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Comparison of Some Parameters in Patients Infected with Urinary Tract Infection According to Age and Gender in Al- Najaf Governorate

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Abstract

Three biomarkers namely; cathelicidin, neutrophil gelatinase associated lipocalin (NGAL), and Interleukin-22 (IL-22), are used as prognostic indicators for urinary tract infection (UTI). This study involved sixty patients diagnosed with UTI who attended to (AL-Sader medical city and private laboratories) in AL-Najaf province, during the period from November 2021 and January 2022. Patients were divided according to their age, gender (male and female), and control group (apparently healthy). Thirty samples exhibited negative urine cultures, all of which matched the patients' ages. The results revealed a significant increase in cathelicidin, NGAL-2 and IL-22 levels in urinary tract infection patients when compared to the control group, as well as other age groups (30-39), (40-49), and (50-59). Female patients had a significant rise ($p< 0.05$) in all three biomarkers when compared to male patients.

***Keywords*:** cathelicidin; neutrophil gelatinase associated lipocalin (NGAL); Interleukin-22 (IL-22); age; gender.

1. Introduction

Cathelicidin (LI-37), the most significant Antimicrobial Peptide (AMP), plays a function in microbe defense and has broad spectrum action. It belongs to the (AMP) family, which consists of tiny molecular peptides (R-100 amino acids) with two terminals. Cathelicidin, an N-terminal prosequence followed by a C-terminal, plays a crucial function in the natural immune system [1-3]. On the other hand, Neutrophil gelatinase associated lipocalin-1 (NGAL-1) is a component of the innate immune system that is expressed in many cells and is highly regulated during infection, injury, tubular injury, and cancer. It participates in many cellular functions such as apoptosis, cellular proliferation, bacteriostatic, and differentiation [4, 5].

Also, NGAL-1 belongs to the lipocalin superfamily and is a 24 KDa protein bound to neutrophil with 178 amino acids. It is a great potential marker for early UTI diagnosis [6]. Besides, Interleukin-22 (IL-22) is an antimicrobial cytokine produced by immune cells. It belongs to the IL-10 family and is secreted by lymphoid lineages such as innate lymphoid cells (ILC3), natural killer T-cells (NKT), and T-helper 22 (Th22), as well as macrophages and neutrophils [7]. The signal has a great affinity for IL-22 R1 and is strongly expressed in renal tubular epithelial cells and the urine bladder. IL-22 contains two receptors or two heterodimeric subunits IL-22 R1 and IL 10 R2. IL-22 is involved in a variety of biological processes, including urinary tract and gastrointestinal mucosal homeostasis, as well as epithelial cell repair, restoration, and regeneration [8-10].

Urinary tract infection (UTI) is an infection in any part of human urinary system; kidney, ureters, bladder and urethra and usually causes a significant burden among the individuals and are associated with high health care and social costs [11]. Women are at greater risk than men of developing UTI as it affects half of all women in their lifetime and one-fourth have recurrent infections. These infections can be painful to bladder and serious consequences if occurred and spreads to kidneys. [12, 13] Most of

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TUI treatment treated with antibiotic due to bacterial infections.

Urinary tract infection (UTI) is one of the most common infections caused by the predominant organism *Escherichia coli* (*E. coli*) and other pathogens, and it is a major public health concern because of the rise in bacterial resistance to major broad and wide spectrum antibiotics. More than half of all women will have at least one UTI during their lifetime [14-15].

According to many researchers on UTI disease, women have a higher prevalence of disease than men because they have a shorter urethral length and a moist peri urethral environment, so infection begins with uropathogenic contamination, then colonization and migration by flagella and pills to the bladder and kidney, which may be associated with highly anti-diabetic symptoms.[16, 17]

So, according to above survey, the main aim of this study is investigation of three biomarkers namely; cathelicidin, neutrophil gelatinase associated lipocalin (NGAL), and Interleukin-22 (IL-22), as prognostic indicators for urinary tract infection (UTI).

2. Materials and Methods

Three biomarkers namely; cathelicidin, neutrophil gelatinase associated lipocalin (NGAL), and Interleukin-22 (IL-22), were used as prognostic indicators for urinary tract infection (UTI). This study was diagnosed with sixty patients between November 2021 and January 2022 in AL-Najaf province (AL-Sader medical city and private laboratories). Patients were divided by age, gender (male and female), and control group (apparently healthy). Thirty samples exhibited negative urine cultures, normal leukocyte counts, and normal CRP levels, and they all matched the patients' ages.

2.1. Inclusion criteria

In this investigation, two kits were used for each marker (urine/gender sample and urine/serum sample, together with haematological and biochemical parameters such as WBC count, CRP level, and creatinine, and our selected three biomarkers Cathelicidin, NGAL-1 and IL-22).

2.2. Diagnosis

The following criteria were used to diagnose all of the patients. More than 100,000 colony forming units (CFu / millilitres) of uropathogenic bacteria found in urine; additionally, from 1,000 to 100,000 colony forming units (CFu / millilitres) may contain infections. This study included appositive cultures with ten or more urinary white blood cells per high power field, as well as some clinical signs such as cloudy urine, new back pain, abdominal pain, and fever of more than 38°C, pain after or during urination, worse incontinence, and appositive cultures without any of the above criteria [18].

2.3. Urine collection

After an hour, urine samples were centrifuged at 12,000 rpm for 10 minutes and deposited in an Eppendorf tube in 80^{o}C, while other tiny samples were placed on nutrient agar plates and incubated for 24 to 48 h. [17].

Three biomarkers' kits for detection in serum and urine

a. **Cathelicidin ELISA kits:** Two kits, that purchased by Hycult Biotech /Holanda, are used for evaluation of cathelicidin level in serum and urine . [3]
b. **Neutrophile Gelatinase associated lipocalin:** Two kits were supplied by Dianova / German company for serum and Urine. [19]
c. **IL-22 ELISA kits:** Two kits were supplied by Biotechn R&D system / USA.

2.4. Statistical analysis

The Statistical Package for Social Science version 23 for Windows Software was used to enter and analyse all of the findings from the current investigation. For quantitative data, descriptive statistics were produced in the form of mean and standard deviation (Mean SD). Numbers and percentages were used to convey qualitative data. The statistical comparison between the two groups was evaluated using the Student T-test. $p<0.05$ (*) was used to indicate statistical significance. A p-value of less than 0.001 was deemed extremely significant (**), whilst a p-value of more than 0.05 was deemed inconsequential.

3. Results

3.1. Cathelicidin level

Mean serum cathelicidin levels in patients with UTI and the controls were 3.53 ±1.26 and 0.44 ± 0.100 ng/mL, respectively. Cathelicidin expression in UTI was significantly higher than that in control tissue ($p\leq 0.05$). (Figure 1). [19, 20]

a. Comparison between cathelicidin levels of urinary tract infection in serum and urine

Also, a significant increase also showed between cathelicidin in level in urine (5.73 ± 1.60) in comparison with control group (0.54 ± 0.169). The

level of cathelicidin was higher in urine in comparison in serum. Results of Figure 1 appeared a significant increase ($p \leq 0.05$) in cathelicidin levels.

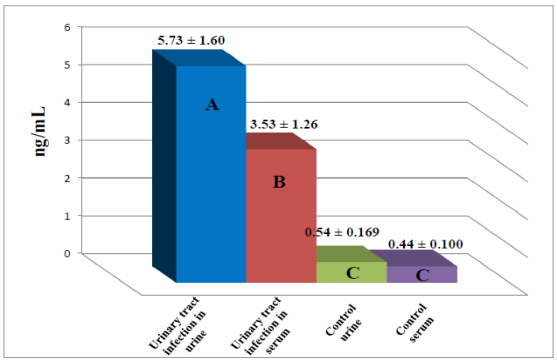

Fig. 1. Comparison between cathelicidin levels in serum and urine of urinary tract infection patients. Different letters refer to significant difference at ($p \leq 0.05$), similar letters refer to non-significant difference.

b. Comparison between cathelicidin level in patients according to age

Current results that depicted in figure 2 revealed a significant increase in cathelicidin level at advanced age 60-69 years in serum and urine respectively, (5.053 ± 0.548) ,(7.595 ± 0.543) in comparison with other age 50-59 years 3.513 ± 0.522 , 6.2 ± 0.302 and 40-49 years 2.613 ± 0.354, 5.08 ± 0.624 and 0-39 years 1.9± 0.188, 3.3 ± 0.516. The markers showed a significant increase in urine in compare with serum of urinary tract infection.

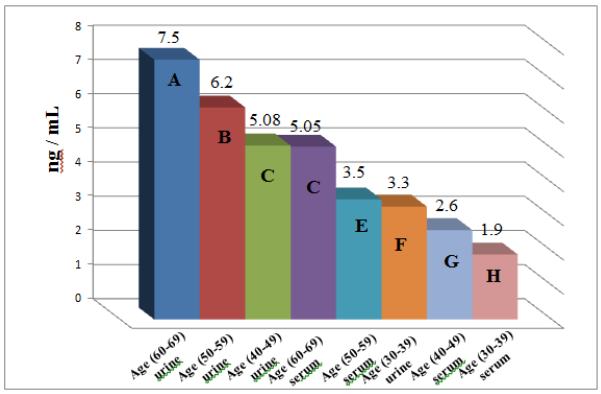

Fig. 2. Means level of cathelicidin in urinary tract infection patients according to ages, Different letters refer to significant difference at ($p \leq 0.05$).

c. Comparison between patients according to gender

In contrast to male serum and urine (2.64 0.619), (6.84 0.938), the current results showed a significant rise (p0.05) in female serum and urine (4.380 1.136), (4.580 1.515) in compared to male serum and urine (2.64 0.619), (6.84 0.938). In female urine, there was a substantial rise (p 0.05) when compared to serum.

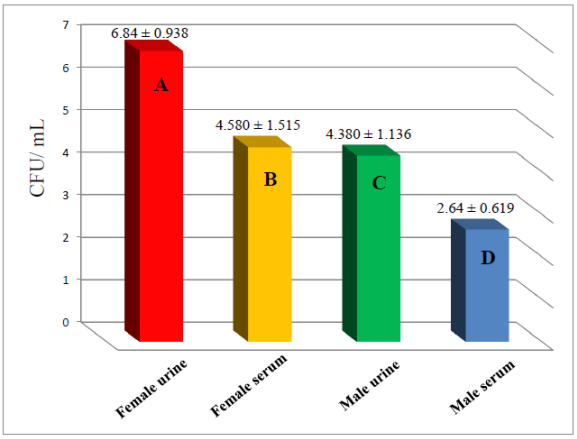

Fig. 3. Means and standard deviation of urinary tract infection in serum and urine according to gender. Different letters refer to significant difference at ($p \leq 0.05$).

3.2. Neutrophil gelatinase associated lipocalin-1 comparison between urinary tract infection patient and control in urine and serum[21]

Neutrophil gelatinase associated lipocalin-1 with patients were significantly elevated in serum, urine and control as depicted in figure 4 which means (220.416 ± 32.918, 47.4 ± 8.461, 245.41 ± 26.96 and 49.6 ± 7.497 Pg/ ml) respectively in patients ($p \leq 0.05$) which $p \leq 0.001$. Higher Neutrophil gelatinase associated lipocalin-1 level was highly increase in urine in comparison with serum.[22, 23].

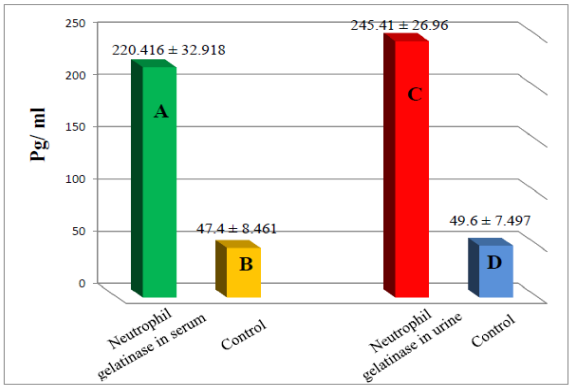

Fig. 4. Means of Urinary tract infection according to Neutrophil gelatinase associated lipocalin-1 in serum (A and B), while (C and D) Means of Urinary tract infection according to Neutrophil gelatinase associated lipocalin-1 in urine. Different letters refer to significant difference at ($p \leq 0.05$).

a. Comparison between patients group according to ages

Figure 5 showed a significant increase in UTI with age, (60-69 serum and urine) with values (177 ± 6.946, 210.9 ± 8.11, 201.2 ± 19.87, 224.6 ± 8.19, 222.4 ± 14.97, 247.6 ± 6.54, 257.7 ± 13.21, 274.7 ± 15 CFU/ ml) as compared with other age 50-59, 40-49, and 30-39, respectively.

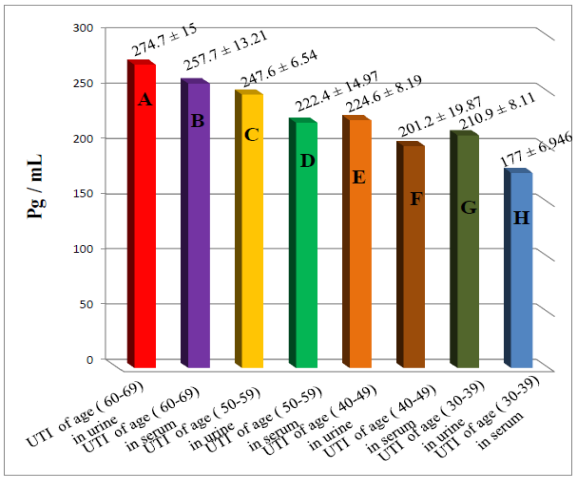

Fig. 5. Means of neutrophil gelatinase lipocalin-1 level of Urinary tract infection compare between patients according to ages. Different letters refer to significant difference at (p≤ 0.05).

b. Comparison between neutrophil gelatinase lipocalin-1 patients group according to gender

As shown in Figure 6 revealed a significant increase in (p< 0.05) in UTI levels with gender type as (Female serum, Female urine as compare with Male serum and Male urine) as (245.16 ± 19.04, 269.4 ± 16.5, 193.2 ± 18.84 and 223.8 ± 12.89) respectively which p≤ 0.001, high Neutrophil gelatinase lipocalin-1 in female and male urine as compare with female and male in serum.

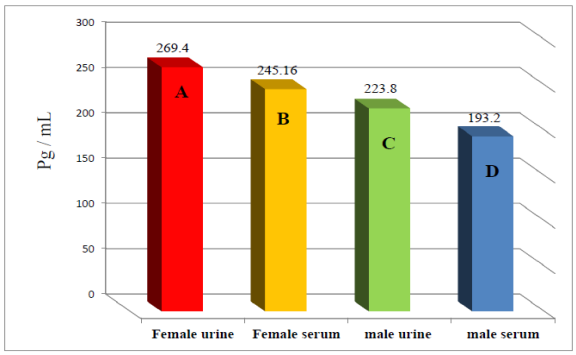

Fig. 6. Means of Urinary tract infection compare between patients according to gender. Different letters refer to significant difference at (p≤ 0.05).

3.3. Interleukin-22 Comparison between UTI patients and control group

Figure 7 indicated a significant increase in (p≤ 0.05) in Interleukin-22 with patients in serum (99.6 ± 16.9) compare with control (16.61 ± 1.45) which p≤ 0.05, also a significant increase in (p≤ 0.05) in Interleukin – 22 with patients in urine (120 ± 14.83) compare with control (18.31 ± 1.7), higher Interlukin-22 level in urine as compare with serum.

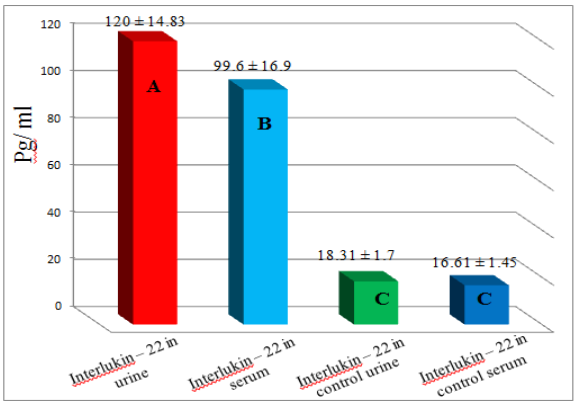

Fig. 7. The means of level of Interleukin-22 in patients compare with control group in serum and urine. Different letters refer to significant difference at (p≤ 0.05).

a. Interleukin-22 levels with Comparison between patients group according to age:

Figure 8 indicated a significant increase in (p≤ 0.05) in Interleukin-22 levels according to age group in serum and urine with (30-39, 40-49, 50-59 and 60-69) which means (77.1 ± 2.33, 98.6 ± 4.57, 87.06 ± 6.78, 104.5 ± 27.9, 103.6 ± 6.9, 121.06 ± 4, 118.3 ± 9.05 and 136.7 ± 5.7) respectively in patients which p≤ 0.001.

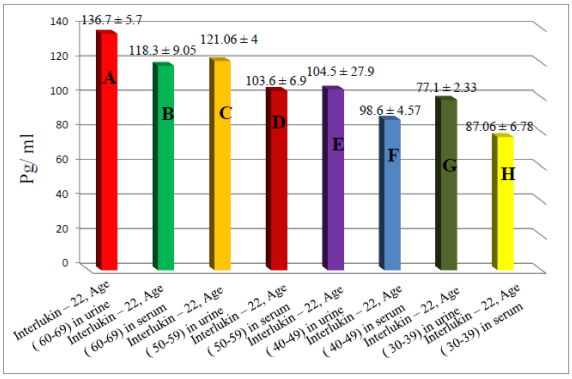

Fig. 8. means of level of Interleukin--22 in patients compare between the results of serum and urine according to ages. Different letters refer to significant difference at (p≤ 0.05).

b. levels of Interleukin-22 with Comparison between patients group according to gender

Figure 9 showed a significant increase ($p \leq 0.05$) in Interleukin-22 levels in patients according to gender in serum and urine with (male serum, male urine, female serum and female urine) which means (86.42 ± 9.39, 108.1 ± 11.6, 114.3 ± 8.7 and 130.4 ± 12.03) respectively, which $p \leq 0.001$.

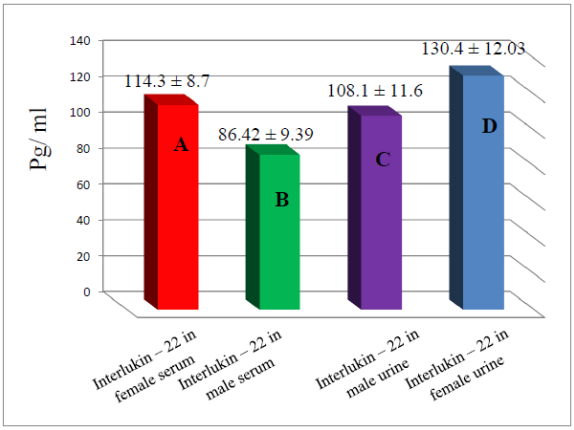

Fig. 9. Means of level of Interleukin-22 in patients with compare between the results in serum and urine according to gender. Different letters refer to significant difference at ($p \leq 0.05$).

4. Discussion

In comparison to the control group, the current investigation found a substantial rise in cathelicidin levels in urinary tract infection patients (Figure 1).

The current findings are consistent with several recent studies that have found a high level of cathelicidin in UTI infection illness as a result of harmful bacteria being killed by cathelicidin produced by immunological and epithelial cells, resulting in lower bacterial loads and tissue damage [24, 25]. Previous research demonstrated the benefit of cathelicidin by employing mice lacking cathelicidin (Camp-1) and demonstrating a rise in bacterial and tissue injury burdens. Antimicrobial action of highly cathelicidin has been shown to protect against bacterial infection in several investigations [26].

One of the most essential properties of cathelicidin is its capacity to operate as an immune modulator, activating or suppressing various immune cells through a chemotactic action, and innate immunity was a primary source of cathelicidin during infection[27, 28]. Other studies have shown that high cathelicidin levels activate mast cells, increase expression of Toll-like receptor-4 (TLR-4) and lead to tissue inflammation by enhancing an important enzyme called myeloperoxidase (MPO) bacteria. Cathelicidin is also considered a proinflammatory in function and leads to tissue inflammation by enhancing an important enzyme called myeloperoxidase (MPO) bacteria [29, 30].

Figure 2 showed that cathelicidin lead levels rise significantly with advanced age (60-69) compared to other ages. Other studies have suggested that role of older ages in urinary tract infection patients and association with high cathelicidin level and disuse as a result of cathelicidin in severity of UTI and decline in production and secretion of antioxidants such as catalase, peroxidase, and superoxide dismutase with low concentration of all antioxidant defense system lead to more survivability[31].

Figure (5) in current results show a high level of NGAL in older age (60- 69) years than other age. The current study indicates in figure (6) significant increase in NGAL in female than male. No previous studies have been found to explain why NGAL level, was higher in female than male in current study but our explanation may depend on that a female gender considered as a risk factor for UTI infection and uropathogenic bacteria infect female and growth rapidly through urinary tract and easy to migrate, colonized and distributed through urinary bladder and kidney.

The Figure (7) referred to significant increase in IL-22 level in urinary tract infection patients as compare with control group, the research data about IL-22 in UTI disease is very little so that the current results agree with very recent studies that explain the role of IL-22 as antibacterial properties in which can limit bacterial infection and after microbiota which has a broad implication for maintenance of homeostasis of mucosal surface in urinary tract infection and gastrointestinal tract [8].

In the current study, the figure (8) shows a considerable increase in IL-22 with older age compared to other age groups, such as cathelicidin and NGAL. Previous research has linked a high inflammatory response to older age by triggering an immune response against a variety of uropathogenic bacteria and a high expression of several cytokines such as IL-22. In addition, many studies have demonstrated the role of older age in women with urinary tract infections due to estrogen depletion and a high inflammatory response [32, 33].

In the current investigation, the figure (9) demonstrated a substantial rise in IL-22 in females compared to males in urinary tract infection patients' urine and serum. The main causes of urinary tract

infection in women are due to pathophysiological changes more than in men. Several researchers have studied these charges and found that changes in vaginal flora due to estrogen deficiency lead to high bacterial growth, particularly gram negative bacteria, and colonization of uropathogens, resulting in an inflammatory response associated with secretion and production of cytokines such as IL-22 [34, 35].

5. Conclusions

From above results, we can conclude that the three biomarkers namely; cathelicidin, neutrophil gelatinase associated lipocalin (NGAL), and Interleukin-22 (IL-22), that used as prognostic indicators for urinary tract infection (UTI) are differ according to gender, age in serum and urine.

6. Conflicts of interest

"There are no conflicts to declare".

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