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Detection of the Serum Level of C3 and C4 Among Patients with Urinary Tract Infection

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Abstract:

The current study aims to determine the prevalence of bacterial urinary tract infection (UTI) and estimate the serum levels of C3 and C4 in UTI patients. This study had been included 350 samples (blood and urine) obtained from outpatients suspected of having UTI from Baquba-Teaching Hospital. The detection of causative Bacteria was done using an automatic VITEK 2 system. A sample of 350 urine was taken, and after diagnoses, it found that only 205 (58.5%) had UTI; 123 (as 60 %) with a positive culture of bacteria, and 82 (as 40%) with negative culture of bacteria. Among the Gram-negative bacteria, mostly isolated were Escherichia coli 44 (resembles 35.7%), K. Pneumonia 18 (resembles 14.6%), P. aeruginosa 8 (resembles 6.5%) 2 (resembles 1.6 %) which was diagnosed as Burkholderia cepacia. Gram-positive organisms included 51 (as 41.4%) of total positive results, divided into 37 isolates (30.08%) of Staphylococcus spp. and 14 isolates (11.3%) of Enterococcus spp. Immunological parameters, which were included the determination of serum levels of C3 with C4 showed that lower levels of both C3 and C4 in UTI patients compared with the control group (18.48±1.3 versus 19.4 ± 2.9pg/ml) and (0.739±0.257 versus 1.205±0.259 pg/ml) respectively. In conclusion, E. coli, K. pneumoniae and S. aureus were found to be the highly isolated bacteria in UTI cases, with decreased levels of C3 and C4 in serum in UTI patients.

Keywords*: *uropathogens, E. coli, C3, C4, ELISA, UTIs, Diyala

1.Introduction

Infection of urinary tract (UTI) is considered the fourth highly common healthcare-associated infection that affects humans. It resembles a major public health issue with a significant financial cost [1]. regarding morbidity and mortality due to ascending renal infections, UTI is a significant public health cause [2]. UTI is characterized by presence of pathogenic microorganisms in UT besides some serious symptoms like urgency and frequency of urination [3]. The bladder

infection is called cystitis, while pyelonephritis is the infection of renal pelvis [4]. UTI includes either symptomatic features of infection with the invasive bacteria resulting in UT inflammation, or asymptomatic bacterial colonization of UT along with the urinary bladder, ureters, kidneys, and urethra [4, 5]. Thus, UTIs are classified as complicated or uncomplicated based on the type or duration of antimicrobial therapeutics of the patient [5]. UTI is mainly common in females, of one out of every three women in the ratio [6]. The urethra is where UTI-causing bacteria usually enter the bladder and can also spread through the blood or lymphatic system to the other parts of the urinary system [7].

UTI is greatly caused via Gram-negative bacterial agents like *Klebsiella* species, Escherichia *coli*, *Pseudomonas aeruginosa*, *Proteus species* and *Acinetobacter* species [8]. Among the Gram-positive bacteria, *Enterococcus* species and *Staphylococcus saprophyticus* are the most common predicted bacteria responsible for UTI [9].

The innate-immune defence has a critical role in antimicrobial elimination, destroying pathogens, determining localization and challenge, and facilitating adaptive immune response [10]. Furthermore, immune system cells, such as phagocytes and antigen-presenting cells, mediate the immune response by expressing PRRs and PAMPs on the surface of these bacterial species [11]. The complement system, which is considered an essential part of the innate system, is a specific network in plasma and its activation results in clearing bacteria by immune cells besides destroying them bacteria directly by some mediators [12]. So, the current study aims to determine the prevalent uro-pathogenic bacteria causing UTI, besides measuring serum levels of C3 and C4 in patients who had bacterial UTIs**.**

2. **Methodology**

This cross study was performed At Baquba teaching hospital from August in 2020 to February 2021. All specimens (blood and urine) from both sexes were included in the study .A sterile universal container was used to collect mid-stream urine specimens, which was then processed according to standard microbiological techniques (13). All urine specimens were examining by microscope and only specimens had infection cultured on blood and MacConkey agar plates and bacterial isolates were diagnosed by VITEK-2 system [14]. Patients' blood samples (5ml) were collected and used to obtained serum by placing them in a clean plain tube with gel and allowing them to clot for 30 minutes at 37oC before centrifugation. The tubes were centrifuged for 5 minutes at 6000 rpm, the serum was collected, and the C3 and C4 levels were measured by sandwich ELISA technique made by Bio-Rad Germany company [15].

3. Results

Bacterial isolation and identification

A total of 350 urine specimens were collected; only 205 (58.5%) were confirmed to have UTI infection depending on symptoms and microscopic examination; 123 (60 %) of positive culture of bacteria, and 82 (40 %) of negative culture. Out of 205 patients, 84 (40.9%) were male, while 121(59.02%) were female. The group of 31-40 years ages ranked the highest rate of infection (17.7%) of 35 patients with urinary tract infection, followed by the age group 41-50 years with 30 (14.6%) patients, and the age group of 10-20 years was the least with 9 (4.3%) only as showed in Table (1).

Table (1): Distribution of UTIs according to age range

Ages (years)	Bacterial growth				Total	
	G - ve		G + ve			
	No.	%	N0.	%		
10-20	5	2.4	4	1.9	9	4.3
21-30	6	2.9	5	2.4	11	5.3
31-40	21	10.2	14	6.8	35	17.07
41-50	15	7.3	15	7.3	30	14.6
51-60	9	4.3	9	4.3	18	8.7
61-70 and more	16	7.8	4	1.9	20	9.7
Total	72	35.1	51	24.8	123	60.0

The Gram-negative bacterial cultures ranked the prevalent UTI pathogens in 72 isolates (58.5%), whereas the Gram-positive cultures were in 51 isolates (as 41.4%). Gram-negative bacteria were most common, 72 (58.5%) of the total isolates. *Escherichia coli* was 44 (35.7%); while 18 (14.6%) were *Klebsiella pneumoniae*, 8 (6.5%) was *P. aeruginosa*, and only 2 (1.6%) were *Burkholderia cepacia*, as shown in Table (2).

Gram-positive organisms constituted 51 (41.4%) of total isolates, including 37 isolates (as 30.08%) of *Staphylococcus* besides 14 isolates (as 11.3%) of *Enterococcus spp*. *Staphylococcus Spp.* of two main groups including 17 (13.8%) *S. aureus*, with 20 isolates (of 16.2 %) as coagulase Negative *Staphylococcus* (CONS) strains.

Table (2): Distribution of UTI patients according to gender and culture types.

Culture type	Bacterial Isolates			Total	
				No.	%
G – ve bacteria	*E. coli*			44	35.7
	Klebsiella pneumoniae			18	14.6
	Pseudomonas aeruginosa			8	6.5
	Burkholderia cepacia			2	1.6
G + ve bacteria	*Staphylococcus Spp.*	*Staph. aureus*		17	13.8
		CONS	*Staph. haemolyticus*	7	5.6
			Staph. epidermidis	3	2.4
			Staph. vitulinus	1	0.8
			Staph. sciuri	4	3.2
			Staph. homins	3	2.4
			Staph. lentus	1	0.8
			Staph. warneri	1	0.8
	Enterococcus spp.	*Enter.faecalis*		7	5.6
		Enter.faecium		5	4.06
		Enter. avium		2	1.6

C3 and C4 detection among UTI patients

Sera serum levels of C3 and C4 in UTI patients were determined. The Immunological findings showed that serum C3 concentrations of UTI patients were lower than those of the healthy control group (18.481.3 versus 19.4 2.9 pg/ml), as shown in figure (1). The obtained immunological results clarified patients' C4 concentrations were highly lower than in the healthy control group (0.7390.257 versus 1.2050.259 pg/ml).

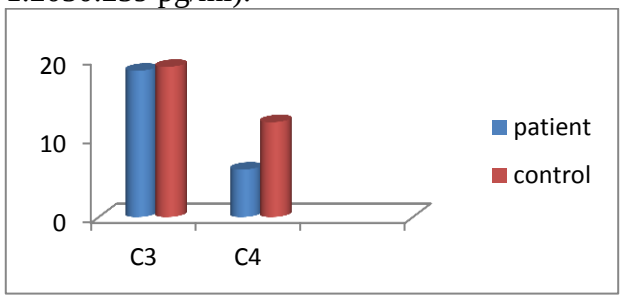

Figure (1): Levels of C3 and C4 in patients with UTI and the control group

The gender distribution in patients and controls is included (figure 2). The numerical ratio between females and males in the current study, the female number, was 71, while male number was 52. Mean± S.D of C3 concentrations in female patients. was18.696±1.439, while it was 18.200±1.240 in male patients. The results showed no significant difference between the healthy control and female patients and significant between the healthy control and female patients, and significant between difference between the healthy control and female patients and significant with male patients, the *p* -value of them 0.075 and 0.007, respectively. The mean± S.D of C4 concentration in female patients was 0.785±0.224, while in male patients, it was 0.676±0.286 and it was 1.205±0.259 in controls, with high significance at p-value 0.0001.

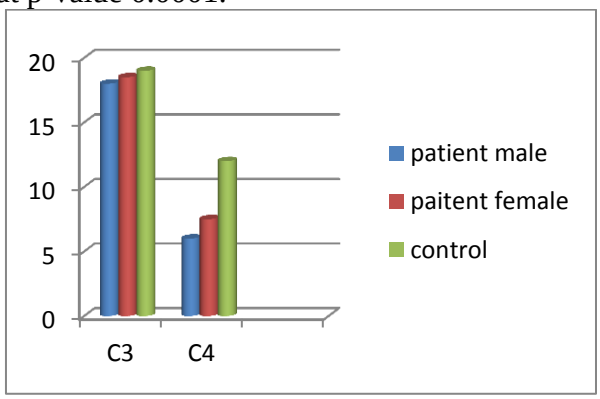

Figure (2): Comparison of the level of C3 and C4 between gender of patients control.

The C3 level of different age classes is shown in Table (3) as the study group has been divided into six groups based on the age range: groups A (10-20 years) old, groups B (21-30 years) old, groups C (31-40 years) old, group D (41-50 years) old, groups E (51-60 years) old, and groups F (61-70 years) old.

Table (3): The comparison C3 level among different age groups of patients and healthy controls.

Group / Age		control		Patients		P.value
		NO.	Mean ± SD	NO.	Mean ± SD	
A	10-20	6	21.200±2.069	8	17.438±0.903	**0.005****
B	21-30	13	19.200±1.740	12	18.158±1.621	**0.135***
C	31-40	21	19.771±1.386	33	18.923±1.216	**0.026****
D	41-50	12	18.276±5.587	30	18.600±1.173	**0.761***
E	51-60	4	18.875±1.065	22	17.891±1.234	**0.164***
F	61-70 and more	3	19.300±2.406	16	18.906±1.757	**0.808***

according to the age range, the age six types showed significant relation to the level of measured serum C4 of both patients and healthy groups (table 4).

Table (4): comparison of the level of C4 between patients and healthy control according to age groups.

Group / Age	control		Patients		P.value
	NO.	Mean ± SD	NO.	Mean ± SD	
10-20	6	1.100±0.297	8	0.633±0.361	**0.022****
21-30	13	1.264±0.232	12	0.733±0.287	**0.0001****
31-40	21	1.143±0.243	33	0.743±0.250	**0.0001****
41-50	12	1.278±0.239	30	0.826±0.140	**0.0001****
51-60	4	1.407±0.383	22	0.717±0.270	**0.031****
61-70 and more	3	1.043±0.160	16	0.655±0.323	**0.021****

Table (5): Statistical significance of C3 and C4 levels in patients infected with UTI patients

C3	**Control**	**Patients**	
		G-ve	**G+ve**
NO.	59	70	**51**
Mean ± sd	19.402±2.9176	18.829±1.4470	**18.002±1.1120**
P.value	Control vs G-ve		**Control vs G+ve**
	0.146*		**0.002****

C4	**Control**	**Patients**	
		G-ve	**G+ve**
NO.	59	70	**51**
Mean±sd	1.205±0.259	0.760±0.235	**0.710±0.284**
P.value	Control vs G-ve		**Control vs G+ve**
	0.0001**		**0.0001****

As shown in Table (5), a low level of C 3 was found in UTI patients with Gram negative bacteria (18.829±1.4470) mg/ ml and gram-positive bacteria (18.002±1.1120) mg/ ml compared with the control group (19.402±2.9176) mg/ ml. was highly significant for gram-positive *v*ersus control and the P - value of them was 0.002** ($P < 0.05$).

The results showed that a low level of C 4 was found in UTI patients with Gram-negative bacteria (0.760±0.235) mg/ ml and gram-positive bacteria (0.710±0.284) mg/ ml compared with control group (1.205±0.259) mg/ ml. was highly significant for gram-negative *v*ersus control and for gram-positive *v*ersus control the p-value of them was 0.0001**.

4. Discussion

A total of (205) urine and clinical blood specimens collected from patients who suffered from UTI cultured on differentiated media. Results illustrated were 123 (60.0%) gave a positive cultural with 52 (42.2%) of males, and 71 (57.7%) of females. While she was 82 (40.0 %) gave a negative growth as shown in Table (4-1) might be due to other pathogens such as fungi or viruses [14]. These findings are similar to [15], in which the rates of bacterial presence in urinary tract infections were 81.4%. The current results are similarto the [16, 17] findings, as the percentages were 32.6% and 32.6%, respectively, for positive cultures.

The current research found that females had a higher rate of UTI 121 (59.02%) than male patients who were 84 (40.9 %). According to evidence from multiple epidemiological studies, UTI was more prevalent in females than males [19, 20]. Other findings recorded by [21] revealed a high increase rate of females' UTI, which was 268 (68.2%) in females and 124 (31.8%) in males. This might be due to many factors, including a short urethra in females besides its close proximity to the anus, decreased normal flora in the vagina, less pH level of acidity of vaginal endothelial, poor hygienic habits and other anatomical and physiological factors [22].

According to age ranges, the results proved that the age range of 31- 40 years had the most prevalence among patients with UTI **(**35 patients as 17.07 %) followed by age group 41-50 years old of 30 patients (as 14.6%). This finding was in agreement with [23] which showed that the age group 31-40 has more UTI incidence than the other age groups.

E. coli, *K. pneumoniae* besides *S. aureus* were the common prevalent bacteria in UTIs of 35.7%, 14.6% and 13.8%, respectively (table 3). A previous study in Erbil province showed that E.coli reported the highest bacterial percentage of 41 isolates (58.5 %) [24]. Pathogenic bacteria that cause UTIs have more aggressive virulence factors that improve their attachment to host cell and colonization of UT lining to strengthen the bacterial invasion abilities. These bacteria can avoid the host's immune system using virulence factors like lipopolysaccharides, lipopolysaccharide layer, and a variety of other cellular components with capsules [25].

C3 is the most important host immunity element and resembles a UTI risk factor [26]. Several studies have been carried out to determine the C3 role in infections. Complement activation was found to cause damage to the tissues of the host urinary system in studies, inhibiting complement activation and decreasing inflammatory response resulting in tissue damage [27]. The current study also found that an acute UTI did not raise C3 levels in chronic UTI patients. Since C3 is involved in activating both alternative and classical complement pathways, the activation of the complement mechanism is poor in UTI. Other potential causes include the formation of immune complexes, which can cause complement levels can decline within few hours [28]. C3 is involved in the complement cascade, immune complex solubilization, bacterial killing through opsono-phagocytosis, and complex formation, which attacks the membrane of urinary lining, with humoral response in the UT [29].

Many studies suggested that the complement component can facilitate the pathogenic *E. coli* to worsen UTIs; other documents also showed that it enhances invading pathogens into UT tissues [29]. It has been documented that the mice lacking the C3 is resistant to colonization of E. *coli* in uroepithelial cells. Furthermore, opsonization of *E. coli* pathogenic for the extra-intestinal tract by C3 facilitates bacterial attachment and invasion of uroepithelial cells [30]. Harmful bacteria developed many mechanisms to evade immune system that demonstrates complement's importance in the clearance of invading microbes [31]. Capsule production, LPS modification, recruitment of human complement (C4BP, factor H, with FHL-1) regulators to the bacterial surface, in addition to production of proteases enzyme that cleave components of complement [32]. Bacteria can also generate a specific inhibition of complement which blocks many steps in cascade of complement; in short, pathogenic bacteria frustrate C1 activation in UT and block C3 with C5 convertases, besides blocking C5 cleavageand preventing C5aR activation then inhibiting formation of MAC [33]. Simultaneously, C4 is included in activation of classical and lectin pathways against microbial infections. It has a critical role in the prognosis of UTI patients, so it can be a useful predictor in their sera [43, 35].

Conclusion

The findings revealed that *E. coli*, *K. pneumoniae*, and *S. aureus* are the most prevalent pathogenic bacteria responsible for UTI. It was also discovered that the infection of UT was lower in males than females, with significant prevalence in the age range of (31-40) years old. According to immunological parameters, patients with bacterial UTI have lower levels of C3 and C4 in their serum than in the control group.

Ethical approval:

All subjects involved in this study were informed, and the agreement will be obtained verbally from each one before collecting samples.

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