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Incidence and sensitivity of bacterial uropathogens among diabetic patients

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ABSTRACT

Urinary tract infections (UTI) are common in diabetic patients. This investigation was based to evaluate the incidence of UTI in patients with DM. All urine samples were processed in the lab following standard laboratory protocol. Commonly recovered UTI isolates were E.coli, K.pneumoniae, Pseudomonas sp. and S. aureus. UTI was alarming in diabetic patients belonging to the lower socioeconomic status. In type 1 diabetic patients E.coli (38.09 %) was the most prevalent cause of UTI. Varieties of factors are responsible for UTI in diabetic patients which include genetic susceptibility, and damaged immune response. The sensitivity of the isolates of 13 antibiotics was tested. The results showed a variance as far as their resistance to these antibiotics. Imipenem is the most effective antibiotic on the studied bacteria isolates. On the other hand, bacteria isolates showed high resistance to Penicillins and Cephalosporins antibiotics represented Cefotaxime (62%), Cephalexin (74%), Amoxicillin (77%), and Piperacillin (64%).

Key words: UTI, diabetic, uropathogens, antibiotics

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by increased blood glucose level resulting from defects in insulin secretion, insulin action, or both(1) .The chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Diabetes mellitus has long been considered to be a predisposing factor for urinary tract infection (UTI) and the urinary tract is the principle site of the infection in diabetics with increased risk of complications of UTI (2, 3) .

The incidence of diabetes mellitus is increasing markedly throughout the world and is becoming a serious public health threat particularly in the developing countries. Diabetes mellitus is associated with many complications and in the long run it has some major effects on the genitourinary system which makes diabetic patients more liable to UTI, particularly to upper urinary tract infections (4,5) .

Diabetes mellitus has a number of effects on urinary system. Patients either with Type1 DM or Type 2 DM are at increased risk for urinary tract infection. Diabetes causes several abnormalities of the host immune system that may result in a higher risk of infections like UTI (6) .

Patients with diabetes have a 10-fold increased risk of UTI when compared to non-diabetics (7) and diabetics have a longer hospitalization then non-diabetics (8). Diabetes has long been considered to be a predisposing factor for urinary tract infection. In females, the urinary tract has an important association with the reproductive organs because of its proximity (9). Women with diabetes have higher risk of UTI because of changes in immune system. Any other disorder that suppresses the immune system raises the risk of urinary infection. The increased frequency of UTIs in diabetic patients is likely due to several factors. Suggested host-related mechanisms are: (a) the presence

of glycosuria; (b) defects in neutrophil function and (c) increased adherence to uroepithelial cells (10). Based on the facts addressed above, the present work was intended to study the prevalence of bacterial Uropathogens among diabetic patient in some of Basrah hospitals .

MATERIALS AND METHODS

Sample collection

A total of 60 urine samples were collected from diabetic patients presenting at Basrah Hospital , Iraq within a period of five months in 2013 . Each patient was asked to collect approximately 10-20 ml of midstream urine into a sterile wide mouth universal container. The urine samples were transported in cooler boxes to the microbiology laboratory, Basrah University for bacterial investigation within 4–6 hrs of collection .Until culture time, the urine samples were stored at 2–8◦C in refrigerator. Diagnosis of diabetes was made based on the WHO criteria (11).

Identification of Uropathogens from urine samples

The urine samples were cultured on Blood agar, MacConkey agar and Cysteine Lactose Electrolyte Deficient Agar (CLED) and the plates were incubated at 37^0C for 24 h. The plates containing more than 10 CFU/ml colonies were selected as significant growth (12). The bacterial isolates were characterized and identified by API system (API 20E, API Staph and API 20-strept). In addition, the cultural and morphological features such as catalase, coagulase, motility, oxidase, Indole, Methyl-Red, Voges-proskauer, citrate utilization, urease, carbohydrate oxidation/ fermentation etc. described by Morella *et al.* (13) .

Glycated hemoglobin (HbA1c) Quantification: HbA1c was quantified spectrophotometrically using HbA1c test kits (Agappe diagnostics, Kerala India). Briefly, hemolysate was prepared from heparin anticoagulant whole blood samples. The HbA1c fraction were then specifically eluted after washing away the HbA1a+b fraction and quantified by direct photometric reading at 415 nM. Poor glycaemic control was defined as HbA1c < 7.0% as recommended by the American Diabetes association (14).

Antimicrobial susceptibility

Antimicrobial sensitivity testing of all isolates was performed on diagnostic sensitivity test plates by the Kirby Bauer method (15) following the definition of the National Committee of Clinical Laboratory Standards (NCCLS, 1999) (16). Bacterial inoculums were prepared by suspending the freshly-grown bacteria in 25 mL sterile nutrient broth. A sterile cotton swab was used to streak the surface of Mueller Hinton agar plates. Filter paper disks containing designated amounts of the antimicrobial drugs obtained from commercial supply firms (Himedia Labs, Mumbai, India) were used. The antimicrobial agents tested were Amoxicillin (10µg), Cephalexin (30µg), Cefotaxime (30pg), Ciprofloxacin (5pg), Norfloxacin (10g) Nitrofurantoin (300µg) Amikacin (30µg), Gentamicin (30 µg), Augmentin (Amoxicillin /clavulanic acid) (20/10µg), Imipenem(10 µg), Trimethoprim(SXT) (5µg), Piperacillin (100 µg),and Aztreoname (30 µg).

Statistical analysis: This was carried out using SPSS-16. The association between glycaemic and UTI was assessed. A *p*-value<0.05 was said to be significant.

RESULTS

The results obtained for the selected factors that may interfere with UTIs in diabetic patients as mentioned in methods were statistically analyzed by Chi square test: 1-Gender: out of 60 diabetic patients, 21(35%) of them had bacteriuria irrespective of gender. Amongst 40 females and 20 males, the percentage of UTIs among females (42.5 %) in which was higher than that of the males (20 %) and it is statistically significant (P< 0.01) (Table 1). 2-Age: (Table 2) represents distribution of UTI among different age groups in both genders, the percentage of UTI among patients of age (31-40) was (46.15%) higher than the other rest group of ages, but statistically not significant.

Table-1- : Distribution of UTI among diabetic patients (males and females)

Diabetic Patients	Male No.%	Female No.%	Male and Female. No.%
Positive UTI(significant bacteriuria)	4 (20 %)	17 (42.5 %)	21 (35 %)
Negative UTI (non significant)	16 (80 %)	23 (57.5 %)	39 (65 %)
Total	20	40	60

Significant difference between males and females,((P<0.01) .

Table-2-: Distribution of age among UTI diabetic patients

Diabetic patients	17-30 Years No. (%)	31-40 Years No. (%)	41-50 Years No. (%)	51-60 Years No. (%)	≥61 Years No. (%)	Total No. (%)
Positive UTI	2 (40)	4 (57.1)	14 (50)	9 (42.8)	2 (22.2)	21 (35)
Negative UTI	3 (60)	3 (42.85)	14 (50)	12 (57.1)	7 (77.7)	39 (65)
Total	5	7	28	21	9	60

The prevalence of the uropathogens in diabetic patients is shown in (Table-3). The data analysis of reports of the patients showed a considerably high prevalence of *Escherichia coli* infections (38.09%, 8 cases). Among other Gram negative bacilli, 4 (19.04%) were *Klebsiella pneumonia*, 3(14.2%) were *Pseudomonas* sp. and 2(9.5%) were *Proteus*. In addition 3(14.2%) out of 21 isolates were *Candida albicans* (4.7 % ; 1 cases).

Table-3-: Numbers and percentages of the types of microorganisms causes UTI in diabetic patients (n=60)

NO.	UTI Pathogens	NO.	(%)
1	*E.coli*	8	38.09
2	*Klebsiella pneumoniae*	4	19.04
3	*Pseudomonas* sp.	3	14.2
4	*Staphylococcus aureus*	3	14.2
5	*Proteus* sp.	2	9.5
6	*Candida albicans*	1	4.7
Total		21	35

The results showed a variance as far as their resistance to these antibiotics. In concerned, Imipenem was the most effective antibiotic on bacteria isolates (gram negative and positive), (figure-1) showed percentages resistance bacteria of antibiotics. The percentages of resistance of all isolates to the antimicrobial agents were: 62% to Cefotaxime, 74% to Cephalexin, 77%to Amoxicillin (AX), 64% to Piperacillin, 50% to Aztreoname, 43% to Gentamicin, 3l% to Amikacin, 24% to Ciprofloxacin, 28% to Norfloxacin, 62% Augmentin, 40% Trimethoprim (SXT), 44% to Nitrofurantoin and 4% to Imipenem.

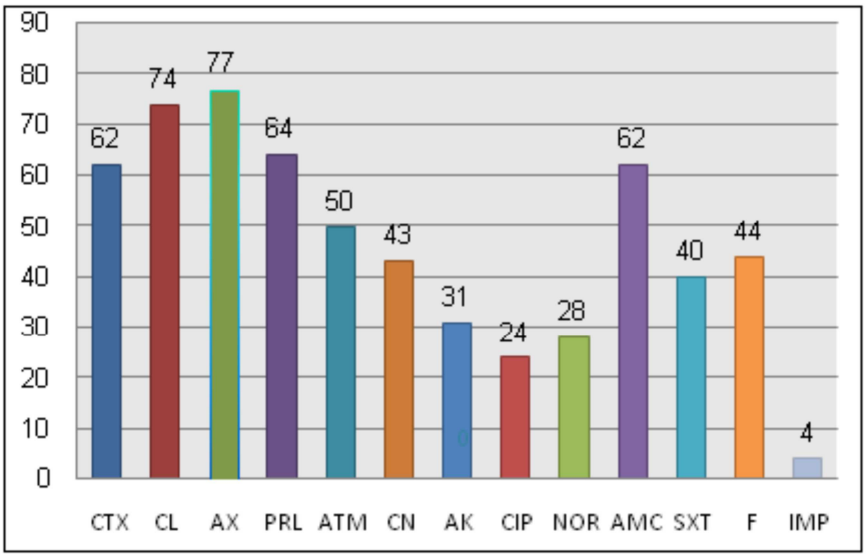

Fig. 1: Percentages resistance of bacteria for antibiotics

DISCUSSION

Several different factors accompanied with UTIs are thought to be linked with diabetic patients. The results of this study showed that among these factors; gender and age are likely to be the most effective factors. These factors are vary in their occurrences comparing with people who are non-diabetic. Therefore, it is necessary to shed a light upon each single factor in order to estimate, in details, the correlation between two parameters , UTIs and DM (diabetes mellitus). As mentioned in many studies, women are more prone to UTI compared with men (17). Different groups of diabetic and non-diabetic participants showed that UTIs women are forming higher ratio, whereas the lowest was

for men. Women infected with UTI via different ways including anatomical and behavioral, (Table1). In previous works, the most convenient susceptible age of UTI in diabetic patients was below 44 years old. Yass *et al* (18), have found that the susceptible age for UT infection was between 10-30 in males and over 51 in females (both were non-diabetics). Other signs indicate to other condition such as age over 65 years old. A matter indicates that age is not exerting a dramatic influence neither in UTI nor in DM, (Table2).

In the present study we have attempted to determine the distribution of various bacteria causing UTI among the diabetics, an immunocomprised population, and their antibiotic susceptibility pattern . In the general population, most urinary tract infections are caused by *Escherichia coli* and affect mainly women because of sexual activity and pregnancy. Prevalence in women is also due to decrease of normal vaginal flora (*Lactobacilli*), less acidic pH of vaginal surface, short & wide urethra, proximity of urethra to anus and poor hygienic conditions (19).

The prevalence of UTI among the diabetic patients was found to be 35 %.such high prevalence were also observed by Saleem M & Daniel B (20) and other studies by Njunda AL et al and patil NR et al have also demonstrated comparatively prevalence of 34.4% & 36% of UTI in diabetics respectively (21). Contrary to our findings, Geerling et al have reported a prevalence of 26.0% of UTI in diabetic diabetic patients (22).

Bacteria colonizing the perineum and vagina can enter the bladder and further ascend to the kidney. The essential step in the pathogenesis of UTIs is the adherence of uropathogens to the bladder mucosa. Adhesins are therefore important virulence factors. Although virulence factors have been characterized best in *E. coli* (the most common uropathogen), but many of the same principles may be applicable to other uropathogens; for example *Klebsiellae pneumoniae* (23).The present study showed different uropathogens with different percentages, (Table 3).

The resistance of bacterial isolates under study for Quinolones antibiotics which included Ciprofloxacin and Norfloxacin were proportion of resistance (24%) and (28%), respectively of the total isolates under study, that cause of resistant isolates under study for Quinolones antibiotics used could be due to a change in the target site fora link to antibiotics on enzyme, as it even in the change (GyrA), one of the structural blocks of an enzyme (DNA gyrase) (24) .

While the antimicrobial resistance group Aminoglycoside and involved in Gentamycin, Amikacin and that the ratio of their resistance (43%) and (31%), may be attributed cause of bacterial resistance to antibiotics Aminoglycoside three mechanisms: modification by enzymes modified such as Adenylating, Phosphorylating Acetylating or mutation such as chromosomal mutation in the gene coding for the target protein in under small unit ribosome 30S, causing the loss of affinity to link target protein and reduce the permeability of bacterial cell of the antibiotic (25). On the other hand , as for Imipenem which belongs to the group Carbapenems showed isolates sensitive large and the rate of resistance (4%). The cause of the resistance has to developments in the mechanisms of resistance of bacteria

All the isolates in this study showed resistance to at least 5different antibiotics, indicating the presence of strong selective pressures from the antibiotics in the community. Brown *et al*. (26) have reported that horizontal gene transfer is a factor in the occurrence of antibiotic resistance in clinical isolates and suggested that the high prevalence of resistance to a particular antibiotic does not always reflect antibiotic consumption as previously suggested by Nwanze *et al*.(27) .

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