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Bacterial Profile and Evaluation of Cxcl10 Level in Urine Among People Suffering from Urinary Tract Infections

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Abstract

A common bacterial infection that affects millions of people worldwide every year are infections of the urinary tract. The most frequent causes of urinary tract infections, both simple and serious, are *Escherichia coli* and *Klebsiella pneumonia*. *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, and saprophylococcus *saprophyticus.* In the early 1970s and late 1980s, chemokines were discovered. These positively charged cytokines have molecular weights ranging from 8 to 10 kDA. Since they control immune cell infiltration and inflammatory mediator release., they are essential to the immune system. The 10 kDa interferon-gamma inducible protein of the inflammatory chemokine IP-10, (IP-10) is too referred as C-X-C motif chemokine ligand 10 (CXCL10). Assess the concentration of CXCl10 in the urine of individuals with urinary tract infections. Biomarker (CXCL10), had high-level in-patient group compared to control group which suggests an inflammatory state in these patients. the Sensitivity % of (CXCL10) marker 55.6% and the Specificity 82.2% can be castoff as markers of inflammation, progression, and complications in patients with UTI.

البيانات البكتيرية وتقييم مستوى الكيموكاين 10 في الادرار بين الاشخاص الذين يعانون من التهاب المسالك البولية

علاق علي عبد الحسين، مي الجيلاوي، مسار رياض رشيد

عدوى المسالك البولية هي عدوى بكتيرية شائعة تؤثر على الملايين من الناس في جميع أنحاء العالم كل عام. تعتبر الإشريكية القولونية والكلبسيلة الرئوية الأسباب الأكثر شيوعًا لعدوى المسالك البولية، سواء كانت بسيطة أو خطيرة. تشمل أيضًا البكتيريا المسببة لعدوى المسالك البولية بروتيوس ميرابيليس، الزائفة الزنجارية، المكورات المعوية، المكورات العنقودية الذهبية، والمكورات العنقودية الرمية. في أوائل السبعينيات وأواخر الثمانينيات، تم اكتشاف الكيموكينات. هذه السيتوكينات موجبة الشحنة تتراوح أوزانها الجزيئية بين 8 إلى 10 كيلودالتون. نظرًا لأنها تتحكم في تسلل الخلايا المناعية وإطلاق الوسطاء الالتهابيين، فهي ضرورية لجهاز المناعة. بروتين IP-10 الكيموكيني الالتهابي ذو الوزن الجزيئي 10 كيلودالتون (IP-10)يُعرف أيضًا باسم الكيموكين ذو الدافع C-X-C (CXCL10). تم تقييم تركيز CXCL10 في البول لدى الأفراد المصابين بعدوى المسالك البولية. وُجد أن هذا المؤشر الحيوي (CXCL10) مرتفع في مجموعة المرضى مقارنة بمجموعة التحكم، مما يشير إلى حالة التهابية في هؤلاء المرضى. كانت حساسية مؤشر CXCL10 (55.6%) ، والخصوصية 82.2%، ويمكن استخدامه كعلامة على الالتهاب، وتقدم الحالة، والمضاعفات لدى المرضى المصابين بعدوى المسالك البولية.

1. Introduction

A common bacterial infection that affects millions of people worldwide every year are urinary tract infections (UTIs) (De Gaetano et al., 2023) . Bacteria are the main cause of UTIs, while viruses and fungi have also been shown to cause infections on occasion (Mancuso et al., 2023). Bacteria classified as Gram-positive or Gram-negative contribute to the development of infections. T The most frequent reasons of urinary tract infections, both simple and serious, are *Escherichia coli* and *Klebsiella pneumonia. Proteus mirabilis, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus,* and staphylococcus saprophyticus (Flores-Mireles et al., 2015) .UTIs typically affect the bladder. The bladder epithelium has strong barriers, and BECs have antimicrobial properties. Despite its benefits, UPEC frequently ignores BECs and the bladder epithelium(Wu et al., 2017) . Naturally, the cytokine response of the cells in response to an appropriate stimulus can serve as a means of both acquired and innate immunity to destroy or protect against pathogens (Pirdel and Pirdel, 2022). Early in the 1970s and late in the 1980s, chemokines were discovered. These positively charged cytokines have molecular weights ranging from 8 to 10 kDA. Since they control immune cell infiltration and inflammatory mediator release, they are essential to the immune system (Li et al., 2023). The activation, adherence, and recruitment of many types of white blood cells to areas of inflammation rely on chemokines and their corresponding receptors. Activation of type-1 helper (Th1) in an inflammatory environment leads to the production of IFN-γ and TNF-α. Consequently, several cells including lymphocytes, erythrocytes, keratinocytes, fibroblasts, neutrophils, monocytes, and endothelial cells release CXCL10. (Gao et al., 2020). Both IP-10 and CXCL10 refer to the same inflammatory chemokine, IP-10, which is an inducible protein of 10 kDa. IP-10 is linked to infectious agents such as fungi, viruses, bacteria, and parasites. (Hussein, 2021).

2. Material and Method

2.1. Ethical Approval

Before the sample was collected, written permission was obtained from each study participant, and all subjects involved in this experiment were informed. The University of Kerbala's College of Medicine's Publication Ethics Committee gave its approval to this work.

2.2. Study Design

Between October 2023 and January 2024, a total of 100 urine samples from both sexes (male and female) who were hospitalized and visited Al-Hussein Teaching Hospital / Laboratory Microbiology in Karbala, Iraq, with urinary tract infections were collected.

2.3. Clinical Samples

Usually, patients with UTIs provided the specimens for collection. Urine samples from midstream were collected and stored in sterile screw-cap containers. Following inoculation on culture media, the urine samples were cultured aerobically at 37°C for duration of 24 hours.

2.4. Phenotypic Identification of The Isolates

The isolated from pure colonies was phenotypically identified using GN cards (ID) and GN cards (ID) of the VITEK 2 system (Biomérieux, France), based on morphological, cultural, and biochemical properties(Bitew et al., 2017).

2.5. Measurement Chemokine 10 (CXCL10)

Chemokine 10 levels in urine were evaluated using ELISA research kits and a conventional sandwich-ELISA method (Human CXC -chemokine Ligand 10, CXCL10 ELISA KIT, BT LAB, China, CAT.No. E3800Hu).

2.5.1. Sandwich ELISA Method Principle

ELISA, when combined with an antibody, could be the most efficient immunosorbent method for detecting antigens. because their sensitivity is typically two to five times higher than methods where the antigen is directly attached to the solid surface. To detect antigen, test solutions containing antigen are incubated after a specific (capture) antibody is coated on micro titer plate wells. Following the removal of the unbound antigen, incubation is continued using a second antibody specific to the antigen that has been coupled to an enzyme (developing reagent). After removing the unbound conjugate, substrate is added. Following a second incubation, the degree of substrate hydrolysis is evaluated. There is a direct correlation between the amount of hydrolyzed substrate and the amount of antigen present in the test solution (Hussein, 2017).

2.5.2. Test Principle

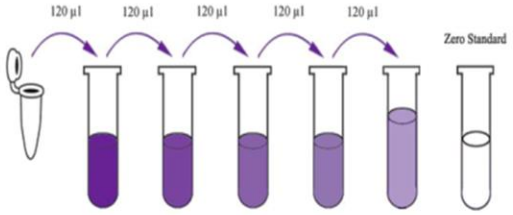
The plate has been pre-coated with a human CXCL10 antibody. Upon introduction into the sample, CXCL10 forms a binding interaction with the antibodies that have been immobilised on the surface of the wells. Subsequently, the sample's CXCL10 was attached by introducing biotinylated human CXCL10 antibody. The biotinylated CXCL10 antibody was subsequently bound by the addition of streptavidin-HRP. After incubation, any streptavidin-HRP that was not bound was eliminated using a washing process. Upon the addition of the substrate solution, the colour evolved in direct correlation to the concentration of human CXCL10. The process was halted by introducing an acidic stop solution, and the absorbance at 450 nm was subsequently quantified, consider specifying that the absorbance was measured using a microplate reader.

2.5.3. Assay Procedure

1. Before beginning the assay procedure, all of the reagents were prepared Fig.1.
2. Each sample well received 50µl of standards, 40µl of sample, and 10µl of biotinylated antibody; the standard well did not get biotinylated antibody since it was present in the standard solution.
3. Each well (standard and sample wells) received 50µl of Streptavidin-HRP reagent; the blank well received no additions. The wells were then covered with a seal plate, gently shaken, and incubated for 60 minutes at 37C.
4. The color developed as follows: 50µl of Substrate Solution A was added to each well first, followed by the addition of 50µl of Substrate Solution B to each well. Shake well to combine. incubated in a dark, 37°C environment for ten minutes in order for color to develop.

5. 50µl Stop Solution to each well had been added to stop the reaction (The blue color immediately became yellow.
6. After applying the stop solution, the optical density (OD value) was measured at 450 nm in less than ten minutes.

320ng/L	Standard No.5	120µl Original Standard + 120µl Standard Diluent
160ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
80ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
40ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
20ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
640ng/L	320ng/L	160ng/L	80ng/L	40ng/L	20ng/L

Figure 1: Concentration of Standards of cxcl10

2.5.4. Calculation of Results

The results were calculated according to the standard curve shown in Fig.2.

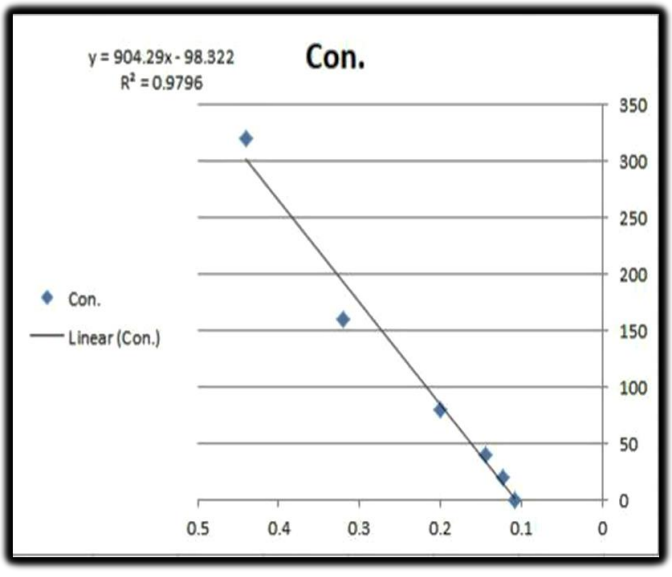


Figure 2: CXCL10 Standard Curve

2.6. Statistical Analysis

The statistical analysis of this study was conducted using IBM SPSS V27.0 software. Data were presented as the Mean ± standard Error of the mean. Chi Square was employed for comparison, and a ROC curve was utilized to identify the appropriate cut-off value for diagnosing the presence or absence of a disease.

3. Results

3.1. Demographic Characteristics of Study

The study samples as presented in Table 1conducted that People who live in the rural areas suffered from urinary tract infections more than those who live in urban areas, where the percentage in rural areas was (66.6) and in urban areas it was (33.3). People suffer from urinary tract infections more in the summer season than in the winter season, where the percentage in summer (68.8) and in winter was (31.1). The distribution of UTI cases based on age is as follows: 42.2% were in the (15-30) year age group, 26% were in the (46-60) year age group, 20% were in the (31-45) year age group, and 11.1% were in the >60-year age group.

Table 1: Demographic Characteristics of Study

Variables		Patients	Percentage
		Count	%
Sex	Male	7	15.6
	Female	38	84.4
	Total	45	100
Residency	Urban	15	33.3
	Rural	30	66.6
	Total	45	100.0
Season	summer	31	68.8
	Winter	14	31.1
	Total	45	100.00
Age	15-30	19	42.2
	31-45	9	20
	46-60	12	26.6
	>60	5	11.1
	Total	**45**	**100.00**

3.2. Bacterial Isolation Percentage

The microbiology data obtained for the current study showed that the Gram-positive bacteria were 23 isolates (51.11%) represented by *Coagulase -ve staphylococcus* species (20%) were the commonest isolated genera, followed by *Staphylococcus haemolyticus* (8.8%), *Staphylococcus aureus* (8.8%), *Staphylococcus epidermidis* (4.4), *Staphylococcus hominis* (2.2%), *Enterococcus faccalis* (2.2%), while 22 isolates (48.88%) were Gram negative bacteria represented by *Escherichia coli*.as in Fig.3.

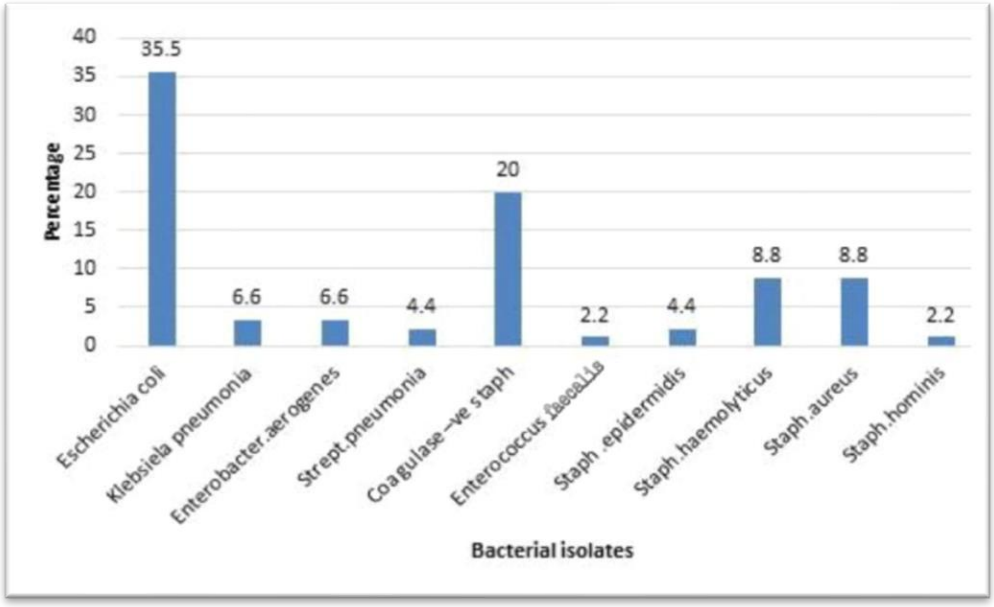


Figure 3: Percentage of Bacterial Isolates Studied

3.3. Study of The Immune System

3.3.1. Level CXCL10 in Patients and Control Group

The results summarized in Table 3. The analysis revealed a statistically significant difference (P < 0.001*) between the total number of controls and the total number of patients.

Table 3: Level CXCL10 In Patients and Control Group

Parameter	CXCL-10 level mean ± SE (pg/ml)		Probability
	Patients	Control	
CXCL10	137.02 ± 6.42	107.28 ± 4.99	P < 0.001*

3.3.2. ROC Curve and AUC Analysis for The CXCL10 In Patients with Urinary Tract Infection Compared to Control Groups

For the CXCL10, ROC curve and AUC analysis were carried out for patients in comparison to the control group. Platelets perform well in predicting such cases, according to the results of the receiver operating curve (ROC) curve and AUC analysis for CXCL10 as a diagnostic parameter; the data are shown in Table 4 and Fig.4. For CXCL10 levels, at a level of 131.795, the sensitivity is 55.6% and the specificity is 82.2%. The AUC's p-value was statistically significant, at less than 0.001. Youden's J statistics were used to validate the Sensitivity and Specificity results for the given values.

Table 4: Receiver operating characteristic curve showing sensitivity and specificity of CXCL10 in patients with Urinary tract infection compared to control groups.

Test Result Variable(s)	**CXCL10**
AUC	71.4%
Sensitivity %	55.6%
Specificity %	82.2%
Youden index	0.378
Cut-off points	131.795
CI (95%)	0.607-0.822
PPV	76%
NPV	65%
Accuracy	70%
P value	**<0.001[S]**
S= Significant, PPV= Positive protective value, NPV= Negative predictive value, AUC= Area under curve, CI= confidence interval	

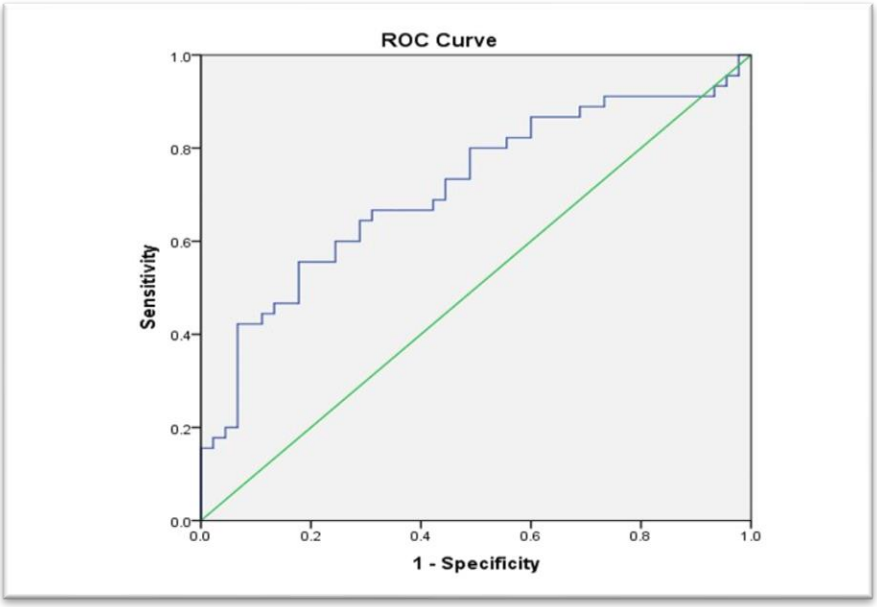


Figure 4: Receiver operating characteristics (ROC) curve analysis was used to compare the levels of CXCL10 in patients with urinary tract infection to those in control groups.

4. Discussion

Urinary tract infections are the most frequent Microbial diseases caused by bacteria encountered by health care professionals (Spaulding and Hultgren, 2016). In current study noticed the Females had a higher prevalence and incidence of urinary tract infections than males The closer distance between the urethra and the anus, the broader and shorter urethra, sexual activity, incontinence, and the surface of the vagina has a lower acidity level and unclean living conditions (Quaiser et al., 2015). Males are more prone to UTIs after the age of fifty, when they are also more likely to experience prostate issues as a result of less prostatic fluid. Additionally, a larger prostate gland may restrict and obstruct urine flow, increasing the risk of infection. According to Nicolle's (2008) observations, males who are not circumcised also have a higher likelihood of contracting UTIs because germs can more readily accumulate in the folds of the additional skin of the penis (John et al., 2016). There is evidence from studies that show how a community's socioeconomic status and geographic location might affect the prevalence of urinary tract infections (UTIs) (Ayoyi et al., 2017).

A higher proportion of individuals residing in rural regions experienced urinary tract infections, with 66.6% in rural areas compared to 33.3% in urban areas. Current study agreement with results of (Seifu and Gebissa, 2018) in Shashemene referral hospital, Ethiopia ,where the percentage of prevalence of urinary tract infections in Urban area (37.1)% ,and in Rural (62.9)%.

Individual-level risk factors for urinary tract infections (UTIs) include sexual activity, female sex, prior history of UTIs, and possibly insufficient fluid intake or dehydration (Simmering et al., 2018) . There may be environmental risk factors for UTIs in addition to risk factors at the human level. Indeed, single-center studies have shown that UTIs are more common during the summer, with a seasonal increase in incidence (Czaja et al., 2007).

In the study finding,the percentage people who sufferd UTI in summer season was higher agreemant with results (Simmering et al., 2018). Studies on infections in a range of age groups were few (Alwan et al., 2023). The present study showed that among males and females, the age group of 15-30 years had the highest number of infected people ,where the percentage 42.2% . According to results (Almukhtar, 2019),58.4% of patients were in the age range of 21-30 years agree with current study. Numerous studies revealed that the Enterobacteriaceae family was the most common cause of urinary tract infections in people (Odoki et al., 2019) These studies are in agreement with current study. In current study showed the E.coli was most common pathogen in UTI,were the percentage of *E.coli* was 35.5%.Result of (Alwan et al., 2023). agreement with current study.Results of disagreement with current study where Klebsiella spp. was the most prevalent microorganism in UTI patients. Cytokines and chemokines produced by the detrusor smooth muscle cells during the inflammatory process in the bladder wall may be discharged into the urine (Yu et al., 2022)According to recent research, a wide range of nonhematopoietic cells, such as urothelium and detrusor cells, express chemokines and their receptors(Bouchelouche et al., 2006). Chemokines are used for more than only inflammatory cell infiltration.

The secreted proteins in the chemokine superfamily have molecular weights between 8 and 10 kDa (Ragnarsdóttir and Svanborg, 2012)and work by interacting with G protein-coupled receptors that are found on

glycosaminoglycans in endothelial cell layers. Chemokines are known for their promiscuity, meaning that they can connect to multiple receptors simultaneously, and that receptors can bind to multiple chemokines(Ragnarsdóttir and Svanborg, 2012) . The result of current study showed elevated CXCL10 level in UTI patient.The result of (Tyagi et al., 2016)showed elevation level of CXCL10 corroborates with current study. No statistically significant differences in the urinary levels CXCL10 were discovered in convalescent phases or, the patients in the acute UTI, or in the healthy controls (Gorczyca et al., 2014).

5. Conclusions

Coagulase-negative staphylococcus was the predominant pathogen among the gram-positive isolates, while Escherichia coli was the most common pathogen among the gram-negative isolates, according to the bacteriological profile of urinary tract infections (UTIs). The biomarker investigation revealed that the patient group had significantly higher levels of the biomarker (CXCL10) compared to the control group, indicating the presence of an inflammatory condition in these patients. The marker CXCL10 has a sensitivity of 55.6% and a specificity of 82.2%. It can be used as an indicator of inflammation, progression, and complications in patients with UTI. Further studies with large sample size should be conducted to confirm the significant association of CXCL10 as indicator of severity UTIs patients. assessment the concentration of CXCl10 in the serum of individuals with urinary tract infections and compare concentration in urine.

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