International Journal of Medical Laboratory 2019;6(4):288-295.

International Journal of Medical Laboratory
Shahid Sadoughi University of Medical Sciences

Original Article

Neutrophil Gelatinase-associated Lipocalin Value in Patients' Urine with Gram Positive Cocci Infection in the Urinary Tract

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ABSTRACT

Article history
Received 26 Apr 2019
Accepted 2 Oct 2019
Available online 10 Dec 2019

Key words
Antibiogram
Gram-positive cocci
NGAL
Urinary tract infection

Background and Aims: This study aimed to investigate the amount of Neutrophil gelatinase-associated lipocalin (NGAL) in patients' urine with Gram-positive cocci infections

Materials and Methods: The microbial culture was prepared from 100 urine samples and the results were recorded. The genus and bacterial were species identified and an antibiogram test was conducted to investigate their resistance. Human Lipocalin /NGAL enzyme-linked immunosorbent assay test was used. To analyze the data, Tukey and One Way ANOVA tests were used.

Results: The highest mean of NGAL in the patient group was related to the men's category with 8.77, and the gender had a significant impact on the mean of NGAL. White blood cells has a significant impact on the mean of NGAL ($p<0.05$). The negative significance of the mean value also indicates the amount of NGAL being higher in the patient group.

Conclusions: These data suggest a relationship between the amount of NGAL with white blood cells value in patients who present urinary infection of Gram-positive cocci. NGAL value plays an important role as an adjuvant and surrogate marker in early diagnosis of urinary tract infections and acute renal damage in line with microbiological investigations.

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[DOI: 10.18502/ijml.v6i4.2012]

Introduction

Urinary tract infections (UTI) are common within the pediatric populations. Recurrent UTIs (rUTI) occur in approximately 12% of the patients following an initial UTI [1]. UTI is one of the most common bacterial infections known as the second factor infection in the human body [2]. The lack of diagnosis and timely treatment of this type of infection can triger severe disadvantages including urinary tract disorders, blood pressure, kidney disorders, uremia, and in pregnant women, premature delivery and even abortion [3, 4]. Urinary tract infections usually occur more frequently in women than men, and half of the women suffer from at least one infection during their life. The reoccurrence of the disease is a common issue. In women, urinary tract infections are the most common form of bacterial infections, and the urinary tract infections raise is about 10% [5, 6]. About 40 percent of women have at least one urinary tract infection during their lifetime, and in a significant number of these women, the infection will recur again [7]. Recurrent infections are generated by different microorganisms [8].

The lower urinary tract infection is also called bladder infection. The most common symptoms include burning when urinating, and the need for frequent urination (urgency for urinary) in the absence of vaginal flow and significant pain [9]. These symptoms may be moderate to severe [10], and in healthy women, they on average, last six days. Some pains may occur above the pubic bone [11]. The people who are involved in upper urinary tract infection or Pyelonephritis may also experience common symptoms of lower urinary tract infection, fever, or nausea and vomiting (10). Rarely, may the urinary infection be associated with bleeding [11] or a visible pyuria. [12]. The most cases of urinary tract infections are caused by bacteria that are a part of the microbial flora of the intestine, vagina or the skin of the perineum area and, in the following, other enterobacteria such as *Proteus* and *Klebsiella* include the family of *E. coli*. As the most common urinary tract infection bacteria factor of gram-positive bacteria, we can point out to *Staphylococcus saprophyticus (S. saprophyticus)* or *S. aureus* or *Enterococci*. The rarer bacteria such as *Citrobacter*, *Saratya*, *Pseudomonas*, and *Provencia* are more commonly found in hospital infections [13].

In fact, about 80-85% of the major reason for urinary tract infections is related to *E. coli* and also about 5-10% of other cases are related to *S. saprophyticus.* These infections can rarely be connected to viral or fungal infections [14, 15]. *S. saprophyticus* is the main factor for gram-positive cocci and negative coagulase in the genus of *Staphylococcus*. The bacteria are negative in terms of phosphatase test, but in terms of urease and lipase, they are positive. This bacterium is one of the most common causes of urinary tract infections. It is estimated that about 10 to 20 percent of UTI infections are caused by *S. saprophyticus* [16]. In medicine, a biomarker can be used to detect a traceable substance within an organism as a

[DOI: 10.18502/ijml.v6i4.2012]

mean to investigate the function of its members. The presence of a biomarker in the body may indicate a specific disease; for example, the presence of antibodies in the body may indicate an infection. One of the new biomarkers in the diagnosis of renal damage is related to neutrophil gelatinase-associated lipocalin (NGAL) [18]. NGAL, a protein expressed in the uroepithelium [19], functions within the innate immune system by exerting a bacteriostatic effect on gram-negative bacteria through iron chelation [20]. Urinary NGAL (uNGAL) has been shown to be variably upregulated in UTI [21]. This biomarker is a 24 kDa protein bound to neutrophils, which is produced by neutrophils and a variety of cells and found at varying levels in the stomach, colon, kidneys, trachea, lungs, prostate and salivary glands [18, 22, 23]. NGAL rapidly increases in renal ischemic or nephrotoxic lesions [17, 24]. Its origin is the proximal tubules complex [18]. The measurement amount of this protein has increased in clinical trials due to the emergence of new and rapid tests and standard techniques. Many of these results have been obtained from the extent of NGAL measurement in clinical settings, by research-based methods including the enzyme-linked immunosorbent assay (ELISA). Therefore, in this study, the determining evaluation of NGAL in patients' urine with Gram positive coccidia infection was investigated.

Materials and Methods

The case- study population consisted of 100 people. Fifty were in the patient group (Treatment group) and 50 others who did not show UTI, were placed in the experimental group (Control group). From all the case-study people in this research, urine samples were collected. In the next step, from the urine samples, the microbial culture was prepared and then the results were recorded. For patients whom their urine culture test was positive, the genus and bacterial species were identified by performing the specialized microbiological tests, and hence an antibiogram test was conducted to investigate their resistance. Moreover, the amount of 2 mL from the urinary-centrifuged supernatant was obtained from the samples their culture of which was approved to be Gram-positive cocci infection. They were then stored in a freezer with the temperature of -80˚C and NGAL measurements were performed when a sufficient number had been obtained. Measurements were performed quantitatively using the ELISA method (GARNI Medical Engineering Company). This study was approved by the Ethics Committee of Islamic Azad University, Sanandaj, Iran.

Statistical analysis

The statistical package for the social sciences (SPSS) software, v. 16 (SPSS Inc, Chicago, IL, USA) was used for the analysis. In this descriptive study, frequency tables and charts, parameter tests of t-test and variance test as well as Tukey test were used.

Results

According to gender segregation, 16 males and 34 females were placed in the control group and 17 males and 33 females in the patient

[DOI: 10.18502/ijml.v6i4.2012]

group. The mean age of the statistical population was 43.7 years.

The highest frequency percentage in the control was related to the women category (less than 1 with 54 percent), and in the patient group was also related to the women category (less than 8 with 82 percent) (Table 1).

The highest frequency percentage in the control and the patient group was related to the low category with 52 percent (Table 2).

The highest sensitivity to fosfomycin was with the frequency of 44 people. The highest half-sensitivity to Cefazolin was with a frequency of 4 people. Also, the highest resistance to trimethoprim-sulfamethoxazole was with a frequency of 27 people and, the patients' age average was 74.43 years. The highest mean of NGAL in the control group was related to the women category with an average of 3.88 ng/ml, and in the patient group was related to the men category with an average of 8.77 ng/ml. In the control group, gender had no significant impact on the mean of NGAL (p=0.88). As shown in the table 4, in the patient group, it was significant (p=0.01) (Table 4). The highest mean of NGAL in the control group was related to the 1-2 category with an average of 4.17 ng/ml, and in the patient group was related to the 8-12 category with an average of 14.03 ng/ml (Table 5).

The highest mean of NGAL in the control group was related to the low category with an average of 4.23 ng/ml, and in the patient group was related to the high category with an average of 7.9 ng/ml (Table 6).

Table 1. Frequency and white blood cells frequency percentage of control and patient groups

	White blood cells	Frequency	Frequency percentage
Control	<1	27	54
	1-2	15	30
	>2	8	16
Patient	<8	41	82
	8-12	6	12
	>12	3	6

Table 2. Frequency and frequency percentage of number of bacterial in the control and patient groups

	Number of bacteria	Frequency	Frequency percentage
Control	Rarely	24	48
	Low	26	52
Patient	Medium	22	44
	High	2	4

[DOI: 10.18502/ijml.v6i4.2012]

Table 3. Sensitivity frequency to antibiogram

Sensitivity	Frequency
Ciprofloxacin	5
Cotrimoxazole	1
Clindamycin	1
Fosfomycin	44
Gentamicin	1
Oxacillin	3
Vancomycin	15
Penicillin	11
Cceftriaxone	7
Trimethoprim-Sulfamethoxazol	16
Doxycycline	3
Cefazolin	7
Cefotaxime	4
Ofloxacin	13
Cephalexin	3
Cephalothin	4
Norfloxacin	6
Colistin	4
Cefepime	2

Table 4. The neutrophil gelatinase-associated lipocalin amount (ng/ml) in the control and patient groups based on gender

Group	Gender	Mean	Standard deviation	P-value
Control	Male	3.73	3.45	0.88
	Female	3.88	3.19	
Patient	Male	8.77	7.85	0.01
	Female	4.73	2.94	

Table 5. Mean and standard deviation of the neutrophil gelatinase-associated lipocalin amount (ng/ml) by white blood cells

	Variable	Mean	Standard deviation
Control group	<1	3.74	2.9
	1-2	4.17	4.01
	>2	3.52	3.14
Patient group	<8	4.88	2.98
	8-12	14.03	10.48
	>12	6.93	7.01

Table 6. The neutrophil gelatinase-associated lipocalin amount (ng/ml) by the number of bacteria

	Variable	Mean	Standard deviation
Control group	Rarely	3.4	2.63
	Low	4.23	3.73
Patient group	Low	7.18	6.93
	Medium	4.66	2.69
	High	7.9	2.82

Discussion

Urinary tract infections are one of the most common causes of the patients' referral to clinics and hospitals. Women, children and the people who suffer from an underlying disease

[DOI: 10.18502/ijml.v6i4.2012]

are the groups which are exposed to greater risks. The most common serious bacterial infection in children is related to urinary tract infections. The description of urinary tract infections has been available since ancient times and its first documented description has come into *Ebers Papyrus* dating back to 1550 BC, when the Egyptian people called it as "Transferring the heat of the bladder" [25]. The effective treatment of this disease was not undertaken until the availability of antibiotics in the 1930s, when prior to its use, the application of herbs, phlebotomy, and resting were recommended [26]. In renal nephrons damages, NGAL is a specific index. In a research which we performed, similar results were obtained; according to the results, the evaluation amount of NGAL could be effective in early diagnosis of urinary tract infections and acute kidney damage. In 1992, Flemingham and his colleagues investigated the available *Enterococcus* strains in the patients who were suffering from UTI. The amount of these strains increased from 4% in 1971 to 12.6% in 1990 [27].

In 2013, Urbschat et al. found that the NGAL biomarker can act as a factor in early detection of urinary tract infections. In patients who had upper UTI, the amount of this biomarker increased [28]. In Hjortrup studies, the number of case study patients were 222 people and a difference was found compared with our research. We found similar results regarding the amount of NGAL in renal damage. In this case, the amount of NGAL had a direct correlation with UTI in Hjortrup studies [29]. Previous studies have shown that NGAL increases in acute infections of the urinary tract and the uNGAL levels demonstrates a dose-response relationship to the bacterial colony counts [21, 30].

Urbschat also achieved similar results in similar research. In Urbschat's investigations, about 30 patients with upper UTI, 29 patients with lower UTI and 38 others without any infection were investigated. The amount of urine penetration in the patients increased rapidly, which is in line with the results we obtained. Moreover, Urbschat examined urinary kidney injury molecule biomarker, which induced no change in the amount of urinary tract infection [28]. Although the amount of urinary kidney injury molecule was not investigated in our research, there was no contradiction in the results obtained. Another important finding is the positive correlation between serum NGAL level and the acute inflammation markers white blood cells, neutrophils and C-reactive protein. Allegra et al. also showed a positive correlation between serum NGAL levels and leukocyte (white blood cells) and neutrophil numbers [31], in agreement with the results in our study showing that the relation between leukocytes and NGAL in the kidney injuries can be very important.

Conclusion

Based on the results obtained and comparison with similar studies which were conducted, a direct relationship can be found between NGAL biomarker and kidney damage. This means that this biomarker can be used as a factor for the early detection of UTI and

[DOI: 10.18502/ijml.v6i4.2012]

kidney damage. Undoubtedly, performing further investigations in this field will lead us towards greater recognition of this biomarker.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

The authors thank all the contributors to this research.

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[DOI: 10.18502/ijml.v6i4.2012]