Study of Ciprofloxacin Resistant *Escherichia coli* (CREC) in Type 2 Diabetic Patients with Symptomatic Urinary Tract Infections

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Abstract:

Background: Type 2 diabetes is the most common form of diabetes among diabetic population. *Escherichia coli (E. coli)* remain the predominant uropathogen in diabetics and this pathogen is traditionally associated with urinary tract infections (UTIs) and it has a high resistance for antibiotics. The risk factors for CREC isolates were included: prior ciprofloxacin use, other quinolone use, self catheterization and recurrent UTI.

Objectives: To determine the prevalence of CREC in type 2 diabetic patients with symptomatic UTI, and the prevalence of bacterial infections among those patients.

Patients and Methods: The present study included 100 type 2 diabetic patients with symptomatic (UTIs) attended the National Diabetes Center (NDC) of Al-Mustansiria University, and 20 with age matched non-diabetic patients with symptomatic(UTIs) association with *E. coli*. Data regarding age, sex, plasma glucose (Random or Fasting) and urine samples for direct examination and cultures were investigated. Serum levels of glucose were determined by using auto-analyzer system (Abbott). The diagnosis of bacteria isolates and their in vitro susceptibility to antibiotics were evaluated by Kirby Bauer method.

Results: Out of 100 diabetic patients with symptomatic UTI, 50 patients had positive growth culture, of those 34 patients had pathogenic *E. coli.* The percentage of ciprofloxacin resistant *E. coli* was 70.6%. There was a significant difference between the diabetic patients with ciprofloxacin resistant *E. coli* and control group ($P< 0.01$).

Conclusions: High resistant activity of *E. coli* to ciprofloxacin was recorded in this study among diabetic group compared to non-diabetic group. The increase in ciprofloxacin resistance might be attributed to the diabetes associated with UTI and considering diabetes as one of the risk factor.

Key words: Type 2 diabetes, Urinary tract infections, ciprofloxacin resistance and *E coli*

Background

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia and other signs. The World Health Organization(WHO) recognizes three main forms of diabetes: type 1, type 2, and gestational diabetes,which have similar signs, symptoms, and consequences, but different causes and population distributions.Type 2 diabetes is the most common form of diabetes accounting for 85-90 % of diabetic population. [1]

Type 2 DM which is previously known as adult-onset diabetes, maturity-onset diabetes, or non-insulin-dependent diabetes mellitus (NIDDM), is due to a combination of defective insulin secretion and insulin resistance or reduced insulin sensitivity, which almost certainly involves the insulin receptor in cell membranes.[2]

The urinary tract is the principal site of infection in diabetics. A changed bacterial adhesion to the uroepithelium, partly as a result of a changed and lowered Tamm Horsfall Protein, and granulocyte dysfunction, possibly as a result of an abnormal intracellular calcium metabolism, are involved in the pathogenesis of urinary tract infections in diabetics.[3]

Escherichia coli (E. coli) remain the predominant uropathogen in diabetics and this pathogen is traditionally associated with UTI particularly because of antimicrobial resistance. *E. coli* is a gram-negative bacterium of the *Enterobacteriaceae* family, normally colonizes the large intestine. In fact, colonization of mucosal surfaces by *E. coli* is common, but most strains of the bacterium are not invasive. *E. coli* causes the vast majority of UTIs, ranging from uncomplicated cystitis to sepsis [4].

The symptomatic UTI is requiring specialized treatment strategies. There is a greater likelihood of UTI affected by antimicrobial resistance or atypical uropathogen. Therefore, pre or post therapy urine cultures are indicated. This situation is further complicated by the fact that accurate diagnosis depends on both the presence of symptoms and a positive culture. [5]

Ciprofloxacin is a fluoroquinolone that has been in clinical use for 15 years. Ciprofloxacin is recommended for the empiric treatment of urinary tract infection UTI. However, recent studies also have demonstrated an increase in the isolation of Ciprofloxacin-resistant *Escherichia coli*. [6]

Laino[7] found that, the risk factors for CREC isolates were included: diabetes, prior ciprofloxacin use, other quinolone use, self catheterization and recurrent UTI.

Objectives:

The aims of this work are to determine the prevalence of CREC in type 2 diabetic patients with symptomatic UTI, and the prevalence of bacterial infections among those patients.

Patients & Methods

The present study included 100 type 2 diabetic patients with symptomatic (UTIs) attended the National Diabetes Center (NDC) of Al-Mustansiria University.

Data regarding age, sex, serum glucose (Random or Fasting) and urine samples for direct examination and cultures were obtained. The diagnosis of patients with symptomatic UTI was depended on the clinical features and the present of pus cells in direct microscopic examination (≥ 10 Pus cells/HPF). The diagnosis of diabetes mellitus (DM) based on the following criteria: history of diabetes, age of onset and the use of glucose lowering medications (oral agents or insulin).

The control group included 20 non diabetic age matched patients with symptomatic UTI attended the Urologic Clinic in Al-Yarmook Teaching Hospital. Those entire subjects showed positive *E. coli* cultures.

A mid-stream urine (MSU) samples were obtained for microbiological laboratory for direct examination (Microscopic urinalysis) and culture. Pyuria was defined as the presence equal or more than 10 Pus cells /HPF), this mentioned number was a more reliable breakpoint for making the diagnosis. [8]& [9]

The diagnosis of bacteria isolates and their in vitro susceptibility to antibiotics was evaluated. The urine samples were cultured on blood agar and MacConkey agar media, the growth of pathogenic *E coli* was confirmed by using IMViC and *Api* 20E tests, then all pathogens were tested for sensitivity test on Muller-Hinton agar by using Kirby Bauer method, the diameter of the inhibition zone was measured by using standard table of antibiotic susceptibility. Other tests, such as oxidase and urease enzymes were also used in the diagnosis of some pathogens [4].

All blood samples were obtained by vein puncture. Then, serum levels of glucose were determined by using auto-analyzer system (Abbott).

The data were presented in simple measure of frequency, percentage, mean and SD. chi-square (X^2) was used for testing the significance of difference of the qualitative data. P value ≤ 0.05 was used as the level of the significance.

Results

A total number of 100 patients with type 2 diabetes were enrolled in this study .The mean age group of diabetic patients was 51.07+8.01 with a range 37 t0 73 years, the female and male numbers were 74(74%) and 26(26%) respectively. The mean age of diabetic patients with *E. coli* was 59.0+9.21 with a range of 39 to 73 years. The females to males numbers was 31(91.2%) and 3(8.8%) respectively. The highest percentage of age group was within 40-49 range was 40% and 38.2% for total diabetic and diabetic patients with *E. coli* (Table 1). The mean serum glucose was examined statistically (Table 2) and shows significant difference between diabetic group 174.62+69.90 and control group 92.20+9.52 (P<0.0001).

The control group was included 20 non diabetic patients with mean age of 46.85+7.77 with a range of 33 to 64 years.

Table -1- Age and sex groups among the total number of diabetic patients and diabetic patients with *E. coli*

		Diabetic Patients		Diabetic Patients with *E coli*	
		NO.	%	NO.	%
Sex	Females	74	74.0%	31	91.2%
	Males	26	26.0%	3	8.8%
Total		100	100.0%	34	100.0%
Age (years)	30--	6	6.0%	1	2.9%
	40--	40	40.0%	13	38.2%
	50--	37	37.0%	11	32.3%
	60--	17	17.0%	9	26.5%
Total		100	100.0%	34	100.0%

Table-2- The mean of serum glucose level among diabetic and non diabetic groups

Test	Diabetic patients (n=34)	Non diabetic patients (n=20)	T;P
Mean Serum glucose mg/dl	174.62+69.90 (85-423)	92.20+9.52 (74-108)	5.25;0.0001*

Table 3 shows the number patients with pathogenic isolates. Out of 100 patients with symptomatic UTI, 50 patients had positive culture growth, of those 34 patients had pathogenic *E coli*, 3 patients had *Enterococcus faecalis*, 3 patients had *Proteus* sp., 2 patients had *klebsiella* sp, 2 patients had *Pseudomonas* sp and 6 patients had growth of *Candida* sp.

The different antibiotic sensitivity patterns for *E coli* were shown in (Table 4) and (Figure 1).The percentage of resistance for *E coli* were 70.6% to ciprofloxacin, 70.6% to gentamicin, 47.0% to nitrofurantion, 88.0% to co-trimoxazole, 76.5% to tetracycline, 85.3% to ampicillin, 82.4% to cefixime, 58.8% to cephotaxime and 70.6% to nalidixic acid. There was a significant difference between the diabetic patients with ciprofloxacin resistance *E coli* and control group ($P< 0.01$).

Table-3- Microorganisms isolated from diabetic patients with symptomatic UTIs

Culture growth	Diabetic patients	
	NO.	%
E coli	34	34.0%
E faecalis	3	3.0%
***Klebsiella* sp**	2	2.0%
***Proteus* sp**	3	3.0%
***Pseudomonas* sp**	2	2.0%
***Candida* sp**	6	6.0%
No growth	50	50%
Total NO.	100	100.0%

Table-4- Different antibiotic sensitivity patterns for *E coli* among diabetic and non diabetic patients with symptomatic UTIs.

		Diabetic patients with *E coli* (n=34)		Non diabetic patients (control group) (n=20)		χ^2;d.f;P
		NO.	%	NO.	%	
CF	**R**	24	70.6%	7	35.0%	6.52;1;0.011*
	S	10	29.4%	13	65.0%	
GM	**R**	24	70.6%	-	-	
	S	10	29.4%	-	-	
FT	**R**	16	47.0%	-	-	
	S	18	53.0%	-	-	
CO	**R**	30	88.0%	-	-	
	S	4	12.0%	-	-	
TE	**R**	26	76.5%	-	-	
	S	8	23.5%	-	-	
AM	**R**	29	85.3%	-	-	
	S	5	14.7%	-	-	
CFM	**R**	28	82.4%	-	-	
	S	6	17.6%	-	-	
CE	**R**	20	58.8%	-	-	
	S	14	41.2%	-	-	
NA	**R**	24	70.6%	-	-	
	S	10	29.4%	-	-	

Ciprofloxacin (CF), Gentamicin (GM), Nitrofurantion (FT), Co-trimoxazole (CO), Tetracycline (TE), Ampicillin (AM), Cefixime (CFM), Cephotaxime (CE), Nalidixic acid (NA)

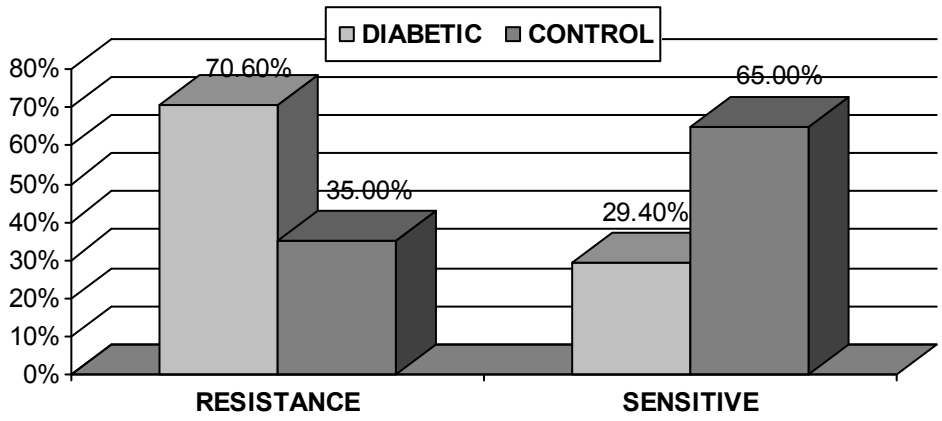

Figure-1- Comparison between the percentages of ciprofloxacin resistance *E coli* between diabetic and non diabetic patients (control group)

Discussion

Urinary tract infections are among the most common diseases in the world. While short course antibiotic therapy is highly effective in out-patients

with uncomplicated UTI, treatment of complicated UTI is more likely to result in clinical failure. Key factors that may contribute to reduced antibiotic efficacy in complicated UTI include higher baseline patient risk such as diabetes, broader spectrum of causative pathogens and higher likelihood of infection with an antibiotic resistant bacterial pathogen [10].

(Table 1) shows the highest percentage of age group was found within 40-49 range was 40% and 38.2% for total diabetic and diabetic patients with *E. coli*. The mean serum glucose level in diabetic patients is high (174.62%) as shown in (table 2), this is indicated that most of those patients were under uncontrolled program, therefore it is necessary to maintain normal or near normal glucose levels [1].

The results of this study indicated that out of 100 diabetic patients (type 2), 50 (50%) were found to be with positive urine culture (Table 3). It was found that *E. coli* was the most frequent pathogen isolated (68%) (34 out of 50). This result is in agreement with that reported by Bonadio *etal*[11]. The urinary tract is the principle site of infection in

diabetics, in which diabetic patients run an increased risk of complications of UTI [3].

The sensitivity pattern of *E. coli* in patients with type 2 DM (Table 4 and Figure 1) showed that 70.6% of isolates were resist to ciprofloxacin antibiotic compared to 35% of isolates from non diabetic patients (control group) with a high significant difference ($P< 0.01$) checked by X^2 method. (Table 4) also showed that resistance percentages of *E. coli* to other types of antibiotics applied in this study. This table revealed that ciprofloxacin has a considerable percentage of resistance compared to the other antibiotics and this percentage was higher than that quoted by *Killgor etal*.,[6] and Medscape[12]. The problem of bacterial antibiotic resistance emerged as soon as the first antibiotics become available for clinical use. The antimicrobial sensitivity pattern of *E. coli* isolated from patients with complicated and uncomplicated UTI revealed an increased resistance to the usual drugs of choice for UTI such as amoxicillin, ampicillin and co-trimoxazole. The most common underlying conditions identified were diabetes mellitus and pregnancy [13].

In the present study, the highest resistance of *E. coli* was noted to co-trimoxazole antibiotic 88.0% (Table 4). This result is in agreement with that found by Laino [7], who reported that (92%) of *E. coli* isolates were resist to co-trimoxazole, which has been the most widely used therapy for UTI over the past decade, the likelihood of treatment failure is increased significantly in UTIs caused by an antibiotic-resistant uropathogen [10].Whereas, the lowest resistant reported in our study to Nitrofurantion was (47%), this result is in agreement with the same reference mentioned before [7].

The sensitivity profile of ciprofloxacin to *E. coli* is still contradictory since some literature stated that the resistance of uropathogenic *E. coli* in non-hospitalized women with DM is not higher than that seen in routine isolates of *E. coli*, this suggest that diabetes in itself is not a risk factor for resistance[14]. This result disagreed with our result and also contradicted with the results of other workers, which indicate high resistance of ciprofloxacin by *E. coli* isolated from diabetic patient group. In univariate analysis, the risk factor for ciprofloxacin-resistant *E. coli* infection that were identified included prior ciprofloxacin use, other quinolone use, other antibiotic use, self catheterization, recurrent UTI and diabetes[7].

Other workers [15] reported that *Klebsiella* sp. in nosocomial UTI of diabetics showed an overall increase in resistance to antibiotics, and antibiotic resistance in the organisms isolated from nosocomial UTI is greater than that related to community-acquired UTI in diabetic patients. This work suggests that bacteriologic pattern of UTI (i.e.nosocomial or community acquired UTI) is a determinative factor beside diabetes.

Gupta *etal.,*[16], stated that resistance of *E. coli* to ciprofloxacin remained low throughout 5 years evaluation study, while Schaeffer[17] stated that potential disadvantages of fluoroquinolones include cost and risk of development of resistance. Goettsch *etal.*[18]suggested that resistance to fluoroquinolones maybe expected to increase in the future.

The cause of the contradictory data considering evaluation of diabetes as a risk factor for increasing *E. coli* resistance to ciprofloxacin may be related to the types of patients involved in each study such as geographical distribution, severity of UTI, age and prior antibiotic use.

Conclusions

High resistant activity of *E. coli* to ciprofloxacin was recorded in this study among diabetic group compared to non-diabetic group. The increase in ciprofloxacin resistance might be attributed to the diabetes associated with UTI and this lead to suggest that diabetes could be considered as one of the risk factor.

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