Determination of Cathelicidin in UTI patients of Basrah province

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Abstract

Cathelicidin is important components of the innate defense in the urinary tract. The aim of this study was to characterize whether these anti -microbial peptide are important for developing urinary tract infections (UTIs). This aim was investigated by comparing blood urinary peptide levels of UTI patients' infection to those of controls. A case- control study was conducted at Basrah province (Basrah general hospital and Al-Sadr Educational hospital) during the period from 18 November 2018 to 15 April 2019. 60 patients with confirmed UTI and 30 healthy controls without UTI. Plasma and urine levels of cathelicidin were determined using an enzyme linked immunesorbent assay (ELISA) kit. The mean concentration of anti-microbial peptide cathelicidin (AMPccl37ng/ml) was highly significant P≤0.001, P≤0.000 in urine and sera respectively, with no significant difference correlation between the type of bacterial infection and concentration of ccl37 in urine and sera. Conclusion Urinary cathelicidin is microbial markers that may assist the diagnosis of UTI in woman.

Keywords: UTI, Cathelicidin, AMPs, ELISA

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1-Introduction

Urinary tract infection (UTI) is a term that describes any infection involving any part of the urinary tract that in cludes the upper (kidney and ureter) and lower (bladder and urethra) tracts[1].The mechanisms of the immune system comprise innate and adaptive immunity are activated by the invasion of microbial pathogens[2].

The defense of urinary tract infection may depend primarily on specific soluble cellderived epithelial mediator, one of which is inducible antimicrobial peptides, such as α-, βdefensins and cathelicidin[3].

Antimicrobial peptides (AMPs) are short peptides with positive charges that are secreted by both epithelial and hematopoietic cells that interact with bacterial membranes and may be chemotactic for certain immune cells[4].Cathelicidin plays a major role in the bactericidal cycle and in maintaining the integrity of the urinary tract. In addition, certain types of cells can induce the development of chemokines and cytokines [5,6,7]. The sources of cathelicidin are circulating neutrophils, renal cells, and uroepithelial cells in urinary tract. In previous studies, positive correlation has previously been observed between cathelicidin level and pyuria [8]. Human cathelicidin (LL-37) is encoded by the CAMP gene and expressed in neutrophils, myeloid bone marrow cells and epithelial cells in circulation. It has antimicrobial activity against both bacteria and viruses, and serves as a chemoattractor for neutrophils and monocytes by communicating with them their N-Formylmethionone-leukocyte-phenylalaninefMLP-receptors.

Cathelicidine is expressed in the proximal tubule and urothelium of the renal pelvis and ureter. Uroepithelial ce lls secrete cathelicidine into the urinary space (tubular lumen) when they are infected with uropathogenic *E. coli [*8, 9]

The objective of this study was to identify cathelicidin as markers of UTI in woman.

2-Materials and Methods

Samples collection The urine and blood samples were collected from (315) suspected urinary tract infection (UTI)patients with age (10-55) year from 18 November 2018 to 15 April 2019 in Basra province (Basra general hospital and Al-Sadr Educational hospital).Patients were separated according to their residency ,marital status ,age and type of bacteria. Urine sample from each patients were considered as a positive UTI patients

after cultivation for bacterial isolation and general urine examination (GUE). The positive patients were included in present work, in addition urine and blood samples were collected also from control people who have a negative result in GUE and cultivation.10ml urine and 5ml of blood were collected from each patient and sera were separated by centrifugation of blood for20 minutes at 1000xg.

Patients

A case- control study was conducted on 60 patients with confirmed UTI and 30 healthy controls were healthy without UTI. The level of high-sensitivity C-reactive protein (hsCRP) was measured by ichroma™ using kit supplied by Boditech. Plasma and urine levels of cathelicidin were determined using an enzyme linked immunesorbent assay (ELISA) kit is commercially available by My bio source. The experiments were conducted and analyzed as factorial experiments with three replications, and compared of differences between the averages by using the less significant difference (LSD).

Statistics

SPSS for window (version 16.0) was used for statitical analyses. Students'-test and $\chi 2$ were used to compare the continuous variables (when normally distributed) and proportions between the patients and controls, respectively. The levels of LL-37 were not normally distributed and were
compared between the cases and controls by Mann- Whitney U test. Logistic and linear regression was performed with UTI (logistic) and log of LL-37 level (linear); these were the dependent variables. Spearman correlation (non-parametric) was performed between the
plasma and urine levels. $P < 0.05$ was considered statistically significant.

3-Results

3-1: Causative bacteria in UTI patients

In Sixty of total patients with uropathogenic bacteria the isolated type were: 42 strains (69.9 %) were gram negative and 18 strains (18%) were gram positive. Summarily, *Escherichia coli* was (n=37) the most common of gram negative and the second *Staphylococcus aureus* was (n=5), whereas the gram positive bacteria, *Klebsiella* was the most often isolated (n= 18) (Figure 1).

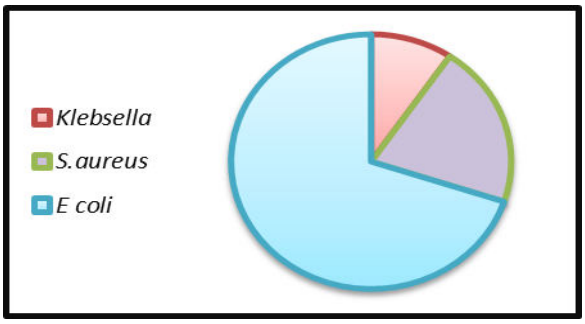

Figure (1) causative bacteria in studied UT patients

3-2: Distribution of patients according to marital status.

Figure 2 showed the demographic distribution of the marital status groups in UTI patients, 73% were married, and meanwhile 27% were unmarried, with highly significant differences ($P \leq 0.000$)

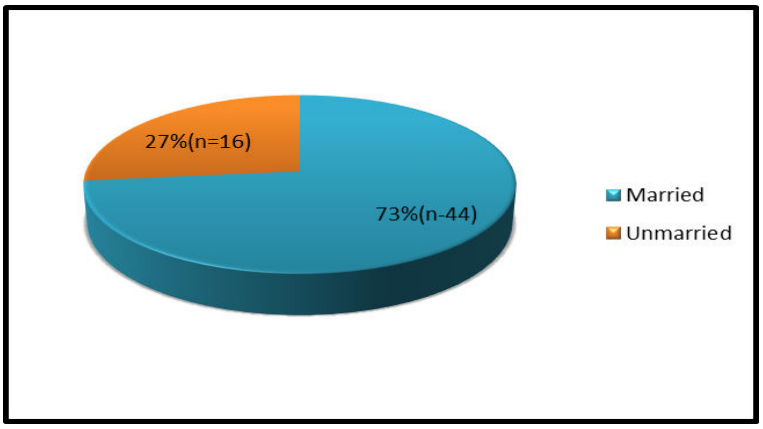

Figure (2) Distribution of patients according to marital status

3-3: Distribution of patients according to residence.

Present data revealed that the distribution of UTI patients were highest in rural regions 53%, while 47% were in town, without any statistical differences p≤0.606 ,figure 3 .

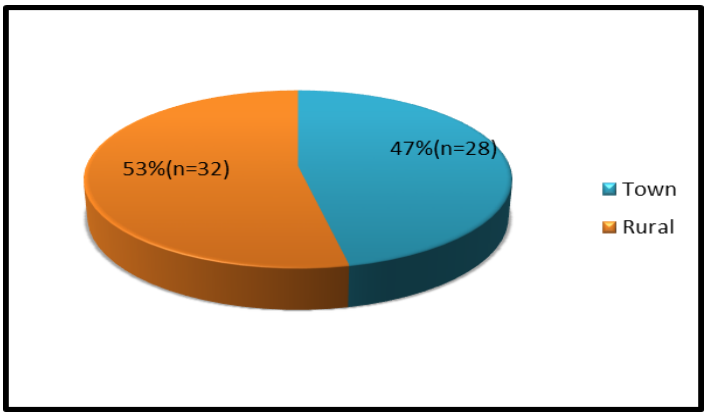

Figure (3) Distribution of patients according to residence.

3-4: Distribution of patients according to age groups

Recent work documented the highest percentage with ages (20-29), whereas the lowest percentage with UTI patients with (50-59) age with highly significant differences P ≤ 0.01, table 1and figure (4).

Table (1) Distribution of patients and control according to age

Groups	Age range groups				
	10-19	20-29	30-39	40-49	50-59
Patients (n=60)	9 (15%)	25 (41.7%)	8 (13.3%)	12 (20%)	6 (10%)
Control (n=30)	2 (6.7%)	5 (16.7%)	13 (43.3%)	9 (30%)	1 (3.3%)

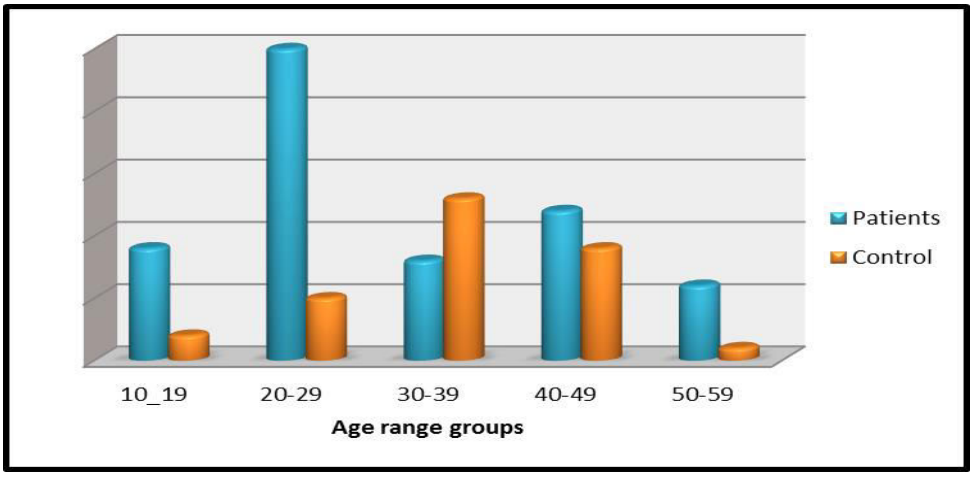

Figure (4) Distribution of patients and control according to age groups.

3-5: Determination of cathelicidin in urine and serum.

Cathelicidin concentration was measured into two studied groups patients and control in urine and serum. The results showed highly significant differences (p< 0.000) in concentration of parameters under study between the two groups, figure (5)and table (2).

Table (2) Cathelicidin concentration in urine and serum of patients and control group

Groups	N	Mean of ccl37 ng/ml		Range		SD		SE		P value	
		urine	Serum	urine	serum	urine	serum	urine	serum	urine	serum
Patients	60	2.074	0.914	0.222-2.074	0.05-7.887	2.169	1.641	0.280	0.212	0.000	0.0
Control	30	0.529	0.251	0.101-0.882	0.069-0.644	0.148	0.101	0.027	0.018		

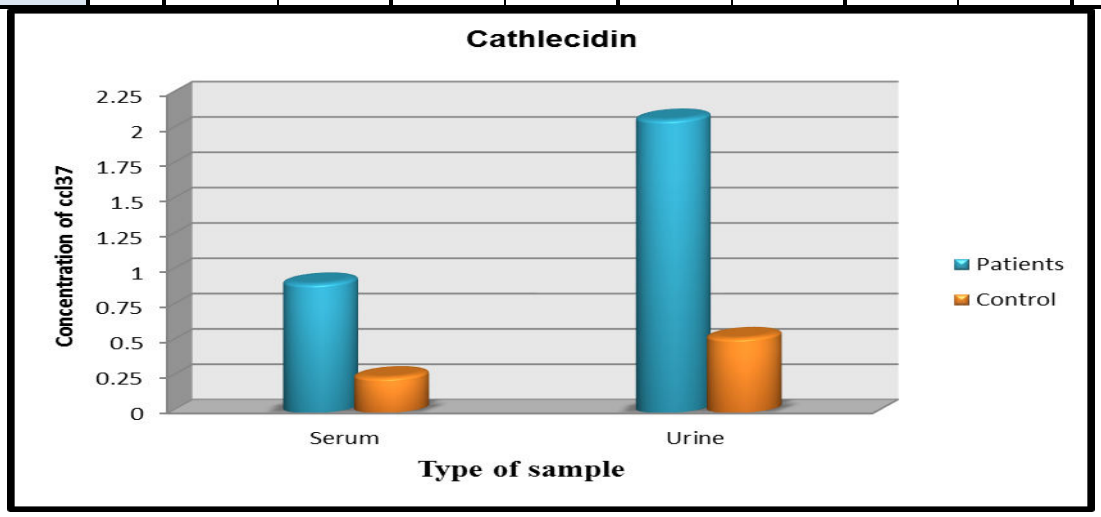

Figure (5) level in urine and serum of patients and control groups

3-6: Cathelicidin level in urine and serum according to type of bacteria.

Present study showed that there were no significant differences between the type of bacteria in both serum and urine with concentration of cathelicidin, p≤ 0.881 and p ≤ 808 respectively, table(3) and figure (6).

Table (3) Cathelicidin levels in urine and serum according to the type of bacterial infection.

Bacterial type	Urine				Serum			
	Mean	SD	SE	P value	Mean	SD	SE	P value
E.coli (n=36)	2.136	2.582	0.430	0.881	0.982	1.789	0.298	0.808
S.aureus (n=19)	2.024	1.540	0.353		0.839	1.595	0.366	
Klebsilla (n=5)	1.613	0.656	0.293		0.480	0.377	0.169	

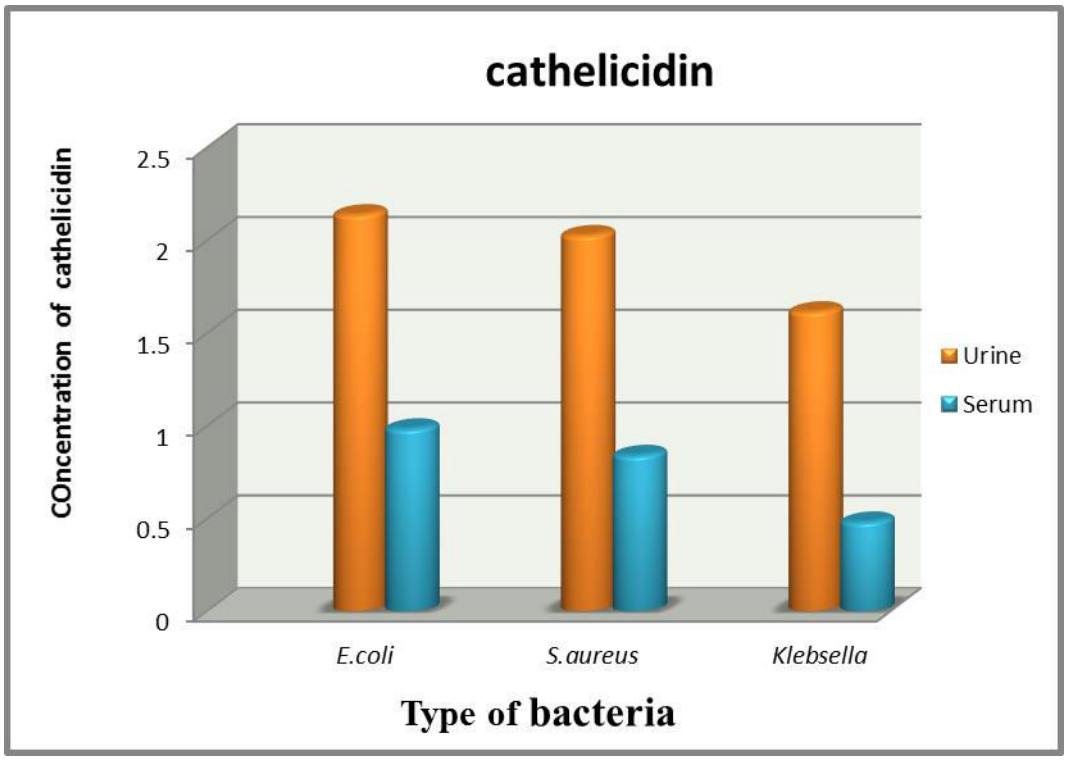

Figure (6) Cathelicidin levels in urine and serum according to the type of bacterial infection .

4- Discussion.

4.1: Distribution of the study groups according to bacterial species

Although UTI's etiology has changed over the last few years.E coli has been shown to be the most common urinary pathogen encountered in this study.These results are well correlated with many studies conducted in different countries, either regionally or internationally.

A study in Egypt found that the most common UTI-causing organisms were *E.coli* 47.5%, *proteus* species

8.4%,*klebsiella* species 17.1% and *pseudomonas species* 10.4%[10]. Strom *et al* . noted that causative uropathogens included *E.coli86%* and *staphylococci* 4%,*klebsiella* species 7.4% and *proteus species* 6.2% [11].

A communityacquired UTI study in Jordon found that *E. coli* was the most frequently isolated organism and oc curred in 55% of patients [12].

According to a study of 49 patients with UTI and 25 apparently controlled in the province of ThiQar / Iraq, the most common bacteria causing UTI were *Escherichia coli* 53.06%; 18.37% Pseudomonas aeruginusa.14.29% *Klebsiella pneumonia;* 8.16% *Proteus* and 6.12% *Klebsiellaoxytoca*[13].

Another study of communityacquired UTI patients showed that the most frequently recovered microorganism was *E. coli* 82%, *Klebsiella* species 7.3 % , *Proteus* species 6.2 %[14

The distribution of identified uropathogen in a study in Gaza city/Palestine was *E.coli* 30% followed by *Klebsiella* species 21%, *Proteus* species 15.3%, *Psedomomas* species 4.7% and *Staphylococci* 2.4% [15]

According to a study conducted in northwestern Iran, *E.coli* was the most common etiological agent of UTI 74. 6%, followed by *Klebsiella* species 11.7%, *Staphylococcus saprophticus* 6.4% and *Pseudomonas aeraginosa* 2. 2% [16].

4.2: Distrubition of patients according to marital status.

All subjects in the current study were female divided into two groups; married 73%, unmarried 27%. The preva lence and frequency of UTI in married women is higher, which is likely due to several clinical factors like anat omical differences, hormonal effects, and behavioral patterns [17].

In this study, UTI were confirmed by symptoms, urinalysis result and culture results (>100,000 colony forming unit/ml) .

4.3: Distribution of UTI patients according to residence and age.

The present study showed that the highest proportion of UTIs was detected among women aged 20-35 years with high parity. This is in line with Krcmery *et al* study[18] who said that.

Women's risk factors for UTI include: sexual intercourse, earlyage first UTI, and maternal history of UTIs. UT I is commonly seen in the current study as the gestational age rises, which coincides with Sheik *et al* results [19] .
UTIs are widely spread infections seen in hospital settings and the second most common infections seen in the general population [20].
In another study, the prevalence of UTI among the pregnant women studied was 47.4%. These results were al most consistent with those of research workers in other countries, with minor differences, This could be due to changes in the environment, the social habits of the community and the standards of personal hygiene and education [21]. Similar studies in our region have shown a prevalence of 38.0 % in Iraq, 28.5 % in Pakistan,[22] and 10.6 %in Turkey.[23,18] .

4.4: Mean concentration of cathelicidin in urine and serum of UTI patients

Present data indicated an increasing in the concentration of ccl37 (AMP) compared to control, this results in agreement with [24] Babikir *et al* whom showed a high significant concentration of cathelicidin in the urine of UTI patients

Present study are consistent with reported results of[25] whom said that urinary ll37 was significantly higher during infection than post-infection, while post-infection ll-37 levels were significantly lower in UTI patients than in control patients

Similar results have been shown by Chromek *et al*[6] . They researched urinary cathelicidin in healthy children as well as in children with UTI and found that ccl-37 is expressed in the urinary tract.

Chromek et al referred that the direct contact with microbes stimulates urinary epithelial cells significantly increase cathelicidin production and secretion, shielding the urinary tract from adherence

During 2019 Awadallah et al in Egypt confirmed that there was increasing in urine ll37 in UTI patients this is accordance with present study [26].

No significant differences in the amount of urinary ll37 between the UTI children and the control group were observed in another study[27]. Present study not recorded any correlation between the type of bacterial infections and the concentration of cll37 in urine and serum, and this results was in the same with results of Vander *et al* 2015)[28] whom showed that cathelicidin may be produces with or without bacteremia.
Unlike to recent work Caterino *et al*.2015 [29] indicated that cll37 was not increased with positives cultures.
In addition Hachamdiglu *et al*.2016 [30] found that the ll37 urinary levels in the children with UTI showed no significant differences when compared with control groups, and they proposed that.

Conclusion

Urinary tract infection raises cc L-37 rates
The increased level found was not only in urine in patients, but was also detected in patients' plasma during the time of urinary tract infection. Detection of elevated LL-37 levels can help to differentiate subjects with suspected UTI .cc L-37 could therefore serve as a good marker for the diagnosis of UTIs

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