

Study of some microbial and Immunological parameter in patients with Urinary Tract Infection in Al- Diwanyia city

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الخلاصة

تم جمع 392 عينة من المرضى المصابين بالتهابات المجاري البولية من مستشفى الولادة والأطفال في مدينة الديوانية. أعطت زرعاً مختلطاً أو وضحت النتائج وجود ارتفاعاً معنوياً بحدالات الإصابة بين النساء 276 (68.2%) وبالذكور 124 (31.8%)، وكان المسبب الرئيسي للإصابة هو *E. coli* 276 (70.4%) من العدد الكلي للحدالات، وكان المسبب الثاني هو بكتيريا *Proteus spp* والي 62 حالة بنسبة (15.8%) في حين كانت المسببات الأخرى *Candida spp* 24 (6.12%) و *Staph. aureus* 17 (4.33%) و *Pseudomonas aeruginosa* 6 بنسبة (1.53%) و *Streptococcus spp* 3 (0.72%) و *Klebsiella spp.* 2 (0.5%) و *Enterobacter spp.* 2 (0.5%).

شملت الدراسة المناعية تحديد التراكيز المصلية للأضداد المناعية IgG و IgM و IgA وكذلك معمل تنخر السرطان ألفا وبيتا النتائج التي تم لحصول عليها وجد زيادة معنوية في تركيز ضد IgG عند المرضى (7/65) دولية عما هو عليه في مجموعة السيطرة (13.89) وحدة دولية. سجلت قيم الأضداد (1.35) نقطة دولية، بين كانت قيم IgM المرضي منخفضة معنوياً (3.76) وحدة دولية عما هو عليه في مجموعة السيطرة (15.73) دولية مقارنة بمجموعة السيطرة (2.86) وحدة دولية. أما قيم معامل تنخر السرطان ألفا فقد سجلت هي الأخرى ارتفاعاً في مجموع المرضي (37.5) لعمو غرام/مل، و عليه بمجموعه السيطرة (10.5) بيكو غرام/مل، وكذلك كانت قيم معامل تنخر السرطان بيتا مرتفعة أيضاً وبشكل معنوي (213.11) بيكو غرام/مل، و عليه في مجموعة السيطرة (14.8) بيكو غرام/مل. رالذي يؤكد أهمية الدراسات الوبائية المرضية للتهابات المجاري البولية وضرورة استخدام المعايير المناعية لتحديد مسار الإصابة وفعالية العلاجات المستخدمة.

Abstract

From 392 isolate of UTI inpatients were collected from maternity and pediatrics teaching hospital in Al-Diwanyia city, from September 2008 to June 2010. About 37 samples gave a positive cultures. Results also showed a high increment rate of UTI in the females that males 268 (68.2%) females & 124 (31.8%) males. *E. coli* was the major pathogen of UTI 276 (70.4%) of total cases, the second important pathogen was *Proteus spp.* (15.8%),

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while the other causes of UTI were *Candida albicans* (6.12%), *Staph. aureus* (4.33%), *Pseudomonas aeruginosa* (1.53%), *Streptococcus* spp. (0.72%), *Klebsiella* spp. (0.5%) & *Enterococcus* spp. 0.5% .

Immunological findings included the determination of serum levels of IgG, IgM and IgA , Tumor necrosis factor alpha and beta were also documented . Results showed that IgG concentrations significantly increased 7.765 in patients in comparison to health control group which was 1.25 , While IgM concentrations was decreased in patients (3.76) in comparison to healthy control (13.89). IgA concentrations was increased too in patients (15.73) while in control group was 2.86 .

Tumor necrosis factor alpha levels was clearly increased (237.5) pg/ml in patients in comparison to control (10.5) pg/ml. Tumor necrosis factor beta concentration was also increased in patients (213.11) pg/ml and in control was (14.8) pg/ml. That's explain the importance of studies on UTI and the importance of using an immunological parameters to monitor the disease development and effectivity of the antimicrobial chemotherapy against such diseases.

Introduction

The urinary tract is one of the most commonest sites of bacterial infection. The incidence of UTI is higher among females 10-20% than males, although the majority of infections are acute and short lived, they contribute to significant amount of morbidity in the population, severe infections result in loss of renal function and serious long term consequences (1). In females the distinction is made between cystitis, urethritis and Vaginitis, but . symptoms, often overlap(1,2).

Principle pathogens of urinary tract infection UTI are Enterobacteriaceae with a high predominance of *Escherichia coli*(1,2) bacterial infection is usually acquired by the ascending route from the urethra to the bladder and may proceed to the kidney(1,3,4).

Other Member of enterobacteraceae are *Proteus mirabilis* is often associated with urinary stones (calculi)(2,4). *Klebsiella*, *Enterobacter*, *Serratia*, and *Pseudomonas aeruginosa* are frequently found in hospital acquired UTI while *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, and *Enterococcus*(1,2,5,6,7).

In addition to these genera *Streptococcus faecalis*, *Citrobacter freundis* and *Acentobacter* were been documented by(6). The most frequent causes of UTI in Al-Diwanyia city children were *E. coli* 36.09%, *Klebsiella* spp. 18.25%, *Enterobacter* spp. 13.27%, *Pseudomonas aerugenosa* 2.48%, *Enterococcus faecalis* 1.65 %, *Acentobacter* 0.8%, *Proteus* spp. 17.42% and *Staphylococcus aureus* 5.08%(5). The most frequent causes of UTI in Babylon city children was *Proteus* spp. 7.28 %, *E. coli* 30.71%, *Klebsiella* spp. 11.44% and *Enterobacter* spp. 6.62%(7,8).

Immunological analysis of serum of cytokines represent a helpful tool to evaluate the overall patho-physiological status and also represent an important parameters of activity of many antimicrobial chemotherapy (9). Tumor necrosis factor one of the most potent pro-inflammatory cytokines, which was documented in several separated previous studies (10,11). Cytokine type determine the course of immune response and the effectiveness of defensive mechanisms(12). Immunoglobulines are group of serum proteins with a crucial antimicrobial activity, IgM represent an indicator of recent infection, IgG levels increased in chronic infections, while IgA contain secretory part which help in reducing the mucosal and secretory based infections(10,11). This work was aimed to characterize the microbial causes of UTI in Al-Diwanyia city and evaluate the immune response in patients by determination of levels of IgG, IgM and IgA. Also levels of TNF-alpha and beta.

Materials & Methods

Microbial samples were isolated from 392 inpatient which were positive bacteirurea of Maternity and pediatrics teaching hospital of Al-Diwanyia. Routine diagnostic test were used as a tool for microbial identification, starting by patient history, gross(Macroscopical) appearance, to more presumptive tests which included haemolysis on blood agar, Oxidase, Catalase, Coagulase, Indole, Monitol, Methyl red, Urase, H₂S production & Vogas proskauer tests(5). Also carbohydrates fermentation include five types of sugar; Glucose, Sucrose, Lactose, Maltose and Fructose(6).

Immunological finding included the evaluation of serum concentrations of TNF- α and TNF- β (Sigma diagnostics)(St, Louis,Mo.), also the levels

of IgM, IgG and IgA (bioMerieux)(France).Levels of immunoglobulines and TNF- α and TNF- β were estimated according to manufacture. Elisa techniques were used in the testing of sera samples by using enzyme labeled antibodies and the appropriate substrate (sandwich Elisa in the cases of TNF- α and TNF- β) then standard curves were made to estimate the concentrations of the immune parameters. Statistical analysis included the use of the specialized statistical software SPSS to determine the values of Least significant differences (LSD) in t-test and data was subjected in Excel to draw the paragraphs(13).

Results

From 392 isolate of UTI inpatients about 37 samples gave a mixed cultures, results showed a high increment rate of UTI in the females 268 (68.2%) females than males 124 (31.8%) (fig-1). *E. coli* was the major pathogen of UTI about 276 (70.4%) of total cases, the second important pathogen was *Proteus* spp. About 62 (15.8%) in total, while the other causes of UTI were *Candida albicans* 24 (6.12%), *Staph. aureus* 17(4.33%) , *Pseudomonas aerugenosa* 6 (1.53%) , *Streptococcus* spp. 3 (0.72%) , *Klebsiella* spp. 2 (0.5%) & *Enterococcus* spp. 2 (0.5%) (fig-2).

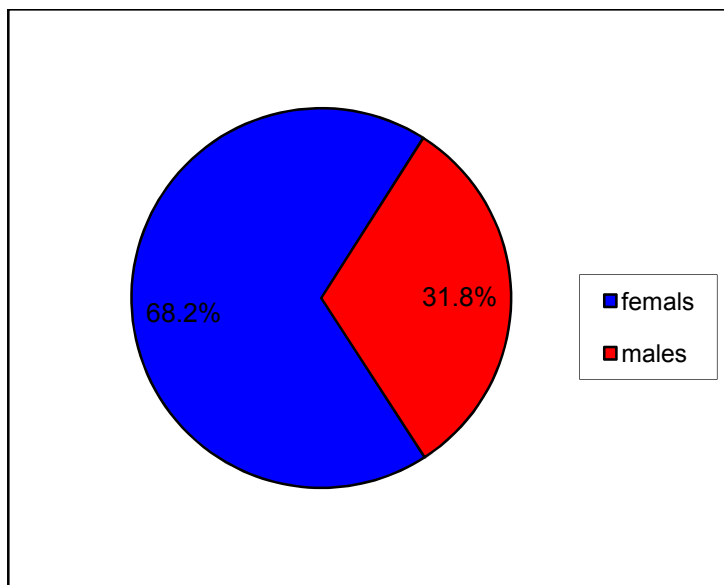


Figure 1:UTI in males and females in Al-Diwanyia

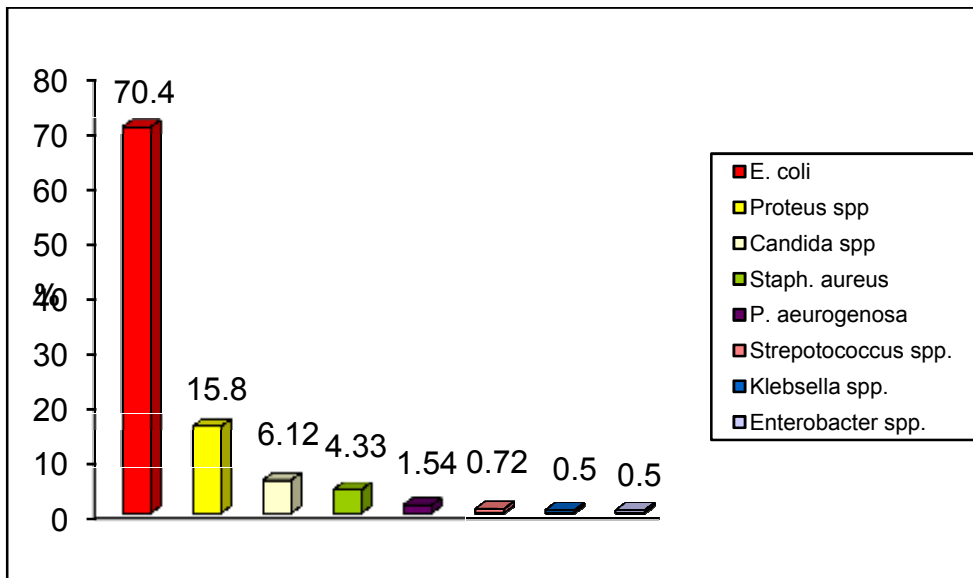


Figure 2: Microbial causes of UTI in Al-Diwanyia

Immunological findings included the determination of serum levels of IgG, IgM and IgA , Tumor necrosis factor alpha and beta was also documented ,obtained results showed that IgG concentrations significantly increased 7.765 IU in patients in comparison to health control group which was 1.25 IU (fig-3). results also showed that IgM concentrations was decreased in patients group 3.76 IU in comparison to healthy control group IU 13.89 (fig-4) . IgA concentrations was increased too in patients group 15.73 IU while in control group was 2.86 IU (fig-5).

Tumor necrosis factor alpha levels was clearly increased 237.5 pg/ml in patients group in comparison to control group 10.5 pg/ml (FIG-6). Tumor necrosis factor beta concentration was also increased in patients group 213.11 pg/ml and in control group was 14.8 pg/ml (FIG-7).

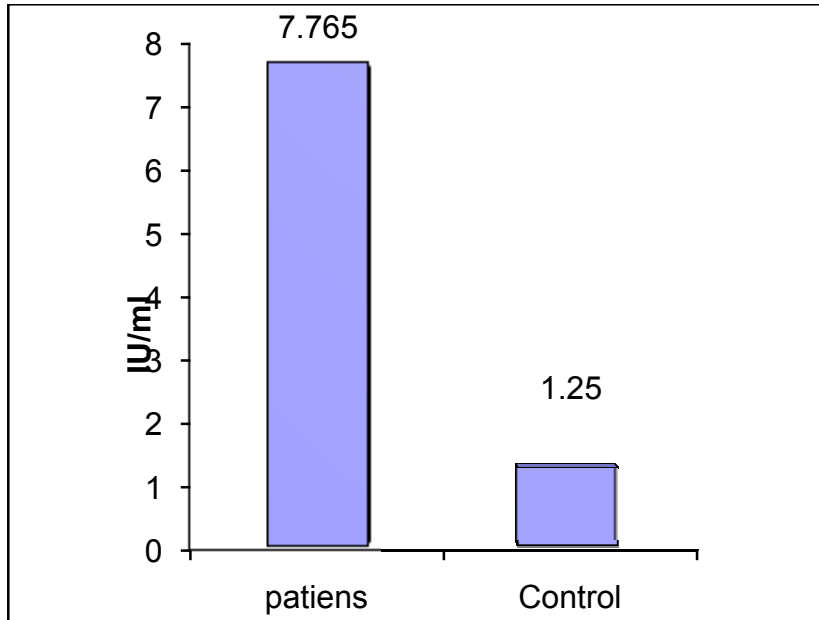


Figure 3: Levels of IgG in serum IU/ml

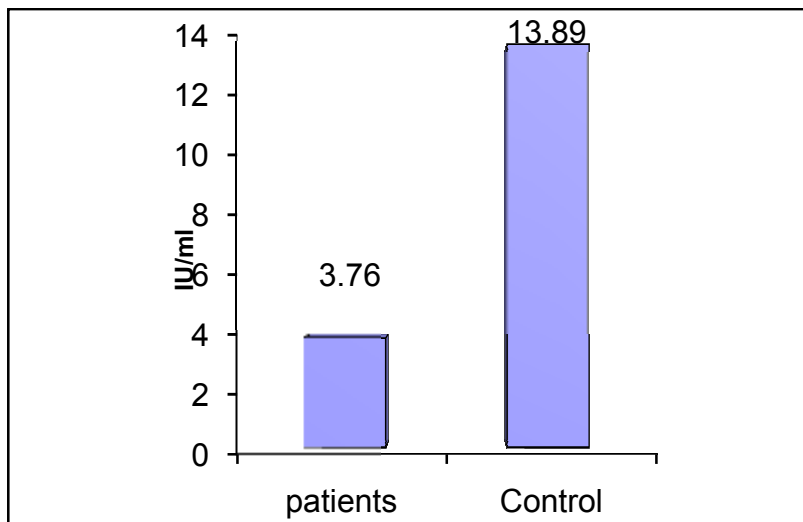


Figure 4: Serum concentration of IgM IU/ml

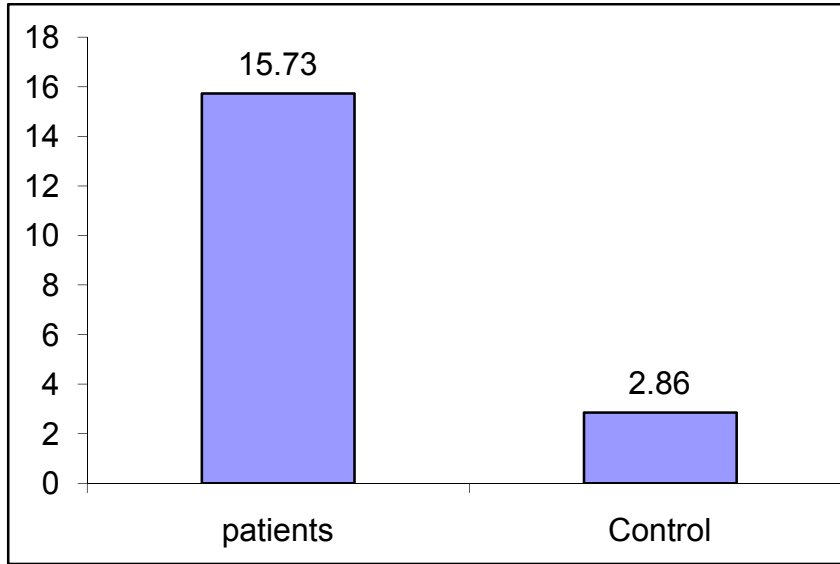


Figure 5: Serum concentration of IgA IU/ml

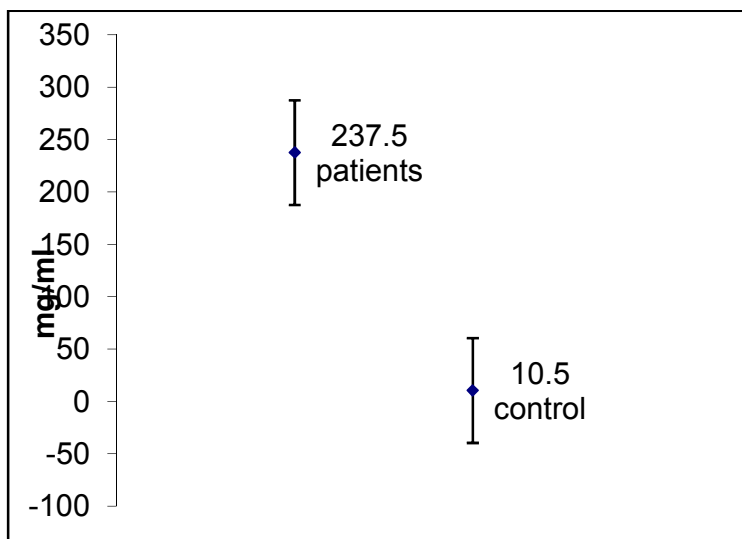


Figure 6: Serum concentration of TNF- α

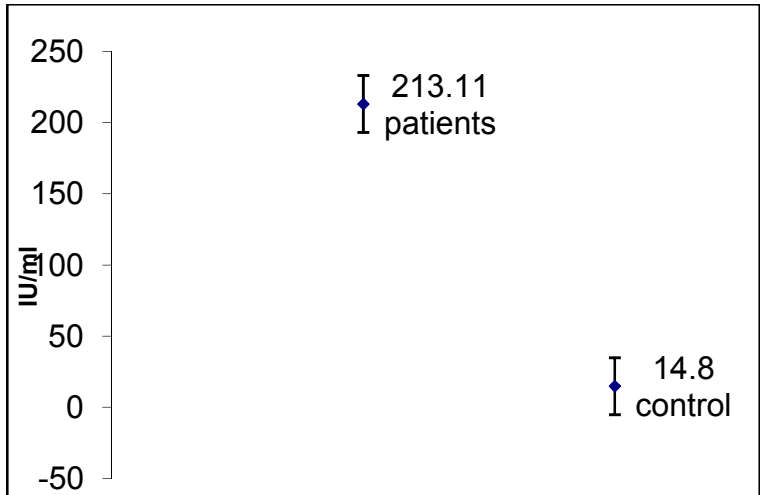


Figure 7: Serum concentrations of TNF-β

Discussion

Most urinary tract pathogens originate in the fecal flora but only the aerobic and the facultative species such as *E. coli* possess the attributes required to colonize and infect the urinary tract(2,9). *E. coli* was the main cause of UTI in this study about 70.4%(fig. 2), the ability to cause infection of the urinary tract is limited to certain serotypes of *E. coli* (e.g. 01.02, 06.07 and 075), the success of these strains may be due to their ability to colonize the peri-urethral areas (3,2,10) *E. coli* have been shown to have particular types of Fimbriae (pili) which enable them to adhere to urethral and bladder epithelium(4,9). Other features of *E. coli* appear to assist in localization of organisms in the kidney and in renal damage, the capsular acid polysaccharide (K) antigens are associated with the ability to cause pyelonephritis and are known to enable *E. coli* strains to resist host defenses by inhibiting phagocytosis(1,2,3,11) Haemolysin production by these strains may also be linked with the capacity to cause kidney damage, many haemolysins act more generally as membrane damaging toxins(3,6). The production of urokinase, by organisms such as *Proteus* spp. which was 15.8% of total recorded cases, has been correlated with their ability to cause pyelonephritis and often associated with kidney stones(Calculi)(6,12). One of the most common causes of UTI in hospitalized patients is *Pseudomonas aeruginosa* due to

its ability to resist different types of antibiotics and other chemicals which used in sterilization processes in hospitals(4,5,14,15).

Among the Gram positive species *Staphylococcus* spp. seems to have particular propensity for causing infection in young and active sexually women(1,6,15).

Enterococcus species are more often associated with UTI in hospitalized patients (1,3,5,15). Non- bacterial causes of UTI include fungi *Candida* spp. mainly infect immunosuppressed patients and young children(9,12). UTI can also associate with catheter in hospitals that's may explain the significant increment of nosocomial infection(4,10,14).

The early defense against bacterial infections depending on the various mechanisms of innate and adaptive immune response, in which cytokines play a crucial role to initiate the inflammatory response(15,16,17). In UTI several previous studies documented an acute increment in cytokine profile in urine and serum(18) .Upon microbial infection and colonization, microbial possess variable types of virulence factors which enable them to colonize and induce pathogenic effects which in turn induce the inflammatory response (19).

Cytokines mediated this response include IL-1,IL-6, and IL-8 (8,9). in general cytokines induced in the inflammatory response achieve lymphocyte development and differentiation and immunoglobulin production, as well as phagocytes activation (10). Cytokines such as IL-1, IL-2, IFN- γ was also increased in many viral and bacterial infections. Previous studies recorded a remarkable increment in levels of immune mediators such as TNF-alpha in UTI which may be decreased by antibiotic treatment this findings come in conformity with results obtained by this study(9,10,19).That's may explain the self limiting nature of the disease and the acute mode pathogenesis in which cytokines play the key role in controlling and directing the immune response and inflammatory response.(20)

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