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### Relationship among Some Immunoglobulins, Complement Protein C3 and Urinary Tract Infection of Women Caused by Gram Negative Bacilli in Kirkuk, Iraq

Najdat B. Mahdi, Sahla K. Abbas and Khalaf J. Ahmed\*

Department of Biology, Collage of Science, University of Kirkuk, Iraq

\*Corresponding author

#### KEYWORDS

Urinary Tract Infection, *Escherichia coli*, Immunoglobulins, Complement protein C3.

#### A B S T R A C T

The urinary tract is one of the most frequent sites for happening of bacterial infections, especially in women. Common bacteria related with urinary tract infection were isolated and identified from 400 women patients aged from 14 to 60 years in Azadi Teaching Hospital and General Kirkuk Hospital. The levels of immunoglobulins (IgM, IgG and IgA) and complement protein C3 were evaluated in women infected by *Escherichia coli* and non infected women with UTI. The results refer to the dominance of *Escherichia coli* and high significant ( $P \leq 0.001$ ) was observed for levels of immunoglobulins IgM, IgG and IgA that ranged ( $295.91 \pm 12.51$ ,  $1758.77 \pm 28.52$  and  $473 \pm 14.67$ ) mg/dl respectively. Also there was significant increased ( $P \leq 0.001$ ) in level of complement protein C3 ranged ( $195.13 \pm 10.53$ ) mg/dl.

#### Introduction

Urinary tract infection is an infection of different parts of body's excretory system which involve a bladder, urethra, two ureters, and two kidneys. It is the second most frequent kind of infection in the body (Schappert and Rechtsteiner, 2008). About 10.8 million patients in United States visited an emergency development for treatment of UTI (Sammon *et al.*, 2014). Bacterial organisms which cause UTI may involve *Escherichia coli*, *Klepsiella*, *Pseudomonas*, *Proteus mirabilis* and *Enterococcus fecalis* (Mahmood, 2011; Dromigny, 2005).

Women are more prone to develop urinary tract infection because the wide and short urethra and its proximity to anus. Bacteria can simply enter urethra from rectum and attack urethra, bladder, ureters and may even damage kidney function (Kolawole *et al.*, 2009; AAFP, 2004). Urinary tract infection may include the upper and the lower tract or only the lower urinary tract. The term cystitis has been used to express the symptoms involving dysuria, urgency, frequency and occasionally suprapubic tenderness. Acute pyelonephritis shows the

clinical symptoms characterized by loin pain or tenderness or both and fever often associated with dysuria, urgency and frequency (Mandell, 2005).

Presence of bacterial growth more than 100000 organism per milliliter from correctly midstream "clean catch" urine sample indicate infection (Stamm and Schaeffer, 2002). Complement system is part of the innate immune mechanisms which consist of over (30) proteins, including serum proteins and cell membrane receptors (Abbas *et al.*, 2010; Janeway, 2001). These proteins are produced by liver cells, tissue macrophages, blood monocytes and epithelial cells of the genitourinal tract and gastrointestinal tract. The activation of three pathways leads to generate the protease C3 convertase. After series of complement proteins activation (according to type of pathway) lead to form C5b6789 which is represent membrane attack complex (MAC), this complex forms membrane channel which causes osmotic lyses of the target cell (Andreas *et al.*, 2013; Goldman and Prabhakar, 2001).

One of the first defense mechanism to the uropathogen is the local immune system on the mucosa of the urinary tract, which include urinary immunoglobulin (Secretory IgA). It has been express that local immunoglobulin can connect to the bacteria and inhibit their attachment to mucosal surfaces (Kantele *et al.*, 2008; Thumbikat *et al.*, 2006).

This study aimed to determine the gram negative bacilli causing urinary tract infection and evaluation the levels of serum immunoglobulins (IgM, IgG and IgA) and complement protein C3 in women infected with urinary tract infection caused by *Escherichia coli*.

## **Materials and Methods**

### **Collection of serum and urine samples**

This study was included collection four hundred serum and urine samples from non pregnant women aged range (14 – 60) years come to General Kirkuk Hospital and Azidi Teaching Hospital during period from December 2015 to May2016.

### **Collection of urine samples**

Clean-catch midstream urine samples from non pregnant women were collected in a sterile container (4-5ml) and immediately transport to the laboratory. First step was in urine culture, second step after urine centrifugation 3000 rpm for 10 minute, the supernatant was storage at -20 C0 until analysis (McFaddin, 2000; SancheziCarbayo, 2000).

### **Collection of serum sample**

Venous blood samples were collected from non pregnant women; sera were separated by centrifugation of clotted blood at 3000 rpm for 10 minute and stored at deep freeze until analysis (Al-Hakeim, 2008).

### **Isolation of bacteria**

Urine samples were cultured onto the media Blood and MacConkey agar plate by direct streaking method using a calibrated bacteriological loop measuring 0.001ml of urine, the inoculated plates were incubated aerobically overnight at 37C0 and examined growth after 24 hour of incubation ,If no growth was detected, plates were reincubated for another 24 hour before discarding as negative result (Colle *et al.*, 1996).

### Characteristics of culture

Colonies of isolated bacteria on blood and MacConkey agar plates were described depending on their shape, diameter, color, odor, and other traits. Biochemical tests which achieved for the identification of bacteria were accomplished according to references (Tille, 2014; Pangana and Pangan, 2010; Ryan and Ray, 2004; MacFaddin, 2000).

### Diagnostic by API 20E

Accurate identification tests of bacterial isolates were carried out by api 20 system for Enterobacteriaceae (manufactured by bioMerieux/France) depending on the procedure suggested by the manufacturing company.

### Determination of C3 Protein and Immunoglobulins (IgM, IgG, and IgA) by Single Radial Immunodiffusion Plates were provided by Busserio Company (Italy origin)

The checked protein, diffusing in agarose gel involving a particular antibody will form an immune-complex, obvious as a ring around the well. The ring diameter is direct proportion to the level of the studied protein. The proportion relates to the diffusion period in fact, at the end (72) hour.

Detach the plate from its envelope and put to stand at room temperature for few minute in order that any condensed water in the wells can evaporate. Fill the wells with (5 $\mu$ l) of sample and control and wait it has been fully adsorbing before handling the plate. Close the plate and place it in door of the refrigerator for 72 hour. Measure the precipitating ring with an appropriate ruler. Read on enclosed reference table the

concentration value corresponding to the precipitating ring diameter.

### Statistical analysis

Statistical analysis to be achieved using the Statistical Package for the Social Science (SPSS), a t-test was used to measure the difference in means between two groups. A value  $P \leq 0.001$  was considered statistically significant.

### Results and Discussion

The results of bacterial growth were involved in 400 urine sample from non pregnant women suffered from some symptoms of UTI on blood and MacConkey agar media showed that (124) urine samples had positive result (significant bacteriuria) for bacterial growth on cultural media in percentage (31%), while 276 urine sample showed negative result and absence of bacterial growth on cultural media in percentage (69%) from total number. After perform essential biochemical tests and API 20E. Table (1) showed that the highest microorganisms cause UTI was *E coli* 97 isolate (78.226%), the result agree with Chowdhury and Parial (2015) who found that dominant bacteria isolates was *E coli* (85%) and disagree with Mousa *et al.*, (2015) who found that common bacteria isolates was *Escherichia coli* (35%). Incidence of *Klepsiella pneumoniae* in this study was (10.48%), this result agrees with Chowdhury and Parial (2015) who found the frequency of *Klepsiella pneumoniae* was (13.5%). Also the incidence of *Proteus spp* in this study was (6.45%), this result agrees with Mahmood (2011) who found the incidence of *Proteus spp* (5%). Incidence of *Pseudomonas aeruginosa* in the present study was (4.84%), the results agree with Kareem and Rasheed (2011) who found the

frequency of *Pseudomonas aeruginosa* in women with UTI was (8.75%).

The results of this study showed significant increased in mean level of serum C3 of women infected by *Escherichia coli* ( $195.13 \pm 10.53$ ) mg /dl compared with non infected women ( $102.74 \pm 6.26$ ) mg /dl respectively. There was significant difference among the mean levels of complement protein C3 in the serum of infected women compare to the mean levels of complement protein C3 in non infected women ( $P \leq 0.001$ ).

The mean level of serum complement protein C3 in women infected by *Escherichia coli* was highly significant compared with women non infected with UTI, this result was in agreement with Essa *et al.*(2016) who found that level of C3 protein in serum was elevated in pregnant women with UTI, increased C3 protein in

serum patients with UTI may return to production of complement proteins by hepatocytes and a number of extrahepatic tissues that involved golmerular epithelial cells (Sacks *et al.*, 1993), endothelial cells (Sheerin *et al.*, 1997), mesangial cells (Van den dobbelsteen *et al.*, 1994) and human proximal tubular epithelial cells (Selvarangan *et al.*, 2000) the C3b fragment which produced by C3 split binds Dr fimbriae expressing on uropathogen *E coli* then C3b binds with Decay accelerating factor (CD55) has been located on the surface of human renal epithelial cells (Selvarangan *et al.*, 2000) CD46 (membrane cofactor protein) can act as a human epithelial cell receptor for entering of opsonized uropathogenic *E coli* (Li *et al.*, 2006). The raise in serum IgG in patients with UTI may be the source of the observed increase in the complements in patients group.

**Table.1** Numbers and percentages of isolated gram negative enteric bacilli.

Isolated Bacteria	Number of Isolates	%
<i>Escherichia coli</i>	97	78.23
<i>Klepsiella pneumoniae</i>	13	10.48
<i>Proteus vulgaris</i>	3	2.42
<i>Proteus mirabilis</i>	5	4.03
<i>Pseudomonas aeruginosa</i>	6	4.84
Total	124	100

**Table.2** The serum C3 protein level mean among women's group

Women's group	Infected women by <i>E coli</i>	Non infected women with UTI	P value	95% Confidence Interval
Serum C3 level				
Mean ± SE	195.13 ± 10.53	102.74 ± 6.26	0.001	35.66 - 85.12

**Table.3** The serum Immunoglobulins mean concentration among non pregnant women study

Immunoglobulins level	IgM	IgG	IgA
Women's group	Mean ± SE	Mean ± SE	Mean ± SE
Infected women by <i>E coli</i>	295.91 ± 12.51	1758.77 ± 28.52	473 ± 14.67
Non infected women by UTI	(133.83 ± 8.17	1052 ± 16.4	187.46 ± 10.27
P value	0.001	0.001	0.001
95% Confidence Interval	112.50 – 211.67	507.93 – 903.99	96.55 – 275.02

In the current study the results showed significant increased in mean levels of serum IgM, IgG and IgA of women infected by *Escherichia coli* (295.91 ± 12.51, 1758.77 ± 28.52 and 473 ± 14.67) mg/dl

compared with women non infected by UTI (133.83 ± 8.17, 1052 ± 16.4 and 187.46 ± 10.27) respectively. There was significant difference among the mean levels of immunoglobulins in the serum of infected

women compare to the mean levels of immunoglobulins in non infected women ( $P \leq 0.001$ ).

The mean level of serum immunoglobulins (IgM, IgG and IgA) in women infected by *Escherichia coli* were highly significant compared with women non infected with UTI, this result was in agreement with Ethel *et al.*, (2006) who found that immunoglobulins concentrations in serum were increased in women infected with UTI. Increasing in serum mean levels of immunoglobulins due to activation of B cell by antigenic stimulation that causing B cell to divide and differentiates into an antibody producing cell called a plasma cell. Induction of production specific immunoglobulin without the assist of T lymphocytes for example large molecular weight antigen with regular repeating epitopes involve Pneumococcal polysaccharide, flagellar and fimbrial antigen is IgM as major antibody, this type of antigen called T cell independent antigen (Mohanty *et al.*, 2014). Some bacterial antigens can't induce B cell directly for producing immunoglobulins but by helping T lymphocytes called T cell-dependent antigen, the switch to other isotypes for example IgA and IgG, production needs the presence of cytokines and other signals secreted by locally responding T cells, so two source of immunoglobulins production in patients infected with pathogen has been two type of antigen, these may explain increased in immunoglobulins in non pregnant women infected with gram negative bacilli (Mohanty *et al.*, 2014; Sompayrac, 2012).

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