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ORIGINAL ARTICLE

Azurocidin and CXCL9 as Novel Biomarkers in Women with Asymptomatic *Bacteriuria*

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ABSTRACT

Background: *Azurocidin and CXCL9 are two important proteins that play crucial roles in the immune response during urinary tract infections.* ***Objective:*** *This study aims to investigate the immunological role of azurocidin and CXCL9 in women with acute (symptomatic) and chronic (asymptomatic) urinary tract infections.* ***Methodology:*** *The study included 35 healthy women as controls, 35 women complain of acute UTIs, and 35 women of chronic UTIs who were hospitalized in ALNajaf Hospital between 1st June 2024 and the end of January 2025. All women have been diagnosed by a physician. We have used ELISA to assess azurocidin and CXCL9 level in each woman's serum.* ***Results:*** *The results proved that there was a significant increase (P value<0.0001) in serum levels of Azurocidin in women with acute UTIs (19.13 ± 2.834 pg/ml) and chronic UTIs (28.78 ± 4.320 pg/ml) compared with control (7.075 ± 0.6675 pg/ml). The serum levels of Azurocidin in women with chronic UTIs were substantially higher (P value<0.0001) than those infected acute UTIs. The results demonstrated that there was a significant increase (P value<0.0001) in serum levels of CXCL9 in women with acute UTIs (401.1 ± 60.28 pg/ml) and chronic UTIs (541.2 ± 48.18 pg/ml) compared with control (240.5 ± 12.01 pg/ml).* ***Conclusion:*** *Our findings show that azurocidin and CXCL9 have a crucial immunological role in women suffering from acute or chronic UTIs; these markers might be helpful for new diagnosis in women with undiagnosed chronic UTIs.*

INTRODUCTION

Urinary tract infections (UTIs) are a common health concern affecting individuals of all ages, with women being disproportionately affected due to anatomical factors. These infections can be classified into lower tract (cystitis) and upper tract (pyelonephritis) manifestations, and can be either symptomatic or asymptomatic. While the urinary tract is normally sterile, various conditions can lead to bacterial invasion and subsequent inflammation, resulting in complicated or uncomplicated urinary tract infections [1,2].

Acute urinary tract infections often present with symptoms such as dysuria, frequency, and urgency, and are typically caused by uropathogenic *Escherichia coli*. These infections are highly prevalent, with one in five adult women experiencing a UTI at some point in their lives. Recurrent UTIs are also common, particularly in women, and can significantly impact quality of life [3,4].

Complicated urinary tract infections are associated with factors that increase the risk of infection or treatment failure, such as the presence of indwelling catheters, urinary tract abnormalities, immunosuppression, or prior antibiotic exposure. These infections can be more severe and may require more extensive evaluation and treatment [5].

Chronic urinary tract infections are a persistent and challenging health issue that affects millions of individuals worldwide. Urinary tract infections are one of the most common clinical syndromes encountered in general and gynecological practices, with women being twice as likely to develop a UTI compared to men. These infections can range in severity from asymptomatic bacteriuria to life-threatening pyelonephritis, and can be categorized as cystitis, pyelonephritis, and prostatitis [6,7].

Urinary tract infections are a significant cause of morbidity and healthcare spending, particularly among young women, children, and the elderly. Recurrent UTIs, defined as at least three episodes in one year or two episodes in six months, continue to be a major problem, with various antibiotics having been the mainstay of therapy but often failing to prevent future infections [8]. The rising prevalence of multidrug-resistant uropathogens has further complicated the treatment of these infections and necessitates the exploration of alternative antimicrobial approaches.

In most cases, bladder and renal infections are asymptomatic and only detected incidentally through the presence of bacteriuria. However, certain risk factors, such as frequent sexual activity, pregnancy, stone disease, and diabetes, can lead to the development of symptomatic cystitis or pyelonephritis, requiring antimicrobial intervention [9].

Azurocidin is an antimicrobial peptide found in neutrophils that can lyse bacterial cells and prevent

them from binding to the epithelial cells in the urinary tract. CXCL9, on the other hand, is a chemokine that attracts leukocytes, such as neutrophils, to the site of infection, facilitating the rapid removal of invading bacteria. The innate immune system is the first line of defense against urinary tract infections, and it utilizes a variety of mechanisms to combat the invading pathogens [10]. Antimicrobial peptides, such as azurocidin, form an essential part of this innate immune response by directly targeting and destroying the bacterial cells. Additionally, the activation of toll-like receptors by the presence of bacteria triggers the release of various chemokines and cytokines, including CXCL9, which recruit and activate leukocytes to the site of infection [11,12].

Together, these mechanisms work in concert to help eradicate a urinary tract infection, with the rapid innate immune response designed to remove most of the bacteria within 24 hours in an uncomplicated UTI. However, in some cases, the infection may progress to pyelonephritis, a more serious form of urinary tract infection, which can lead to chronic renal injury and failure [13]. This study aims to investigate the immunological role of azurocidin and CXCL9 in women with acute and chronic UTIs.

METHODOLOGY

Women patients and controls

A total of 90 women with the age range (18-60) years were enrolled in this study that was carried out in Department of Nephrology at AL-Najaf Hospital in Al-Najaf City, Iraq from 1st June 2024 to the end of January 2025. 30 women had acute UTIs, 30 women had chronic UTIs and 30 healthy women have a negative history and no clinical evidence of any other disease as control. All women patients have been diagnosed by the physicians.

Ethics Consideration

The Institutional-Ethics Committees of the College of medicine at the University of Kufa and the Scientific-Committee for Research of the Health Department of Najaf both gave their approval.

Measurement of serum levels of azurocidin and CXCL9

Five milliliters of blood were taken from each subject, and two milliliters of serum were recovered by centrifugation at 8000 rpm/10 minutes [14]. The enzyme-linked-immunosorbent-assay was used to quantify azurocidin and CXCL9 based on serum concentration as follow: after the determination of diluted standard, blank, and sample wells, 100 µL each dilution were added, and the micro-ELISA plate was covered by the sealer and incubated for 90 min at 37ºC. After incubation, all liquid was removed from each well, 100 µL of Bio-tinylated detection antibody solution were added to each well, and the micro-plate was covered with a new sealer and incubated for 1 hour at 37ºC . After incubation, all liquid was removed from each well and washed by adding 350 µL of washing buffer to each well (these steps were repeated three times). 100 µL of HRP Conjugate working solution were added to each well, covered by a micro-plate and incubated at 37ºC for 30min.The solution was removed from each well, and the washing step was repeated five times. Then 90 µL of substrate reagent were added to each well, and the micro-plate was covered by micro-plate sealer and incubated for 15 min. at 37ºC. 50 µL of stop solution were added to each well, and determination of the optical density (OD value) was done by ELISA reader at 450nm wavelength, then the results were calculated by plotting the standard curve.

Statistical analysis

A mean value and standard error (SE) were computed for each value, which was done with graph-pad-prism. Statistically significant P values of less than 0.05 were considered in the statistical analysis.

RESULTS

Azurocidin:

A significant increase (*P* value<0.0001) in serum levels of azurocidin in total women with UTIs was higher (24.76 ± 2.832 pg/ml) compared with control (7.075 ± 0.6675 pg/ml) (Figure 1). Also, the results showed a higher serum levels in women infected acute (19.13 ± 2.834 pg/ml) and chronic (28.78 ± 4.320 pg/ml) UTIs compared with the controls, with a significant increase recorded (*P* value <0.0001). Azurocidin serum levels, however, differed significantly (*P* value 0.0466) in women with chronic UTIs and those with acute UTIs (Figure 2).

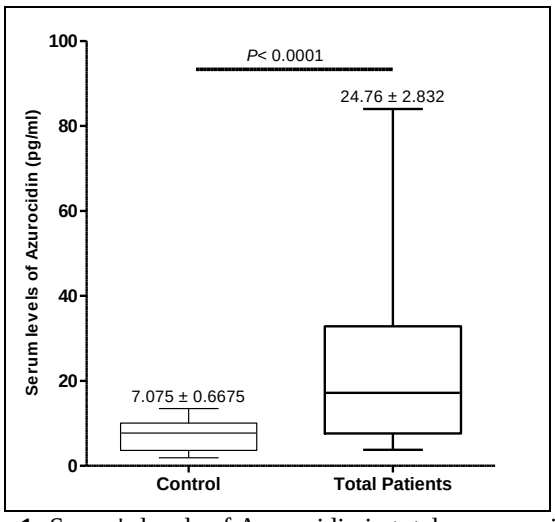

Fig.1: Serum's levels of Azurocidin in total women with urinary tract infections and controls.

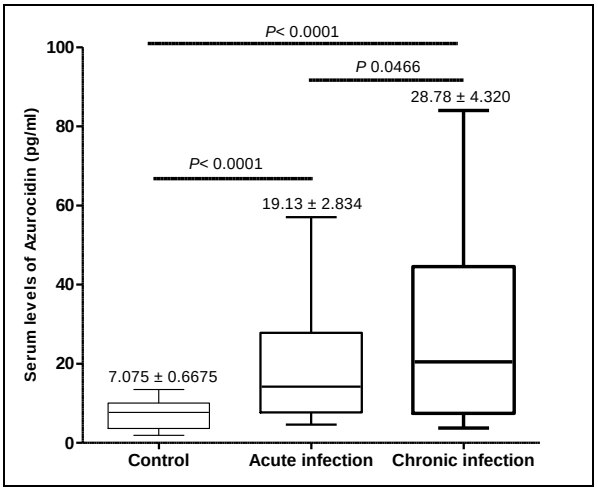

Fig.2: Serum's levels of Azurocidin in women with acute and chronic urinary tract infections compared with controls.

CXCL9:

It was found that the total CXCL9 serum concentration was significantly higher (P < 0.0001) in women with urinary tract infections (482.8 ± 38.43 pg/ml) compared to the control (240.5 ± 12.01 pg/ml) (Figure 3). Also, there were higher serum levels in women with acute (401.1 ± 60.28 pg/ml) and chronic (541.2 ± 48.18 pg/ml) UTIs compared with controls, with a significant increase (P 0.0041, P < 0.0001), respectively. CXCL9 was considerably higher (P 0.0360) between women with chronic UTIs and those with acute UTIs (Figure 4).

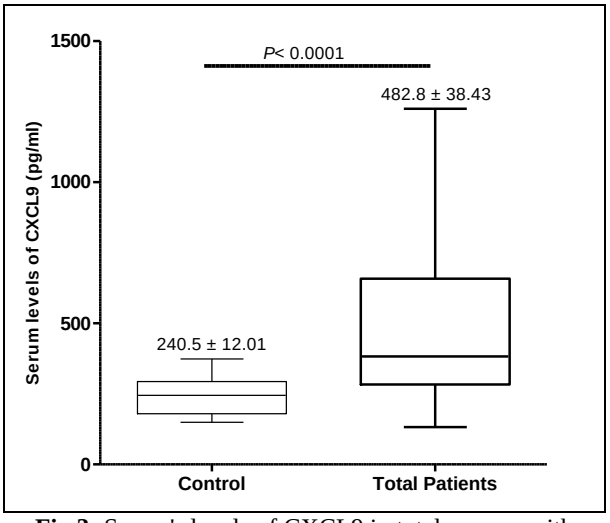

Fig.3: Serum's levels of CXCL9 in total women with urinary tract infections and controls.

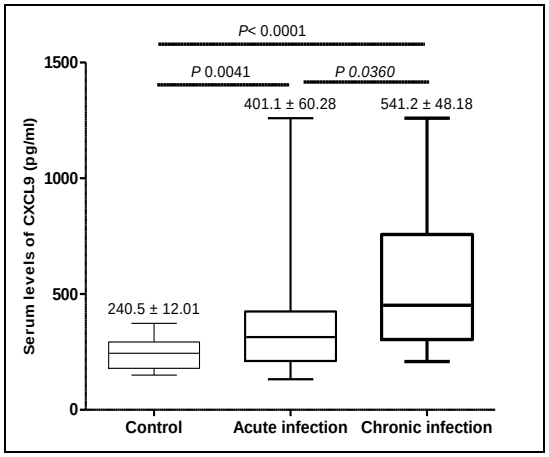

Fig.4: Serum's levels of CXCL9 in women with acute and chronic urinary tract infections compared with controls.

We found that there was a positive correlation (Figure 5) between the two markers (P 0.174). In acute infection, there was also a positive correlation (Figure 6) between the two markers (P 0.0688). In contrast, a negative correlation was observed between the two markers (P 0.0306) in cases of chronic infection (Figure 7).

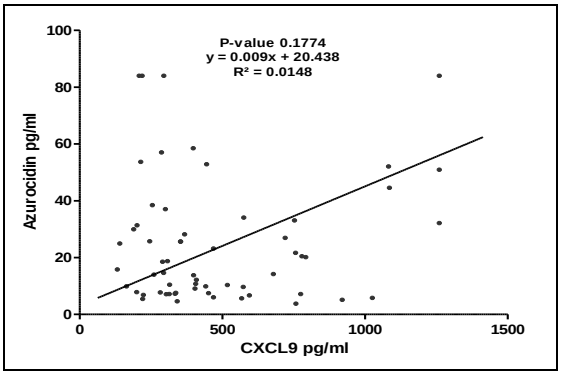

Fig.5: Correlation between total serum's levels of CXCL9 and Azurocidin in women with urinary tract infections.

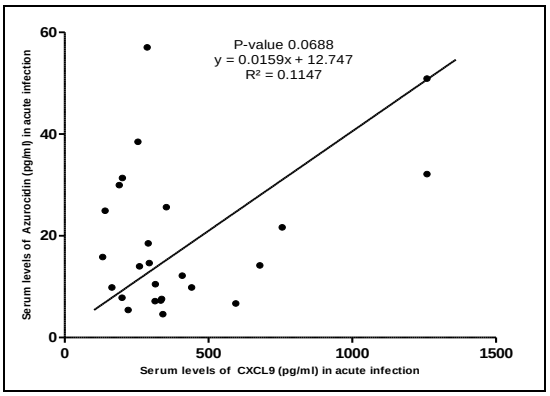

Fig.6: Correlation between serum's levels of CXCL9 and Azurocidin in women with acute urinary tract infections.

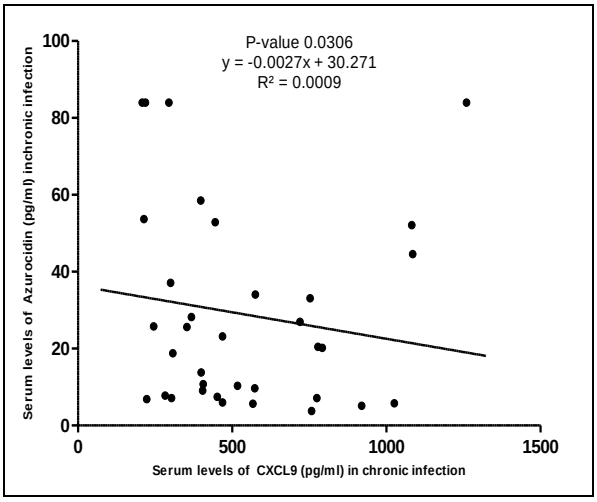

Fig.7: Correlation between serum's levels of CXCL9 and Azurocidin in women with chronic urinary tract infections.

DISCUSSION

Urinary tract infections are a common health concern, with bacterial infections being the most prevalent type. These infections can manifest in different ways, ranging from asymptomatic bacteriuria to more severe conditions like cystitis and pyelonephritis[15]. Asymptomatic bacteriuria is characterized by the presence of actively multiplying bacteria in the urinary tract without any accompanying clinical symptoms. This condition is more common in women, with prevalence rates ranging from 3-20% across different studies. In some cases, the presence of asymptomatic bacteriuria can lead to complications like pyelonephritis and cystitis if left undiagnosed and untreated[16].

In contrast, symptomatic urinary tract infections, such as cystitis, typically present with a range of clinical symptoms, including frequency and urgency of urination, burning pain during urination, abdominal discomfort, and turbid, odorous urine[17]. The distinction between asymptomatic bacteriuria and symptomatic urinary tract infections is crucial, as the former may not require immediate antimicrobial treatment, while the latter often necessitates prompt intervention to prevent potential complications, such as the development of sepsis [18].

Azurocidin, a neutrophil-derived antimicrobial protein, has recently emerged as a potential biomarker for urinary tract infections. Urinary tract infections are a significant health concern, affecting millions of individuals annually [19]. The delayed diagnosis and subsequent empirical broad-spectrum antibiotic treatment have contributed to the rise of antibiotic-resistant pathogens. Biosensors offer a promising solution to this problem, as they can provide rapid and accurate diagnosis of urinary tract infections, enabling targeted antibiotic therapy [20].

Recent studies have demonstrated the potential of azurocidin as a reliable biomarker for the diagnosis of urinary tract infections. Biosensors that can detect and quantify azurocidin levels in urine samples have shown high sensitivity and specificity in identifying uropathogens[10]. This rapid and accurate diagnostic approach can lead to better management of urinary tract infections, reducing the overuse of antibiotics and the emergence of antibiotic-resistant strains. While the development of azurocidin-based biosensors is a step in the right direction, there are still challenges that need to be addressed [21].

CXCL9, also known as Monokine induced by gamma interferon, is a chemokine that plays a crucial role in the immune response during urinary tract infections. It is involved in the recruitment of T cells, natural killer cells, and other immune cells to the site of infection, contributing to the clearance of invading pathogens [22].

The innate immune system mounts a rapid response to these infections, involving the production of antimicrobial peptides, activation of Toll-like receptors, and the release of various chemokines and cytokines.

CXCL9 is one of the key chemokines involved in this process. It is secreted by various cell types, including macrophages, epithelial cells, and endothelial cells, in response to the presence of pathogens or inflammatory stimuli. CXCL9 binds to the CXCR3 receptor, which is expressed on the surface of T cells, natural killer cells, and other immune cells [23].

The binding of CXCL9 to CXCR3 triggers a series of signaling cascades that lead to the activation and migration of these immune cells to the site of infection. This influx of immune cells, particularly T cells and natural killer cells, helps to eradicate the invading pathogens and resolve the infection.

CONCLUSION

Our research indicates that azurocidin and CXCL9 play a vital immunological function in women suffering from acute and chronic urinary tract infections; these biomarkers may assist in the diagnosis of women with untreated chronic UTIs.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article. This manuscript has not been previously published and is not under consideration for another journal.

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REFERENCES

1. Hughes T, Juliebø-Jones P, Saada L, Saeed K, Somani BK. Recurrent urinary tract infections in adults: a practical guide. Br J Hosp Med. 2021;82(12):1-11.
2. Olin SJ, Bartges JW. Urinary tract infections treatment/comparative therapeutics. Vet Clin Small Anim Pract. 2022;52(3):581-608.
3. Al Lawati H, Blair BM, Larnard J. Urinary tract infections: core curriculum 2024. Am J Kidney Dis. 2024;83(1):90-100.
4. Mody L, Juthani-Mehta M. Urinary tract infections in older women: a clinical review. Jama. 2014;311(8):844-854.
5. Kamei J, Yamamoto S. Complicated urinary tract infections with diabetes mellitus. J Infect Chemother. 2021;27(8):1131-1136.
6. Majeed HT, Aljanaby AAJ. Antibiotic susceptibility patterns and prevalence of some extended spectrum beta-lactamases genes in gram-negative bacteria isolated from patients infected with urinary tract infections in Al-Najaf City, Iraq. Avicenna J Med Biotechnol. 2019;11(2):192.
7. Aljanaby AA jabbar J, Gafil FAA. Effect of different antibiotics on aerobic pathogenic bacteria and urinary tract infection in Al-Manathera City, Iraq: a comparative study. Res Chem Intermed. 2013;39:3679-3687.
8. Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol. 2010;7(12):653-660.
9. Luu T, Albarillo FS. Asymptomatic bacteriuria: prevalence, diagnosis, management, and current antimicrobial stewardship implementations. Am J Med. 2022;135(8):e236-e244.
10. Bilsen MP, Treep MM, Aantjes MJ, et al. Diagnostic accuracy of urine biomarkers for urinary tract infection in older women: a case-control study. Clin Microbiol Infect. 2024;30(2):216-222.
11. Gabay JE, Almeida RP. Antibiotic peptides and serine protease homologs in human polymorphonuclear leukocytes: defensins and azurocidin. Curr Opin Immunol. 1993;5(1):97-102.
12. Tinel C, Devresse A, Vermorel A, et al. Development and validation of an optimized integrative model using urinary chemokines for noninvasive diagnosis of acute allograft rejection. Am J Transplant. 2020;20(12):3462-3476.
13. Aljanabi DRA. Estimation of sST2 and CXCR3 levels in serum of patients with chronic and acute pyelonephritis caused by E. coli. Cuest Fisioter. 2025;54(3):455-465.
14. Mohammad AK, Aljanaby ŸAJ. A Key Role of Interleukin 34 in Patients with Chronic Kidney Disease and Lupus Nephritis. Egypt J Med Microbiol. 2025;34(1):289-293.
15. Al-janabi DRA, Aljanaby AAJ. Comparison of Escherichia coli and Proteus mirabilis in patients infected with chronic pyelonephritis in Al-Najaf Governorate, Iraq. In: BIO Web of Conferences. Vol 139. EDP Sciences; 2024:6006.
16. Duncan D. Alternative to antibiotics for managing asymptomatic and non-symptomatic bacteriuria in older persons: a review. Br J Community Nurs. 2019;24(3):116-119.
17. Sendi P, Borens O, Wahl P, Clauss M, Uçkay I. Management of asymptomatic bacteriuria, urinary catheters and symptomatic urinary tract infections in patients undergoing surgery for joint replacement: A position paper of the expert group'Infection'of swissorthopaedics. J bone Jt Infect. 2017;2(3):154-159.
18. Soehnlein O, Lindbom L. Neutrophil-derived azurocidin alarms the immune system. J Leucoc Biol. 2009;85(3):344-351.
19. Ramaiah KB, Suresh I, Nesakumar N, Subramanian NS, Rayappan JBB. Urinary tract infection: Conventional testing to developing Technologies. Clin Chim Acta. Published online 2024:119979.
20. Edens HA, Parkos CA. Neutrophil transendothelial migration and alteration in vascular permeability: focus on neutrophil-derived azurocidin. Curr Opin Hematol. 2003;10(1):25-30.
21. Canney M, Clark EG, Hiremath S. Biomarkers in acute kidney injury: On the cusp of a new era? J Clin Invest. 2023;133(13).
22. Otto G, Burdick M, Strieter R, Godaly G. Chemokine response to febrile urinary tract infection. Kidney Int. 2005;68(1):62-70.
23. Tokunaga R, Zhang WU, Naseem M, et al. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation–a target for novel cancer therapy. Cancer Treat Rev. 2018;63:40-47.