIs, whereas the other 45 (81.81 %) have asymptomatic UTIs. Finally, seven symptomatic UTI girls (20.45%) and 47 asymptomatic with a rate of 79.54%.

The prevalence of UTIs varied among the participants. This is due to the differences in gender anatomy, and data analysis in various seasons and ages (Medina and Castillo, 2019).

According to the means of bacteria and pus cells of all study groups, there was a significant difference between bacteria and pus cells in the male and also in girls (statistically significant) as also shown in a study done by Almaiman et al. (2021). As well in females statistically significant (Neupane et al., 2022). While there was no significant difference between men and women in the case of pus, (Sijad-Ur-Rehman et al., 2022). Male and female bacteriuria differ significantly as determined by Azab, K. (2021). On the other hand, there are relatively few bacteria impacting the urinary tract in adult males. While much more bacteria are affecting the urinary system in females and girls. Adult females reported having a greater mean quantity of bacteria compared to men, and a higher mean amount of bacteria compared to asymptomatic females. This may be due to the different anatomy of urinary tract infections in both genders. Women's urethras are closer to their anuses, and bacteria have greater opportunities to invade. Also, the urethras of women are significantly shorter than those of men, making it simpler for bacteria to enter the bladder (Abelson et al., 2018).

The total WBC count was estimated in this study subjects. In females, there was no significant difference between bacteria and white blood cells as also in the study shown by (AL-Khikani et al., 2019). On the other hand, there is a significant difference between WBC and bacteria in the current study. In children's girls, there was a significant difference between bacteria and white blood cells. The mean total (WBC) count was considerably higher in girls than in the other groups. This could be due to girls being more likely to have UTIs since their urethra (the tube that connects the bladder to where the urine exits the body) is shorter than a man's, bacteria have an easier time getting into the bladder as a result of this (Shormanov et al., 2021). The urethra is closer to the anus in girls, making it a potential source of bacteria that might cause a bladder infection (Zare et al., 2022).

According to Han et al. (2016), lymphocytes can be used as a diagnostic marker in urinary tract infections, women show a significant difference between bacteria and lymphocytes while all other study groups are non-significant.

In the current study, the mean of granulocytes count was found to be the statistically non-significant difference between all study groups ( (Kazmolu et al., 2019) except male statistically significant. Neutrophils (granulocytes), which are cells of innate immunity, are the main cells that are responsible for bacterial elimination once the inflammatory response starts (Incir et al., 2021).

## 5. CONCLUSION

This study demonstrates that biomarkers for urinary tract infections were more prevalent in girls . Significant urinary tract infection biomarkers were predominately neutrophil cells. This demonstrates how the innate immune response to bacteriuria works. Gender is regarded as a factor in the prevalence of UTIs because women are more likely to develop UTIs than men. Hematologic

markers revealed a relationship between urinary tract infections and males having elevated granulocytes marker and females having elevated lymphocytes marker.

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